Research Topics

Food safety in low and middle income countries

Edited by
Delia Grace, Bassirou Bonfoh, Hung Nguyen-Viet and Barbara Häsler

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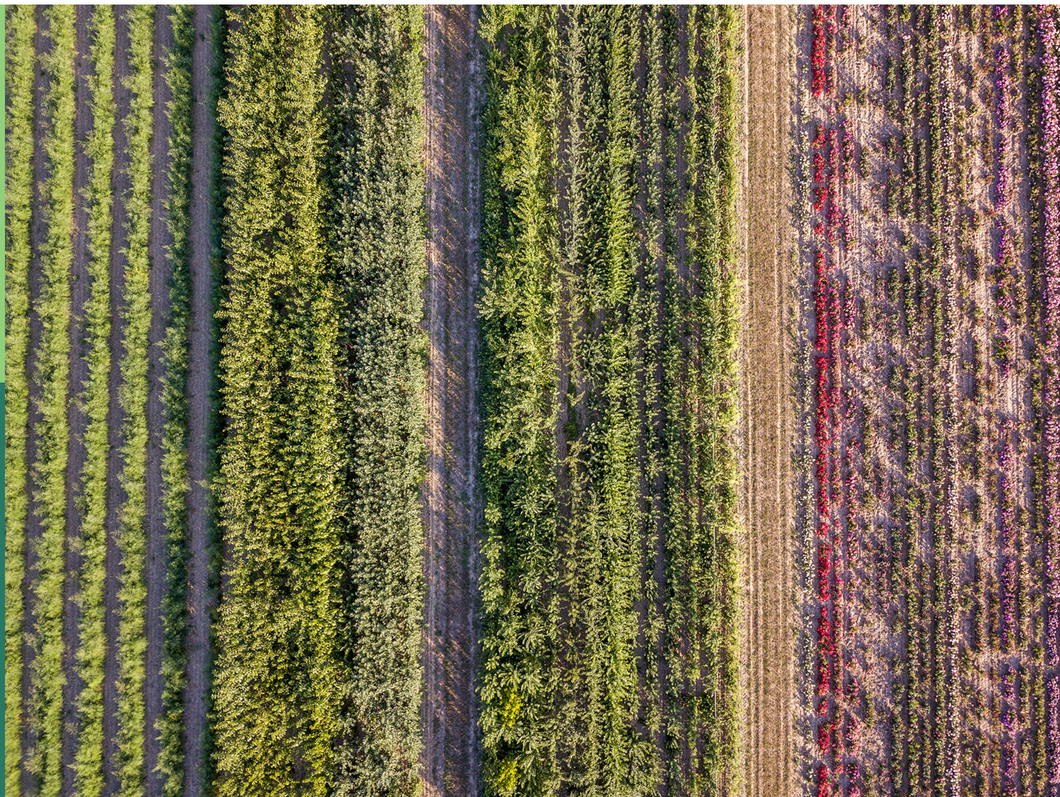

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Food safety in low and middle income countries

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Table of contents

06 Editorial: Food safety in low- and middle-income countries
Delia Grace, Bassirou Bonfoh, Barbara Häsler and Hung Nguyen-Viet

12 Food safety knowledge and risk perception among pregnant women: A cross-sectional study in Jordan during the COVID-19 pandemic
Safaa Almanasrah, Tareq M. Osaili, Anas A. Al-Nabulsi, Nail A. Obeidat, Amer M. Sindiani, Lina Elsalem, Hana Alkhalidy, Maysm N. Mohamad, Sheima T. Saleh, Rameez Al Daour, Ayesha S. Al Dhaheri and Leila Cheikh Ismail

27 The governance of quality and safety in Tanzania's informal milk markets
Emma Blackmore, Alejandro Guarin, Charity Kinyua, William Vorley, Delia Grace and Silvia Alonso

38 Burden of foodborne disease due to bacterial hazards associated with beef, dairy, poultry meat, and vegetables in Ethiopia and Burkina Faso, 2017
Arie H. Havelaar, Amanda C. Sapp, Mirna P. Amaya, Gabriela F. Nane, Kara M. Morgan, Brecht Devleesschauwer, Delia Grace, Theo Knight-Jones and Barbara B. Kowalcyk

53 Antibiotic use, knowledge, and practices of milk vendors in India's informal dairy value chain
Garima Sharma, Eithne Leahy, Ram Pratim Deka, Bibek R. Shome, Samiran Bandyopadhyay, Tushar K. Dey, Naresh Kumar Goyal, Åke Lundkvist, Delia Grace and Johanna F. Lindahl

63 Food safety knowledge, needed and trusted information of pork consumers in different retail types in Northern Vietnam
Trang Thi-Huyen Le, Shonara Jayde Langley, Jordan Gibson Dunham, Sinh Dang-Xuan, Fred Unger, Hai Hoang Tuan Ngo, Luong Nguyen-Thanh, Hung Nguyen-Viet and Jenny-Ann Toribio

75 A review of the roles of men, women, and youth in ensuring food safety in the smallholder poultry value chain in Kenya
Ariel V. Garsow, Erica G. Kim, Kathleen E. Colverson, Sanja Ilic, Catherine Kunyanga, Abdiaziz Bainah and Barbara B. Kowalcyk

83 Quantitative risk assessment of salmonellosis in Cambodian consumers through chicken and pork salad consumption
Chea Rortana, Sinh Dang-Xuan, Hung Nguyen-Viet, Fred Unger, Johanna F. Lindahl, Sothyra Tum, Chhay Ty, Delia Grace, Kristina Osbjer and Sofia Boqvist

96 Quantitative analysis of knowledge, attitude and practice of workers in chicken slaughter slabs toward food safety and hygiene in Ouagadougou, Burkina Faso
Ayalew Assefa, Michel Dione, Guy Ilboudo, Valerie Lallogo, Biruk Alemu Gemeda, Delia Grace and Theodore J. D. Knight-Jones

110 State of the art of breeding, milking, and milk processing for the production of curdled milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products
Gwladys S. Komagbe, Alphonse Dossou, Baké Marie-Thérèse Seko Orou, Philippe Sessou, Paulin Azokpota, Issaka Youssao, Joseph Hounhouigan, Marie-Louise Scippo, Antoine Clinquart, Jacques Mahillon and Souaïbou Farougou

122 Foodborne disease hazards and burden in Ethiopia: A systematic literature review, 1990–2019
Lina Gazu, Silvia Alonso, Florence Mutua, Kristina Roesel, Johanna F. Lindahl, Kebede Amenu, Filipe Maximiano Sousa, Pattama Ulrich, Tadesse Guadu, Michel Dione, Guy Ilboudo, Theodore Knight-Jones and Delia Grace

136 Milk purchase and consumption patterns in peri-urban low-income households in Kenya
Emmanuel Muunda, Nadhem Mtimet, Eric Bett, Francis Wanyoike and Silvia Alonso

146 A glance into traditional pig slaughtering practices in Vietnam and opportunities for zoonotic disease prevention
Nancy I. Ting, Sinh Dang-Xuan, Jeffrey Gilbert, Nguyen Thao Thi Nguyen, Steven Lam and Hung Nguyen-Viet

155 Impact of perception and assessment of consumers on willingness to pay for upgraded fresh pork: An experimental study in Vietnam
Hai Hoang Tuan Ngo, Sinh Dang-Xuan, Mats Målqvist, Phuc Pham-Duc, Phi Nguyen-Hong, Hang Le-Thi, Hung Nguyen-Viet, Trang T. H. Le, Delia Grace, Johanna F. Lindahl and Fred Unger

168 Quantitative assessment of aflatoxin exposure and hepatocellular carcinoma (HCC) risk associated with consumption of select Nigerian staple foods
Anthony Wenndt, Florence Mutua, Delia Grace, Lian F. Thomas and Elisabetta Lambertini

180 Describing capability, opportunity, and motivation for food safety practices among actors in the Cambodian informal vegetable market
Sabrina Mosimann, Keorimy Ouk, Nora M. Bello, Malyheng Chhoeun, Jessie Vipham, Lyda Hok and Paul Ebner

193 Exploring the composition and structure of milk and meat value chains, food safety risks and governance in the Addis Ababa and Oromia regions of Ethiopia
Ndungu S. Nyokabi, Johanna F. Lindahl, Lisette T. Phelan, Stefan Berg, Gizachew Gemechu, Adane Mihret, James L. N. Wood and Henrietta L. Moore

207 **Assessing milk products quality, safety, and influencing factors along the dairy value chain in eastern Democratic Republic of the Congo**
Samy Bashizi Bacigale, Rodrigue Basengere Ayagirwe, Valence Bwana Mutwedu, Yannick Mugumaarhahama, Janvier Zirhumana Mugisho, Zabron Nziku, Mamadou Fofana, Patchimaporn Udomkun and Jacob Mignouna

225 **Using a value chain framework for food safety assessment of edible offals in Nairobi, Kenya**
Anima Sirma, Evans Muthuma, John Kariuki, Agnes Maina, Joyce Thaiya and Obadiah Njagi

230 **Prevalence of extended-spectrum β-lactamase producing Enterobacterales in Africa's water-plant-food interface: A meta-analysis (2010–2022)**
Loandi Richter, Erika M. Du Plessis, Stacey Duvenage and Lise Korsten

242 **Diarrhea illness in livestock keeping households in Cambodia: An analysis using a One Health framework**
Shingo Asakura, Borin Khieu, Sokerya Seng, Samkol Pok, Chhay Ty, Chiv Phiny, Teng Srey, Stuart D. Blacksell, Jeffrey Gilbert, Delia Grace and Silvia Alonso

254 **Heavy metal contamination in retailed food in Bangladesh: a dietary public health risk assessment**
Ruhena Begum, Rahima Akter, Sinh Dang-Xuan, Shariful Islam, Nure Alam Siddiky, ASM Ashab Uddin, Asheak Mahmud, Md Samun Sarker, Delia Grace, Mohammed Abdus Samad and Johanna F. Lindahl

266 **Structure and milk hygiene of dairy cooperative value chains in an intensive production area of Uganda—A bottleneck of intervention**
Yoshiharu Sugino, James Bugeza, David Bahame, Joseph Byaruhanga, Haruka Shimazaki, Masahiko Anzai, Taishi Kayano, William Mwebembezi, Andrew Akashaba, Taku Shimada, Yasukazu Muramatsu and Kohei Makita

279 **Describing food safety perceptions among growers and vendors in Cambodian informal vegetable markets**
Sabrina Mosimann, Keorimy Ouk, Nora M. Bello, Malyheng Chhoeun, Leah Thompson, Jessie Vipham, Lyda Hok and Paul Ebner

292 **Effect of nixtamalization of maize and heat treatment of soybean on the nutrient, antinutrient, and mycotoxin levels of maize-soybean-based composite flour**
Rehema Esther Matendo, Samuel Imathiu, Patchimaporn Udomkun and Willis O. Owino

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Editorial: Food safety in low- and middle-income countries

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foodborne disease, informal markets, Africa, Asia, risk assessment, risk management, animal source food

Editorial on the Research Topic
Food safety in low- and middle-income countries

This is the first special edition on food safety in informal markets in low-and-middle income countries (LMICs). Despite their important public health and socio-economic impacts, foodborne diseases have only recently gained the attention of development institutes and initiatives (Grace, 2023). The is the result of growing appreciation of the enormous burden of foodborne disease in LMICs: the health burden is comparable to that of malaria, HIV/AIDs or tuberculosis and the economic cost is more than 100 billion USD a year (Havelaar et al., 2015; Jaffee et al., 2019). Most of the burden is caused by microbial and parasitic infections and most of these are acquired from fresh foods purchased in mass domestic markets in LMICs (Grace, 2015).

These markets are easy to recognize but hard to define. They have been variously called informal, traditional, wet, embedded, and (more recently) territorial markets (Roesel and Grace, 2014; CFS., 2016). They typically include open public markets, kiosks/small shops, butchers, fruit stalls, street food and small-scale eateries. They often lack infrastructure, waste disposal is poor, and pests are common; much of the food sold is fresh, unpackaged, un-processed or traditionally processed and is cheaper than food sold in the modern sector; live animals (especially poultry and fish) may be sold and in some cases wildmeat and traditional foods such as insects; there is an absence of consistent or structured food safety and quality inspection. Informal markets are often supplied by small-scale farmers; workers are not salaried, lack qualifications and training, and often include women and youth; vendors of similar products tend to sell side-by-side with little differentiation of product or price; ready-to-eat food is often available; customers tend to shop frequently, buy in small quantities and be poorer and less educated than customers who patronize the modern food sector. Trust is a major factor in the customer-vendor relation, and vendors may provide added services such as credit or sale in small amounts. The size, variety, and impacts (both positive and negative) of these markets grow in importance as countries develop and urbanize. During this process food safety gets worse before it gets better (Jaffee et al., 2019).

Despite their importance for health, nutrition, livelihoods, equity and the urban environment, these markets have been largely ignored in the research literature (Grace, 2015). Therefore, this Research Topic aimed to bring together studies on foodborne

TABLE 1 Summary of 24 papers on food safety in informal markets.

Study	Methodology	Actor(s)	Product	Place	Hazard(s) or indicator	Key findings	References
Risk assessment							
KAP	Survey; multiple correspondence analysis	Farmers, processors, traders, consumers	Sour milk and local cheese	Benin	n/a	Poor hygiene along the VC; little training for VC actors; many farmers treat their animals without veterinary help. Different categories of farmers and processors, some with better hygiene.	Komagbe et al.
KAP	Survey	Shops slaughtering and retailing	Chicken	Burkina Faso	All hazards	Most informal (60%); 6% birds die during transport; facilities and practices exceptionally poor. Workers have no training.	Assefa et al.
KAP and prevalence	Survey and lab tests	Vendors	Milk	India	Staph.; E. coli; Klebsiella; Shigella	No vendors had received training. Different categories of vendors. 65% of samples had AMR bacteria.	Sharma et al.
KAP	Survey	Growers and vendors	Vegetables	Cambodia	Microbial and chemical hazards	Most concerned about food safety; considered chemical risks more important; 20–50% used at least one risk mitigation practice.	Mosimann et al. (b)
KAP	Survey (online)	Consumers (pregnant women)	Food in (pregnant women)	Jordan	All hazards	Most received food safety information; least awareness of cross-contamination and temperature control. Improved practices during COVID pandemic.	Almanasrah et al.
KAP	Survey	Consumer	Raw and pasteurized milk	Kenya	n/a	98% purchased raw milk and 17% packaged weekly; informal markets key to nutritional requirements of children	Muunda et al.
KAP	Observation, photography, videography	Slaughter, transport	Pork	Vietnam	Meat borne pathogens	Poor hygienic practices and facilities. Difficult to change behavior. Slaughter un-regulated.	Ting et al.
Prevalence	Literature review	Farmer, retailer.	Irrigation water, soil and fresh produce.	Africa	Extended-spectrum β-lactamase Enterobacterales	Environmental AMR studies rare outside South Africa. 13 studies found multidrug AMR potential pathogens in irrigation water.	Richter et al.
Challenges to food safety	Literature review	Slaughter, distribution, retail	Edible offals	Kenya	All hazards	Risky practices at slaughterhouse; weak enforcement of transport regulations; lack of hygiene and cold chain throughout VC.	Sirma et al.
Hazard prevalence and food quality	Survey and lab tests	Producers, collectors, vendors	Milk (raw, pasteurized), sour milk, cheese	Congo	E. coli, Staphylococcus Salmonella	All raw and pasteurized milk above coliform limits; Salmonella and Staphylococcus in all products.	Bacigale et al.
Hazard prevalence and health burden	Systematic literature review	Population	All food	Ethiopia	All hazards	High levels of microbial contamination in foods; no studies on health burden in people.	Gazu et al.
Hazard prevalence and health burden (diarrhea) and risk factor	Survey and lab tests	Livestock Farmers	All food	Cambodia	STEC; Staph.; Campy; Salmonella; Shigella	Diarrhea prevalence 9%. E. coli and Shigella in human stool; E. coli and Salmonella in animal samples Poor hygiene and WASH risk factors. No link between bacteria in livestock and people in livestock-keeping households	Asakura et al.

(Continued)

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TABLE 1 (Continued)

Study	Methodology	Actor(s)	Product	Place	Hazard(s) or indicator	Key findings	References
Health burden and attribution	Literature and modeling	Population	Animal source Food and vegetables	Ethiopia and Burkina Faso	Campy; ETEC; STEC; Salmonella	Substantial burden of FBD. Campy most cases, Salmonella most deaths and DALYs. Chicken highest burden followed by vegetables.	Havelaar et al.
Health burden	Dietary health risk	Population	Chicken and Fish	Bangladesh	Chromium, Cadmium, Lead	Negligible risk	Begum et al.
Health burden	Quantitative risk assessment	Population	Corn, peanut, rice, soybean, cowpea	Nigeria	Aflatoxin	2.8 cases liver cancer per 100,000 people a year; responsible about 2% of DALYs	Wenndt et al.
Health burden	Quantitative Microbial Risk Assessment	Population	Chicken and pork salads	Cambodia	Salmonella	Around 10–15% fall ill annually from meat salads. Transmission is through cross-contamination not meat.	Rortana et al.
Risk management							
Governance	Survey and KII	Entire value chain	Dairy	Tanzania	All hazards	Government lacks capacity to enforce rules but tolerates the informal sector. Informal actors concerned about safety and mitigate risk.	Blackmore et al.
Governance	FGD, KII and observation	Entire value chain	Meat and Milk	Ethiopia	All hazards	Food safety compliance gap in both formal and informal markets. Government policy of formalization not well suited to food system.	Nyokabi et al.
Interventions that improve safety	Literature review	Entire value chain	Chicken	Kenya	All hazards	Women and youth high participation in poultry VC and hence exposure to hazards but less power. Training, financial support and empowering women can improve food safety.	Garsow et al.
Capacity to implement food safety	Survey	Farmers, distributors and vendors	Vegetables	Cambodia	All hazards	Vendors and distributors has high motivation and capability to improve food safety but less opportunity. Farmers has high motivation and less capability and opportunity.	Mosimann et al. (b)
Technology	Nixtamalizatin maize and heat treatment soybean	Experiment	Maize, soybean	Democratic Republic Congo	Mycotoxins and antinutrients	Nixtamalization effective at reducing mycotoxins; heat treatment improves flavor of soybean and reduces anti-nutritional factors.	Matendo et al.
Training and simple technology	Before and after milking hygiene intervention	Farmer and Collecting Center	Milk	Uganda	Total bacterial counts (TBC)	Mastitis on farm reduced. 97% of milk samples at MCC below standard because of post-farm contamination.	Sugino et al.
Willingness to pay	BDM experiment	Traditional and upgraded shops	Pork	Vietnam	All hazards	Consumers willing to pay 20% more for pork from upgraded shops, sufficient to pay for the improvements.	Ngo et al.
Risk communication							
	Survey	Consumers in modern and traditional markets	Pork	Vietnam	Microbial and chemical hazards	Received few messages on food safety; television and experts most trusted; wished for information on traceability and how to choose safe food; little concerns about animal welfare.	Le et al.

VC, Value chain; n/a, Not available; Staph, Staphylococcus; Campy, Campylobacter; STEC, Shiga Toxin E. coli; ETEC: Enterotoxigenic E. coli; KAP, knowledge-attitude-practice; QRA, quantitative risk assessment; RA, risk assessment, RC, risk communication; RM, risk management; SLR, systematic literature review; Micr, microbiology.

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disease in informal markets at national and sub-national levels with a focus on disease prioritization, risk assessment, management, communication and to develop recommendations for policy, practice and further research.

In all, 32 papers were submitted to the special edition and 24 were accepted. The papers were diverse in topic and geographical focus. Most were from Africa (14), which has the highest per capita burden of foodborne disease, followed by Asia (8), which has the highest overall burden of foodborne disease (Havelaar et al.; Gibb et al., 2019). Most studies were on animal source foods (17) followed by vegetables (5). These fresh products are high risk. Animals are reservoirs for many zoonotic pathogens and both animal source foods and fresh produce provide suitable matrices for pathogen survival and growth: as a result they are important sources of foodborne disease (Hoffmann et al., 2017). There were relatively fewer papers on parasites (but these have been perhaps over-represented in the literature) and chemical hazards (but in terms of human health impacts these are both more difficult to study and overall, less important than biological hazards).

Risk assessment predominated (16 papers), followed by risk management (seven papers) and only one paper focused on risk communication. Among risk assessment papers, seven reported knowledge-attitude-practice (KAP) studies, three microbiological prevalence findings, three were quantitative risk assessment (of which two included laboratory analysis), three were literature reviews, and two were on population disease burden. As such, the papers drew more on risk analysis and food science than on medical epidemiology. The eight papers on risk management included two focused on technologies, two on governance, and one on willingness-to-pay for safer food as an incentive for vendor behavior change. The importance of foodborne disease as a development issue has only been recognized in the last few decades, and it is natural that initial research focuses on understanding the problem and its extent. However, as more and more studies corroborate the importance of food safety, more emphasis on risk management and communication would be welcome.

The CGIAR was the first international research organization to have a major program on food safety in informal markets of LMICs. This started in 2006, and as of 2023, more than 8,000 outputs on food safety in informal markets are listed in the CGIAR repository (CGSpace., 2023). Most of the authors in this edition have links to this program (18 out of 24) as well as all four editors, which may also have contributed to the strong CGIAR representation.

Key findings

While the special edition called for papers on food study in informal markets, some also looked at formal markets either as a comparator or to situate the informal market in the context of food systems. These studies confirmed the predominance of informal markets in Africa and much of Asia. For example, in Kenya 98% of household purchased unprocessed fresh milk at least once in the 7 days prior to the survey, while only 17% purchased packed pasteurized milk (Muunda et al.) and in Tanzania 95% of marketed milk passes through informal channels (Blackmore et al.). Informal and formal markets co-exist and attract different clients. For example, in Vietnam, modern urban consumers trusted less in traditional wet markets whereas traditional urban consumers trusted more in them (Le et al.).

Informal markets are often seen as undifferentiated, yet detailed investigation reveal considerable segmentation. A study on the dairy chain in two Indian states identified five categories of milk vendor (Sharma et al.) while in Benin Komagbe et al. differentiated dairy farmers and producers of local cheese into different categories reflecting different practices. A study in Uganda illustrated the (often) porous boundaries between formal and informal markets: of nine licensed Milk Collection Cooperatives that sold milk to processing plants, seven also sold raw milk to shops and vendors and even individual customers (Sugino et al.). The characterization of different types of actors and customers can help in understanding risk and targeting interventions.

As previous studies have found, "*if you look for hazards you will find them*" (Roesel and Grace, 2014) and all the studies that conducted microbiological studies found hazards were present. Earlier work had also found that while hazards are often present, they are not always at high levels, and hazards may be present yet health risk not high. Several studies assessed disease burden. Three of these were quantitative risk assessments, the gold standard for prediction of disease risk from food. Heavy metals are of consumer concern in Bangladesh, but the risk assessment found there was currently low health risk from consumption of fish and chicken (Begum et al.). A risk estimate for hepatocellular carcinoma from consumption of five commonly eaten foods, estimated 2.8 cases per year per 100,000 people: a significant public health problem but only 2% of the total estimated burden of foodborne disease in the region (Wenndt et al.; Havelaar et al., 2015). This study assessed risk for five different commodities leading to more reliable estimates as there was an upper limit on the total burden. Finally, a quantitative microbial risk assessment from Cambodia established 10%—15% of consumers of chicken and pork salad became ill each year from salmonellosis (Rortana et al.). Importantly, the exposure route was not through the well-cooked meat but from cross-contamination within the household.

Previous studies had also concluded "*informal sector food is not always dangerous and formal sector not always safe*" (Roesel and Grace, 2014). One study on dairy products in the Congo found that both raw and pasteurized milk exceeded the relevant standards and both contained *Salmonella* spp. and *Staphylococcus* spp. and another in Uganda found only 13% of milk sampled from in the Milk Collecting Centers (formal sector) met standards, confirming the difficult of ensuring food safety even in formal markets (Bacigale et al.; Sugino et al.).

A systematic literature review found studies on hazards and burden of foodborne disease in Ethiopia. High levels of microbial contamination in different food value chains were often seen in the small, *ad-hoc*, observational studies that dominated the literature, but there were no reports on the incidence of foodborne disease or its health burden (Gazu et al.). Empirical evidence on foodborne disease is difficult to obtain in LMICs, so risk assessments are a useful tool in estimating health burden. Another study took

a different approach to estimating health burden by updating the World Health Organization Global Burden of Foodborne Disease study and complementing with a dedicated Structured Expert Judgement study to estimate the burden attributable to specific foods in Ethiopia and Burkina Faso. In both countries, the burden of foodborne disease was high and highest burdens were attributable to poultry, followed by vegetables (Havelaar et al.).

Seven studies focused on Knowledge, Attitude and Practice (KAP) of different value chain actors (Table 1). Four looked at more than one node on the "farm to fork" chain although only one took a whole chain approach considered best for understanding where risk is introduced, amplified and mitigated. In general, these KAP studies found low levels of knowledge, poor hygienic practices and that most value chain actors received little or no training. However, studies from Cambodia, Kenya and Tanzania also reported that informal value chain actors are concerned about food safety and actively implement risk mitigation (Blackmore et al.; Garsow et al.; Mosimann et al. (a)). This confirms the hypothesis that many actors are "*well-intentioned but ill informed*" and therefore benefits may be attained by increasing awareness. As found in other studies, there are misperceptions about risk. Especially chemical hazards tend to be feared more than biological (e.g., study on vegetables in Cambodia). The seven KAP studies developed their own instruments and while this allows contextualization, it raises the question as to whether better validity and comparability could be achieved by greater uses of standardized instruments, an instance of the toothbrush problem: "*no self-respecting psychologist wants to use anyone else's*" (Elson et al., 2023).

Some papers also reported methodological innovation. A study of slaughterhouses in Vietnam study used videos and photographs to assess hygiene and sanitation (Ting et al.); a study in Jordan used an online questionnaire to investigate food safety knowledge among pregnant woman (Almanasrah et al.); a Cambodian study used the COM-B framework which considers capability (C), opportunity (O), and motivation (M) as three key factors capable of changing behavior (B) (Mosimann et al. (b)); a study in Benin used multiple correspondence analysis (MCA) to identify different categories of farmer and processor (Komagbe et al.); a study in Vietnam used a Becker-DeGroot-Marshak (BDM) experiment to collect data on WTP for pork from typical and upgraded pork shops (Ngo et al.).

Although food safety is a quintessential One Health issue spanning different sectors and populations, few studies used explicitly the One Health approach. One was a study in Cambodia, which cultured fecal and swab samples from livestock and stool samples from humans in the same livestock-keeping households (Asakura et al.). Another literature review, which took a One Health approach, was the only study to consider environment samples along with water and food commonly sampled (Richter et al.).

Six studies focused on risk management. The emerging "three legged stool" approach to improving food safety in informal markets has been developed by the CGIAR and partners and posits that food safety can be cost-effectively improved if and only if three essentials are met. These are (a) an enabling environment (meaning authorities on-board and minimally acceptable infrastructure); (b) appropriate training and technology for value chain actors; (c) incentives for behavior change (Grace, 2023). Two studies looked at governance and found government lacked capacity to enforce regulations (Nyokabi et al.; Blackmore et al.). Two studies tested training and technology (both effective); two studies looked at incentives (price premium and motivation- both effective). However, none of the studies combined all three aspects and none investigated long-term sustainability and scalability of solutions.

Conclusion

In the last decades, much information has been generated confirming the large health and economic burden of foodborne disease and that most of this burden comes from fresh foods sold in traditional (informal, territorial) markets (Grace, 2023). Knowledge is being generated that allows a better understanding of these risks and ways to manage them within their specific social, cultural, technical and infrastructure contexts. The Research Topic highlights the diversity of informal markets and the differing needs of stakeholders in these value chains, which is a challenge in terms of scalability. Successful interventions are emerging and future research should focus more on solutions especially in terms of scale and sustainability.

Author contributions

DG: Conceptualization, Writing – original draft, Writing – review & editing. BB: Writing – original draft, Writing – review & editing. BH: Writing – original draft, Writing – review & editing. HN-V: Writing – original draft, Writing – review & editing.

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Conflict of interest

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Food safety knowledge and risk perception among pregnant women: A cross-sectional study in Jordan during the COVID-19 pandemic

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Background: Pregnant women are at a higher risk of food poisoning compared to the general population. This can be detrimental to both the mother and the fetus. This study aimed to assess the level of knowledge and risk perception of basic food safety and handling among pregnant women in Jordan amid the COVID-19 pandemic.

Methods: A descriptive cross-sectional quantitative study among pregnant women in Jordan was conducted using an online questionnaire between November 2020 and January 2021. The survey included socio-demographic data, food safety knowledge, and risk perception questions as well as COVID-19 related questions. A total score for food safety awareness out of 50 was derived for each participant based on the sum of scores from all domains. Student *t*-test and Analysis of Variance (ANOVA) were conducted using SPSS (Version 26) to compare the mean sum of correct responses of every section (knowledge score) by sociodemographic characteristics.

Results: A total of 325 participants completed the web-based survey. Most of the participants reported receiving food safety-related information during pregnancy (64.9%). The mean total score for the participants was 23.3 ± 4.6 out of 50 (score percentage 46.6%). Participants were mostly aware of foodborne diseases (82.7%) followed by cleaning and sanitation (51.2%), and personal hygiene (49.1%). The least amount of awareness was observed in the cross-contamination (35.0%), food consumption and safety (35.0%), and temperature control (32.8%) domains. Older participants and those with higher education had significantly higher mean scores ($p < 0.001$). Most participants agreed

that the pandemic had a positive impact on enhancing the measures taken to maintain food safety during the pandemic.

Conclusions: This study identified gaps in food safety-related knowledge. Educational programs for pregnant women need robust reinforcement within the community. Efficient educational approaches related to food safety should be provided by health care providers and local health authorities. While the COVID-19 pandemic persists, pregnant women must be well-educated about the virus and its prevention strategies to avoid being infected and ensure their baby's safety as well as their own.

KEYWORDS

COVID-19, food safety, pregnant women, foodborne disease, knowledge

Introduction

Since the preliminary stages of the COVID-19 pandemic and 2 years after its emergence, the COVID-19 disease continues to ravage global health and the economy. Vulnerable and high-risk groups affected by the SARS-CoV-2 virus tend to show more severe symptoms compared to the rest of the population. These include people who are over 60 years old, who have health conditions such as diabetes or heart disease, and who suffer from conditions that diminish normal immune system function (WHO, 2020c). Pregnancy encompasses a wide range of physiological and immunological alterations that could increase the susceptibility to respiratory diseases (Ramsey and Ramin, 2001). It is well-established that pregnant women may develop more severe complications when being infected with COVID-19 as opposed to non-pregnant women (WHO, 2020b). Moreover, adverse pregnancy and neonatal consequences were found to be more common among infected pregnant women including preeclampsia, preterm birth, cesarean deliveries, and stillbirth (Yang et al., 2020), while mother-to-fetus vertical transmission of SARS-CoV-2 remains controversial to date (Kotlyar et al., 2021).

The concept of food safety revolves around handling, preparing, and storing food in a manner that reduces biological risk (in the form of bacteria or viruses) and chemical risk (heavy metal/pesticide contamination, etc). Foodborne diseases pose a significant threat to public health. Numerous findings have indicated that many foodborne outbreaks are related to serious food safety knowledge gaps and improper practices during meal preparation, especially in home settings (Langiano et al., 2012; Wu et al., 2018). According to the World Health Organization (WHO), almost 1 in 10 people worldwide experience food poisoning (WHO, 2020e). Evidence shows that the improper handling of food and deviation from good practices and the Hazard Analysis Critical Control Point (HACCP) system pose a great risk for consumers (Jevšnik et al., 2008). Consumers' improper practices toward food such as consuming raw or undercooked food and poor hygiene, and other practices originating in the domestic environment may contribute to foodborne diseases (Redmond and Griffith, 2003; Haysom and Sharp, 2005). In the light of the COVID-19 pandemic, several reports highlighted the impact of the pandemic on dietary habits, physical activity, mental health as well as hygiene practices, food safety knowledge, and food purchasing and handling behaviors (Cheikh Ismail et al., 2021; Chenarides et al., 2021).

The main cause of the COVID-19 outbreak was linked by many researchers and scientists to local seafood markets in China (Wu et al., 2020). Moreover, direct transmission through person-to-person contact is identified as the main route of transmission of the virus and there is no evidence up to date confirming direct transmission through food or food packages. However, contaminated food, food packages, and surfaces may be considered carriers of the virus (Duda-Chodak et al., 2020; Han et al., 2021). As per general safety guidelines and the increasing number of confirmed COVID-19 cases globally, food safety and handling has become a priority to the public and has been discussed extensively on media platforms, by governments and health authorities (FAO, 2020; FDA, 2021). A recent study among Chinese consumers indicated a significant positive impact of the COVID-19 pandemic on their food safety knowledge and practice (Min et al., 2020) suggesting that public health issues such as the COVID-19 pandemic could encourage people to enhance their food safety awareness and related behavior.

Literature indicates differences in perceived responsibility based on gender, age, and personal relevance (Redmond and Griffith, 2004). It is well-known that pregnant women become more motivated to enrich their health-related knowledge pertaining to the increased level of concern they have for their health and that of their fetuses (Athearn et al., 2004). Several studies showed that pregnant women are not fully aware of the adverse effects of high-risk food consumption during pregnancy and that improper food handling at home

could lead to the development of foodborne illnesses (Athearn et al., 2004; Trepka et al., 2006; Xu et al., 2017). Moreover, pregnant women may exhibit some food safety and handling malpractices such as temperature abuse, cross-contamination, and improper handwashing (Athearn et al., 2004; Trepka et al., 2006; Taylor et al., 2012; Xu et al., 2017; Cater et al., 2020). Some researchers have demonstrated that sufficient education about the risk of foodborne pathogens, proper food safety practices, and handling of high-risk foods is not emphasized for pregnant women (Taylor et al., 2012; Pereboom et al., 2013; Xu et al., 2017; Cater et al., 2020). More alarmingly, a study among this group observed that more than 70% of pregnant women consumed high-risk foods during their pregnancy (Xu et al., 2017). Thus, assessing knowledge and practices related to food safety among pregnant women is essential before implementing educational interventions.

There is no doubt that pregnant women are at a higher risk of food poisoning compared to the general population which can pose detrimental impacts on both the mother and the fetus (Tam et al., 2010). To the best of our knowledge, there are no food safety-related studies available on this group in Jordan. Therefore, this study aims to assess the level of knowledge and risk perception of basic food safety and handling among pregnant women in Jordan amidst the COVID-19 pandemic.

Materials and methods

Study design and participants

The current study was conducted in a descriptive cross-sectional quantitative manner. It was implemented between November 2020 and January 2021. The target population included pregnant women who were 18 years and older and living in Jordan. The study population was drawn from obstetrics and gynecology/OB-GYN outpatient clinics at King Abdullah University Hospital in Irbid city. A trained researcher explained the study objectives to participants and obtained verbal consent before the commencement of data collection. A web link directing to the online survey was then shared with pregnant women who agreed to participate via WhatsApp™. Participants were free to exit the survey at any point and data was collected anonymously. No incentives were provided for participation. An information sheet explaining the study protocol and objective was present on the first page. An electronic informed consent was obtained from all participants. The study protocol was approved by the institutional review board (IRB) of Jordan University of Science and Technology (JUST) [Number: 100/136/2020].

The minimum sample size was calculated to be 273 using the formula

$$n = \frac{Z^2 * P * (1 - p)}{E^2} \quad (1)$$

With a 95% confidence level and 5% margin of error. The population proportion was set based on population knowledge from previous research among females in Jordan (Osaili et al., 2011). All women who had appointments at the clinic during the study period were invited to participate in the study by the nurse. Out of 350 women invited to take part, a total of 325 pregnant women completed the survey and were included in this study (response rate 93%).

Survey questionnaire

The questionnaire was designed to investigate the level of knowledge and risk perception of food safety and handling among pregnant women during the COVID-19 pandemic. According to the literature on food safety (Trepka et al., 2007; Jevšnik et al., 2008; Saeed et al., 2021) and the five keys to safer food manual by the WHO (2020d) a preliminary draft of the questions was developed by researchers at JUST. The survey draft contained 70 questions on food safety knowledge and risk perception and 15 questions related to food safety during the COVID-19 pandemic. The questions were then reviewed by a panel of experts to assess content validity. The expert group consisted of three professors in food safety and two research associates who provided their consent to take part in the evaluation. The panel was introduced to the aims of the study and the procedures for the assessment before the evaluation. During the evaluation, the questions were checked for overlapping items, clarity, understanding, rationale, and consistency with the Jordanian culture. According to the panel's recommendation, the questions were reduced to 50 for food safety knowledge and risk perception and 8 questions related to food safety during the COVID-19 pandemic. Moreover, the questions that were evaluated as not clear or hard to understand by the panel were modified.

The questions were then translated from English to Arabic by two bilingual researchers and back-translated to English by a third researcher to ensure the equivalency of the two versions. The survey was developed as an open online link via Google Forms, and it was pilot tested with 30 subjects in Jordan. The pilot-testing data were not included in the results of this research and no changes were recommended.

The online survey included 65 items divided into three main sections; socio-demographic information (7 items); food safety knowledge and risk perception questions (50 items) and COVID-19 related questions (8 items). The first section inquired about age, education level, household income level, living in a city or village, number of pregnancies, pregnancy trimester, and if they received any food safety information during pregnancy.

FIGURE 1
Survey food safety awareness domains.

The second section of the questionnaire included six domains based on the safer food manual by the WHO (2020d) as shown in Figure 1; personal hygiene (11 items); cross-contamination (6 items); temperature control (12 items); food consumption and safety (9 items); cleaning and sanitation (4 items); and knowledge of foodborne diseases (8 items).

All the questions were close-ended and designed as multiple choices questions and Likert scale with option responses as strongly disagree, disagree, neutral, agree, and strongly agree. A food safety awareness score was derived for each participant by summing the score in each domain of the survey. The score ranged between 0 and 50, the higher the score the greater the food safety awareness.

Statistical analysis

The online survey responses were exported from Google Forms into an Excel file. The data was then cleaned, coded, and imported into Statistical Package for Social Sciences (SPSS) version 26.0 (IBM, Chicago, IL, USA). Descriptive statistics for the sociodemographic characteristics were reported as counts and percentages. Scores for each test category (i.e., personal hygiene, cleaning and sanitation, cross-contamination, temperature control, food consumption and safety, knowledge of foodborne diseases) were calculated as per the correct responses. A total score for food safety awareness out of 50 was derived for each participant based on the sum of scores from all domains. Mean responses and percentages of responses in each category were computed. Student *t*-test and Analysis of Variance (ANOVA) was conducted to compare the mean sum of correct responses of every section (knowledge score) by age, educational level, family income, area of living, number of pregnancies, current trimester, and receiving food safety information. Binary logistic regression analysis was used to assess the correlation between changed food safety-related behaviors during the COVID-19 pandemic and specific food safety domain scores. Results were significant for $p < 0.05$.

Results and discussion

Participants profile

A total of 325 participants completed the web-based survey. The general characteristics of the study population are presented in Table 1. The majority of participants were between the ages of 21 and 40 years (86.5%). This was followed by 34.8% from ages 31–40 years. The majority had completed a university degree (73.5%). The highest education level of about 19% of the respondents was a high school. Only 7.4% of the respondents were educated at less than a high school level. Around half of the participants reported having an average monthly household income of 400 Jordanian Dinars (565$) (55.4%) followed by 26.1% of the respondents having an income ranging from 600 to 1,000 Jordanian Dinars. The majority reported living in the city (56.0%). Around one in four participants were experiencing their first pregnancy and almost half of them were in their third trimester (48.0%). The majority of the participants reported receiving food safety-related information during pregnancy (64.9%). Out of those who received food safety awareness, 61.4% were educated by a doctor and 49.5% obtained information from the internet. This was followed by family and friends (10.0%).

Food safety and risk perception

The mean total score for the participants was 23.3 ± 4.6 out of 50 (score percentage 46.6%) (Table 2). A box and whisker plot that portrays the range of food safety scores among participants is shown in Figure 2. The majority of participants were aware of foodborne diseases (score percentage 82.7%) followed by cleaning and sanitation (score percentage 51.2%), and personal hygiene (score percentage 49.1%). Participants were not very well aware of cross-contamination, food consumption and safety, and temperature control (score percentage 35.0, 35.0, and 32.8% respectively). Although more than two-thirds of the participants reported receiving food safety-related information during their pregnancy, food safety awareness was relatively insufficient among participants, as evidenced by answering less than half of the questions correctly. The high knowledge of foodborne diseases could be due to awareness that occurred as a result of experiencing some foodborne disease at some period in the respondent's lifetime. A previous study conducted

TABLE 1 Socio-demographic characteristics of the study participants ($n = 325$).

Characteristic	*n*	(%)
Age (year)		
<20	23	7.1
21–30	168	51.7
31–40	113	34.8
Over 40	21	6.5
Educational level		
Less than high school	24	7.4
High school	62	19.1
College or above	239	73.5
Monthly income[a]		
<200	25	7.7
200–600	180	55.4
601–1,000	85	26.1
>1,000	35	10.8
Area of living		
City	182	56.0
Village	143	44.0
Number of pregnancies		
1	83	25.5
2	75	23.1
3	54	16.6
4	49	15.1
5 and above	64	19.7
Trimester		
First trimester	87	26.8
Second trimester	82	25.2
Third trimester	156	48.0
Received any food safety information during pregnancy		
Yes	211	64.9
No	114	35.1
If yes, what were the sources of information ($n =$ 211) [b]		
Doctor	129	61.4
Nurse	11	5.2
Nutritionist/Dietitian	7	3.3
Internet	104	49.5
Friends/Family	21	10.0

[a] Jordanian dinars.
[b] Multiple responses were allowed.

TABLE 2 Food safety score for study participants ($n = 325$).

Domains	Mean ± *SD*	Score percentage
Personal hygiene (out of 11)	5.4 ± 2.0	49.1
Cross contamination (out of 6)	2.1 ± 1.1	35.0
Temperature control (out of 12)	3.9 ± 1.7	32.8
Food consumption and safety (out of 9)	3.2 ± 2.1	35.0
Cleaning and sanitation (out of 4)	2.1 ± 0.7	51.2
Knowledge of Food foodborne diseases (out of 8)	6.6 ± 1.3	82.7
Total score for food safety awareness (Range 0–50)	23.3 ± 4.4	46.6

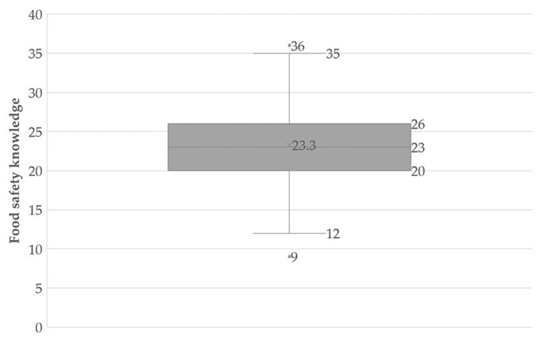

FIGURE 2
Food safety knowledge among participants ($n = 325$).

in Jordanian university female students indicated low level of knowledge related to food safety (Osaili et al., 2011). The high scores in the cleaning and sanitation aspect and the personal hygiene domain could be due to a general practice/self-awareness rather than a strong scientific knowledge base. The information assessed in "cross-contamination," "temperature control," and "food consumption and safety" domains are generally not well-known. In other words, good knowledge of these domains usually requires some awareness/education/self-interest. Providing information or educating pregnant women would increase their awareness and underscore the importance of food safety. This would probably translate to better practice. Care must be taken not to limit awareness interventions to pregnant women only, but rather to include household members to prevent any food safety-related mishaps from occurring.

Most of the studies that investigated food safety knowledge among pregnant women discussed knowledge and practices in different aspects but did not provide an overall score. However, these studies concluded, similar to our study, that food safety awareness in several aspects was insufficient among pregnant women. A study among pregnant women in the USA indicated that participants had high confidence regarding safe food consumption and safe food handling skills while poor skills in using the thermometer (Cater et al., 2020). The total food safety awareness score in the present study was higher than that of college students in Jordan (41.3%) (Osaili et al., 2011) and lower than that of women in Sharjah, United Arab Emirates (UAE) (57.4%) (Saeed et al., 2021), women in Dubai, UAE (53.3%) (Osaili et al., 2022), women at Alexandria university

(67.4%) (Fawzi and Shama, 2009), and women in Lebanon (74.8%) (Haddad et al., 2020).

Personal hygiene

Practicing personal hygiene before and during food preparation is the chief factor for pathogen control and reduction of foodborne disease incidence (Medeiros et al., 2001). Moreover, in light of the COVID-19 pandemic, frequent handwashing is highly recommended to minimize the risk of COVID-19 infection and outbreaks (Haque, 2020). Table 3 shows the query statements and participants' correct responses to personal hygiene questions. Many participants in the present study were aware of the proper practices concerning handwashing after handling raw meat, raw eggs, and disposing of waste (86.8, 80.0, and 81.8% respectively). This was similar to findings among pregnant women from Slovenia (Jevšnik et al., 2008), the USA (Trepka et al., 2007), and Saudi Arabia (Ayaz et al., 2018). Moreover, a large proportion of our participants were aware of the importance of washing hands well before food preparation and after coughing or sneezing during cooking (77.5 and 67.1% respectively). About half of the participants reported washing their hands well after touching any body part while cooking and covering or tying their hair during food preparation (57.2 and 53.5%, respectively). It is possible that the percentage of respondents who reported washing hands would be lower if the study was conducted before the COVID-19 pandemic. Generally, pregnant women were well aware that they were at greater risk to contract COVID-19, and thereby they might have improved their practice of handwashing. However, none of the participants in this study knew that hand washing is not necessary during salad preparation. This suggests a lack of knowledge on some occasions where hand washing is not necessary.

Literature indicates that handwashing for at least 20 s is essential to avoid foodborne diseases (Burton et al., 2011). In the present study, less than a third of the participants were aware of the correct duration of handwashing. An alarming <10% of the participants were aware of the correct way of handwashing and reported wearing gloves during food preparation (6.8 and 2.2% respectively). This was lower than that of pregnant women in Slovenia (Jevšnik et al., 2008), mothers in Saudi Arabia (Ayaz et al., 2018), women in the UAE (Saeed et al., 2021), female university students in Jordan (Osaili et al., 2011), and university students in Greece (Lazou et al., 2012). The majority of participants in these studies knew how to wash their hands properly when handling food. There is a possible risk of infection when handling raw food as microbes can transfer from contaminated food or surfaces to the mouth or nasal area causing infection. Moreover, while wearing gloves during food preparation is an important preventative measure, it should not by any means replace handwashing (Gallè et al., 2020). The

TABLE 3 Query statements and responses[a] of "personal hygiene" domain ($n = 325$).

Query statements	*n* (%)
Wash hands well before starting to prepare or cook food	252 (77.5)
Wash hands well after touching any part of the body during food preparation or cooking	186 (57.2)
Wash hands well after touching raw meat	282 (86.8)
Wash hands well after touching raw eggs	260 (80.0)
Do not wash hands during salad preparation	0 (0)
Wash hands well after coughing and sneezing during food preparation or cooking	218 (67.1)
Wash hands well after disposing of waste	266 (81.8)
Time to spend on washing hands ≥20 s	93 (28.6)
Wash hands the correct way "Wet hands with running warm water, use soap and then wash with running warm water, wipe dry"	22 (6.8)
Wear gloves during food preparation or cooking	7 (2.2)
Cover or tie hair during food preparation or cooking	174 (53.5)

[a]Correct response.

overall score percentage for personal hygiene in the present study was 55.9%, which is lower than that of Saudi mothers (83.8%) and women in Egypt (73.8%) (Fawzi and Shama, 2009; Ayaz et al., 2018). This indeed highlights the crucial need for educational programs reinforcing personal hygiene practices among this group.

Cross-contamination

Findings from the present study indicated a low level of knowledge and risk perception regarding cross-contamination prevention with a score percentage of 35%. Cooking is a practice usually taught by mothers to their daughters in Jordan. Mothers who are not very well-educated regarding food safety may not have had information about "cross-contamination" of foods and thereby would not have transmitted it to their daughters. Basic practices like handwashing and personal hygiene are practices that are usually strictly enjoined. However, habits that prevent cross-contamination may not generally be given much attention. It is imperative to be knowledgeable on how to use and maintain kitchen facilities sanitize to avoid pathogenic growth or cross-contamination and preserve food safety (Langiano et al., 2012). For instance, using the same cutting board and knife for handling raw meat or poultry and vegetables without proper cleaning may lead to foodborne diseases (de Jong et al., 2008). Unfortunately, only one in four pregnant women in the current study believed that to chop vegetables with a previously used knife for raw meat they should wash the knife with water, and soap and use disinfectant before using it (Table 4). This was

TABLE 4 Query statements and responses[a] of "cross-contamination prevention" domain (n = 325).

Query statements	n (%)
Wash knife used to cut raw meat or poultry with water, soap and use disinfectant before using it to chop vegetables	69 (21.2)
Store vegetable salad on the upper shelf in the refrigerator if raw meat or chicken on the lower shelf	102 (31.4)
Store raw eggs separately (protected) from other foodstuffs in a refrigerator	151 (46.5)
Using the same spoon used to stir the food to taste the food during cooking is the least safe way	56 (17.2)
COVID-19 virus cannot be transmitted by the food	65 (20.0)
COVID-19 virus can survive on hard surfaces for days	196 (60.3)

[a]Correct response.

lower than findings from Saudi Arabia and the UAE where most participants reported using different sets of knives and cutting boards for meats and vegetables (82.1%) and washing them after using the same set (96.3%) (Osaili et al., 2011; Ayaz et al., 2018).

Organizing foods depending on their type by separating and storing them in the proper compartments in the fridge is another approach to prevent cross-contamination and allows efficient cooling (Mahon et al., 2006). In the current study about half of the participants stored eggs separately from other food in the fridge and almost a third stored raw meat below vegetables on the fridge shelves. These findings are lower than those in Saudi Arabia and UAE, as more than two-thirds of participants in both studies stored foods according to type and not the available space (Osaili et al., 2011; Alsayeqh, 2015). In another study, pregnant women had stronger beliefs than non-pregnant women about the importance of food separation in the fridge (Jevšnik et al., 2008). These results highlight the importance of educating pregnant women to avoid cross-contamination. In the light of the COVID-19 pandemic, two-thirds of participants were aware that the COVID-19 virus can persist on hard surfaces for days, while a lesser proportion knew that it cannot be transmitted by food (20.0%). This was similar to a recent study among university students in Jordan where 27.0% believed that viruses cannot be transmitted through food (Osaili et al., 2021). Recent systematic reviews indicated that no evidence exists of the transmission of the virus through food; however, the possible transmission may occur from contaminated surfaces and food packages (Duda-Chodak et al., 2020; Han et al., 2021).

Temperature control

Suboptimal knowledge and practices were evident in the temperature control domain in the present study with the lowest score percentage of 32.8%. Purchasing frozen food at the end of grocery shopping shortens the time between shopping and storing the food at home and reduces the possibility of microorganisms' growth which are possible pathogens (Jay et al., 1999). Favorably, this was a common practice among our participants as the vast majority of them reported purchasing frozen food at the end of shopping time (81.2%). This was higher than findings reported in the UAE (Saeed et al., 2021) and Saudi Arabia (Ayaz et al., 2018), and Jordan (Osaili et al., 2011). However, this may not necessarily be due to knowledge of temperature control, rather it could be due to the need to prevent defrosting until the food item is transported home. Jordan is a mostly hot-climate country, thereby food items defrost at a comparatively faster rate. In the present study, participants were knowledgeable about the duration of keeping leftovers in the fridge and the appropriate stage to discard them (76.3 and 65.8%, respectively). This was higher than findings from Saudi Arabia (Ayaz et al., 2018) where less than a third of participants were aware of the proper heating of leftovers and <10% knew when to discard them. On the other hand, most of the participants in the current study had poor knowledge and risk perception regarding how frozen meat and poultry should be defrosted with only 26.5% of them having sufficient knowledge about thawing in the refrigerator. The low-level knowledge could be due to generational transfer of practice amongst family members rather than scientific education. The recommended way to thaw frozen food is in the refrigerator, under running tap water, or in the microwave. These are safe thawing methods that would avoid keeping food within the temperature danger zone which favors the growth of microbes (Badrie et al., 2006). It is thereby of utmost importance to educate women about these practices concerning food safety to prevent foodborne outbreaks/diseases across generations. These findings are in line with similar studies among mothers in Saudi Arabia (Ayaz et al., 2018) and pregnant women in Turkey (Guneri et al., 2017) and lower than those of women in the UAE (Saeed et al., 2021).

Poor knowledge of cooking and holding temperatures for different types of food was evident in the present study. The participants did not have adequate knowledge about the proper storing temperature for chilled ready-to-eat foods, frozen foods, or hot ready-to-eat foods and that meat or poultry are cooked thoroughly based on their temperature (23.4, 15.4, 3.4, and 1.2% respectively) (Table 5). Cooking meat or poultry to an appropriate temperature sufficient to kill food pathogens is a necessary practice as visual inspection of food is not an accurate method of determining doneness (Lyon et al., 2000). Unfortunately, the participants in the current study were lacking knowledge on this matter as only 1.2% of them knew that meats look cooked according to their temperature. Similar findings were reported among women in the UAE and Saudi Arabia as only 18.0 and 22.37% of participants cooked the food according to the recommended time and temperature (Alsayeqh, 2015; Saeed et al., 2021). This might be related to using traditional methods of cooking. A study among pregnant women in the

TABLE 5 Query statements and responses[a] of the "temperature control" domain (n = 325).

Query statements	n (%)
Defrost frozen raw meat or chicken in the refrigerator	86 (26.5)
Reheat leftover until it boils	47 (14.5)
Meat or poultry look cooked by their temperature	4 (1.2)
Put prepared food in the refrigerator, then reheat when ready to eat	103 (31.7)
Discard reheated leftovers immediately	214 (65.8)
Leftovers should be kept in the fridge no more than 2 days	248 (76.3)
Purchase frozen food at the end of shopping time	264 (81.2)
Store chilled ready to eat foods at 1–4°C	76 (23.4)
Store frozen foods at −18°C	50 (15.4)
Store hot ready to eat foods at >60°C	11 (3.4)
Cook chicken till it reaches 73°C	82 (25.2)
COVID-19 virus cannot multiply in the food	95 (29.2)

[a]Correct response.

TABLE 6 Query statements and responses[a] of "food consumption and safety" domain (n = 325).

Query statements	n (%)
Never eat fried or boiled eggs with running yolks	221 (68.0)
Never eat pastries and cakes containing raw eggs	203 (62.5)
Never eat cold deli meat	77 (23.7)
Never eat white cheeses (Nabulsi cheese, Halloumi, etc.)	48 (14.8)
Never eat hot dogs	131 (40.3)
Never eat Shawarma	15 (4.6)
Never eat raw fish (e.g., sushi)	155 (47.7)
Never eat fruits and vegetables without washing	131 (40.3)
Food contaminated with pathogenic bacteria cannot be detected from its appearance or taste	43 (13.2)

[a]Correct response.

USA also indicated malpractices among this group concerning food handling practices (Trepka et al., 2007). Suboptimal knowledge of these practices may subject consumers to the occurrence of foodborne diseases. Furthermore, in the current study, less than a third of participants knew that the COVID-19 virus cannot multiply in food and food products, which was lower than findings reported among university students in Jordan (Osaili et al., 2021). To date, the WHO confirms that there is no evidence that people can get infected from food, nor that the virus can multiply in food (as it needs a human or animal host to grow) (WHO, 2020a). Moreover, the WHO recommends thorough cooking and appropriate food storage to kill the COVID-19 virus (WHO, 2020a).

Food consumption and safety

Concerning food consumption and safety, the participants in this study exhibited a low level of awareness with a score percentage of 35.0%. Table 6 shows the query statements and participants' correct responses to the food consumption and safety questions. Participants were asked about their frequency of consuming certain high-risk food items and only proportions of participants reporting 'never consuming' are presented in the table. Our findings revealed that pregnant women frequently consumed certain foods belonging to the high-risk group for microbial infection such as Shawarma, white cheeses, cold deli meat, unwashed fruits and vegetables, hot dogs, and raw fish (95.4, 85.2, 76.3, 59.7, 59.7, and 52.3%, respectively). However, they reported occasionally consuming foods such as pastries and cakes containing raw eggs and cooked eggs with runny yolks (37.5 and 32.0% respectively). The foods enquired in this study are a staple in the Jordanian diet. Restricting their intake due to pregnancy is not a very practical option which could be a possible explanation for the low scores. Therefore, pregnant women should be well-informed and educated about guidelines that are generally related to food safety. Strategies to ensure food safety while preparing commonly consumed local cuisine should also be explained to ensure their safety and that of the fetus. The results of the current study are comparable to those of pregnant women in Slovenia, where participants less frequently prepared most high-risk food while more occasionally prepared eggs with runny yolks and dishes with raw meat (Jevšnik et al., 2008). Moreover, findings from the USA indicated that pregnant women rarely or less frequently consumed raw or undercooked meat and eggs, and soft cheeses, while more consuming hot dogs or deli meats without heat treatment (Trepka et al., 2007). These findings along with findings from our study are alarming, given the potential increased risk of listeriosis and salmonellosis (Pereboom et al., 2013). A foodborne illness may require medical treatment which could be contraindicated during pregnancy (e.g., certain antibiotics). Untreated cases may result in premature births and abortions. Thereby, it is crucial that pregnant women avoid foods that are high risk, or at least prepare high-risk foods in a manner that would be safe for consumption (e.g., Consuming whole cooked eggs/meat/pasteurized items).

Moreover, a large proportion of the participants in the current study thought that contaminated food can be determined only from appearance or taste (86.8%). As pregnant women are at high risk for foodborne diseases, more attention should be paid to what type of food they consume, and its proper heating and cooking temperatures. The American Dietetic Association has developed dietary guidelines for pregnant women and recommends that pregnant women should abstain

TABLE 7 Query statements and responses[a] of "cleaning and sanitation" domain ($n = 325$).

Query statements	n (%)
Wash fresh fruits and vegetables with cold running water to avoid infections	27 (8.3)
Wash kitchen countertop with cleaning material, then wash it with water, then wipe with disinfectant	255 (8.5)
Sanitize the kitchen sink drain daily	303 (3.2)
The least safe method to disinfect a kitchen sponge by soaking and washing in water	81 (24.9)

[a]Correct response.

from consuming unpasteurized dairy, raw or undercooked meat, eggs, and fish (Kaiser and Allen, 2002).

Cleaning and sanitation

The scoring percentage in the present study for the cleaning and sanitation domain (51.2%) was considerably higher than other domains but lower than that of personal hygiene (55.9%). This could be due to the perception that kitchen sinks/countertops are in more direct contact with the food item rather than the food preparers' nails/hair/clothes etc. Irregular and improper cleaning of kitchen sinks is considered a source of contamination (Josephson et al., 1997). It is recommended to clean kitchen sinks with warm water and soap and then disinfect them (Hillers et al., 2003). Favorably, the vast majority of participants exhibited proper practices regarding the frequency of cleaning and sanitizing the kitchen sink as they reported cleaning it once daily (93.2%) as shown in Table 7. These findings were comparable to those of women in the UAE (Saeed et al., 2021) and women in Saudi Arabia (Alsayeqh, 2015) who reported washing kitchen sinks after every use. Moreover, the majority of our participants were aware of how to properly clean the kitchen counter (78.5%) which was higher than that of women in the UAE (Saeed et al., 2021) and that of mothers in Saudi Arabia (Ayaz et al., 2018).

Furthermore, a used kitchen sponge can harbor a high number of microbes and could be another source of contamination when used without appropriate cleaning (Osaili et al., 2020). In the present study, only one out of four pregnant women were aware of how to properly clean and disinfect the kitchen sponge. Studies among women in the UAE and Saudi Arabia indicated that most women were knowledgeable that kitchen sponges can be a source of contamination (Alsayeqh, 2015; Saeed et al., 2021). In addition, the most appropriate method to clean fresh fruits and vegetables is to clean them under cold running water to diminish the presence of contaminants on their surfaces (Li-Cohen and Bruhn, 2002). Surprisingly, <10% of participants in the current study were aware of this practice which was significantly lower than that of similar studies in the UAE (Saeed et al., 2021), Saudi Arabia (Ayaz et al., 2018), and the USA (Taylor et al., 2012). Although the participants had good knowledge regarding kitchen sink and countertops cleaning, unsatisfactory knowledge in other aspects requires attention as malpractices may lead to the introduction and spread of microbes and increases the risk of infection (Haysom and Sharp, 2005).

Foodborne diseases

Overall, food safety awareness among pregnant women in the present study was the highest in the domain of foodborne diseases (score percentage of 82.7%). Foodborne diseases are a serious issue especially for pregnant women as they are 20 times more susceptible to infection than healthy adults (Athearn et al., 2004). Query statements and responses to the knowledge of foodborne diseases questions are presented in Table 8. Most of the participants were aware that food poisoning is more serious for pregnant women than in other groups (89.9%). On the contrary, surprisingly, pregnant women in the USA did not perceive foodborne diseases as a major problem and did not identify poor handling practices at home as a risk factor for foodborne illnesses (Trepka et al., 2006). This difference in observation could be because of a greater probability of contracting a foodborne disease in Jordan in a lifetime as compared to the USA due to stricter legislation. A person who was diagnosed or experienced a foodborne illness would have higher knowledge about the symptoms of the illness than an individual who has never experienced a foodborne illness. A study among pregnant women in British Colombia indicated that participants were aware of the risks of listeriosis; however, limited knowledge of high-risk foods and proper practices were reported (Taylor et al., 2012). Moreover, an observational study among pregnant women in the Netherlands concluded that participants were not aware of listeriosis and toxoplasmosis, and did not know how to avoid such infections during pregnancy (Pereboom et al., 2013).

In the present study, when asked about symptoms of food poisoning, participants were highly aware that diarrhea, vomiting, and abdominal pain (97.2 and 93.2%, respectively) are common symptoms of food poisoning which were slightly higher than findings among Saudi women (Alsayeqh, 2015), women in the UAE (Saeed et al., 2021) and female students in Jordan (Osaili et al., 2011). Moreover, over two-thirds of our participants were aware that hypertension, low blood sugar, cold, and cough are not symptoms of food poisoning. Understanding the causes and symptoms of foodborne disease is important in minizine the chances of being infected and seeking medical attention promptly. Favorably, the majority of the participants in the current study were aware that COVID-19 holds a higher risk of complications for pregnant women which is in line

TABLE 8 Query statements and responses[1] of "knowledge of foodborne diseases" domain ($n = 325$).

Query statements	*n* (%)
Food poisoning is more serious for pregnant women	292 (89.9)
Diarrhea and vomiting are symptoms of food poisoning	316 (97.2)
Abdominal pain and cramps are symptoms of food poisoning	303 (93.2)
Hair falling is not a symptom of food poisoning	298 (91.7)
Hypertension is not a symptom of food poisoning	225 (69.2)
A drop in Blood Sugar is not a symptom of food poisoning	225 (69.2)
Cold and Cough are not symptoms of food poisoning	221 (68.0)
Pregnant women are at higher risk of complications than other healthy adults if they get COVID-19	271 (83.4)

[a]Correct response.

with recent evidence indicating that this group may suffer from more serious complications that other groups (WHO, 2020b).

Association between the demographic characteristics of participants and their food safety awareness

Table 9 shows the association between the mean total food safety awareness scores and different socio-demographic variables. The present study highlighted that older participants had overall better food safety awareness scores ($p < 0.001$). This was in line with findings among pregnant women in Slovenia (Jevšnik et al., 2008) and mothers in Saudi Arabia (Ayaz et al., 2018) indicating that belonging to an older age group had a significant impact on food safety knowledge. This observation could also be because older participants were more likely to have children and hence were more knowledgeable about food safety. Moreover, significantly higher mean scores were reported for participants with post-secondary school qualifications ($p < 0.001$) which agrees with several studies indicating that high education increases women's awareness of food safety (Alsayeqh, 2015; Ayaz et al., 2018; Saeed et al., 2021). About two-thirds of our participants reported receiving food safety-related information during pregnancy, which had a significant impact on total food safety awareness scores ($p < 0.001$). This highlights the importance and beneficial effects of education for this group.

In the USA, women experiencing their first pregnancy had the lowest food safety scores while women who had multiple children were more knowledgeable and were more likely older (Trepka et al., 2007). No such significant association was recorded in the current study. Literature suggests that the susceptibility of pregnant women increases with the progression of pregnancy due to a reduction in cell-mediated immunity (Lamont et al., 2011). Pregnant women who were in their third trimester were most aware of food safety in the present study. Family income and area of living had no impact on food safety awareness in the current study.

TABLE 9 Association between total food safety awareness score sociodemographic characteristics of participants ($n = 325$)[a].

Characteristics	Knowledge score mean ± SD	*P*
Age (Year)		<0.001
≤20	18.8 ± 4.1	
21–30	23.4 ± 4.2	
31–40	23.9 ±4.0	
Over 40	23.8 ±5.2	
Educational level		<0.001
Primary school and below	19.9 ±4.0	
Secondary school	22.4 ± 4.6	
Post-secondary school	23.8 ± 4.2	
Family income JD		0.117
<200	22.8 ± 3.0	
200–600	23.0 ± 4.8	
601–100	23.4 ± 4.6	
>1,000	24.8 ± 3.7	
Area of living		0.341
City	23.1 ± 4.3	
Village	23.5 ± 4.5	
Number of pregnancies		0.310
1	22.8 ± 4.1	
2	22.9 ± 4.6	
3	23.9 ± 4.0	
4	24.1 ± 3.6	
5 or more	23.1 ± 5.1	
Current pregnancy trimester		<0.001
1st	23.1 ±4.0	
2nd	21.6 ± 4.6	
3rd	24.3 ± 4.2	
Received food safety information		0.001
Yes	23.8 ± 4.4	
No	22.2 ± 4.2	

[a]Student t-test and Analysis of Variance (ANOVA) was conducted to compare the mean sum of correct responses of every section (knowledge score) by age, educational level, family income, area of living, number of pregnancies, current trimester, and receiving food safety information.
Significance set at <0.05.

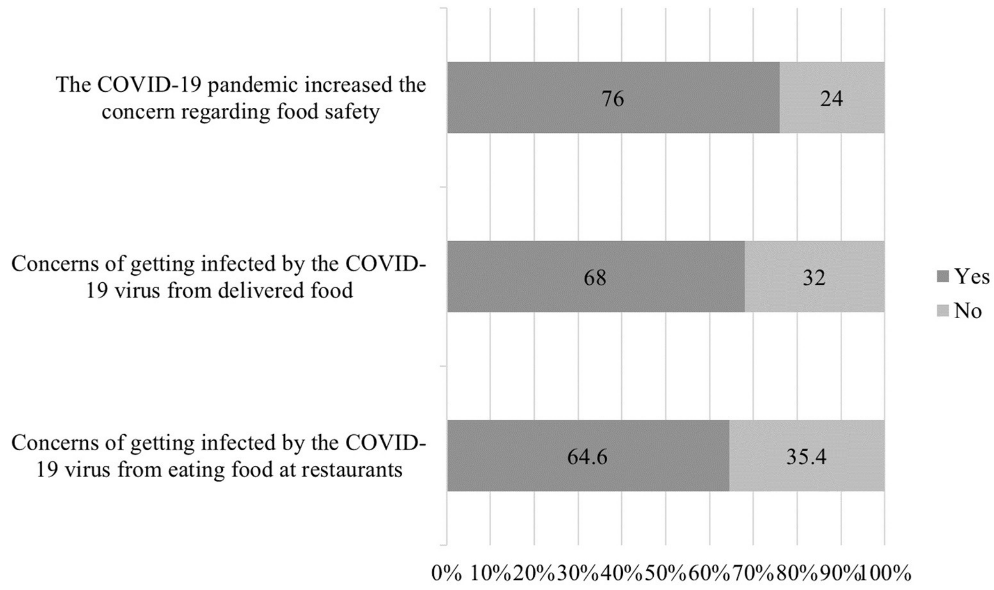

FIGURE 3
Attitude toward food safety and COVID-19 (n = 325).

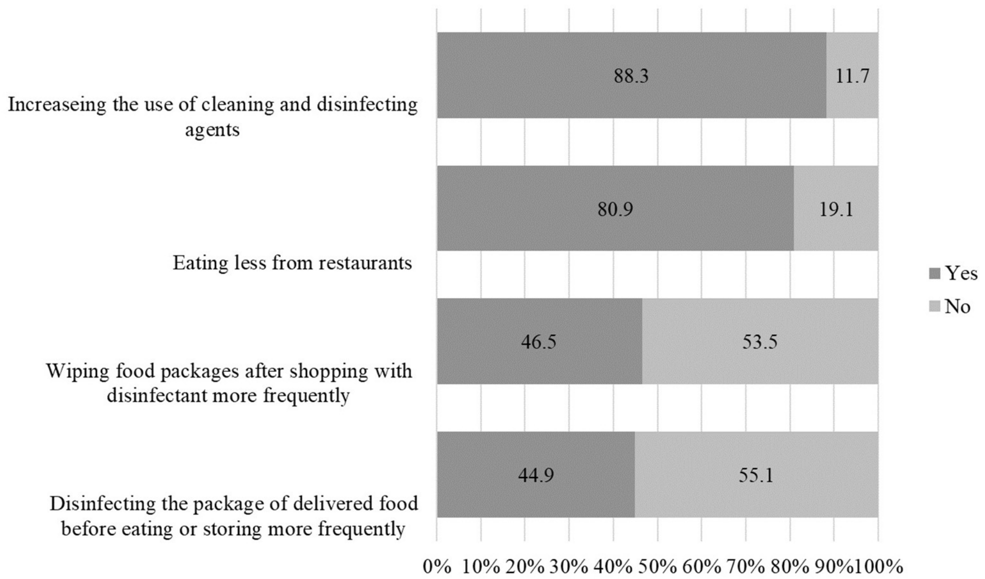

FIGURE 4
Practices during the COVID-19 (n = 325).

Food safety and COVID-19

Attitude and practices

About three in four pregnant women in the present study expressed that the COVID-19 pandemic had increased their concerns regarding food safety as shown in Figure 3. Moreover, about two-thirds of them were concerned about getting infected by the COVID-19 virus from delivered food and from eating at restaurants (68.0 and 64.6%, respectively). These findings are expected as most of the participants believed that pregnant women are at higher risk of complications if they were to be infected with COVID-19. Moreover, since the beginning of

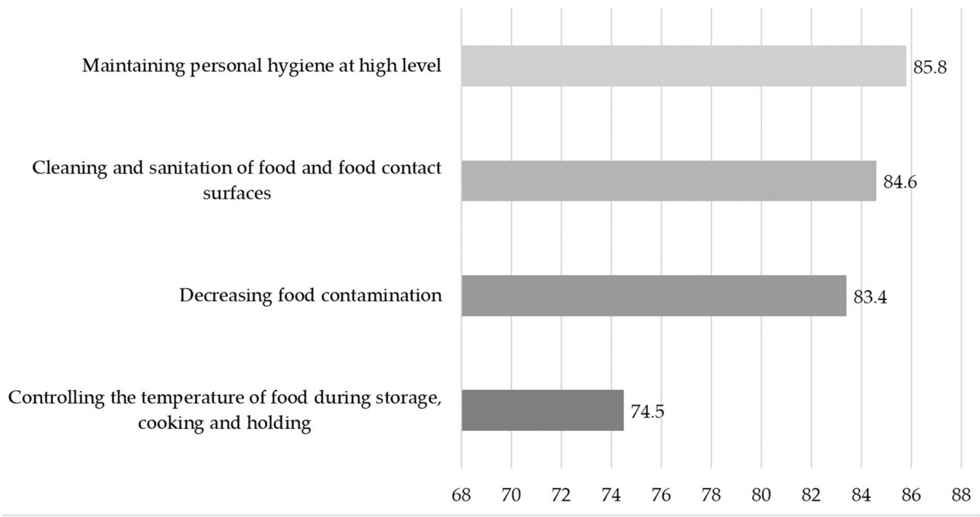

FIGURE 5
Participants answering "Yes" to "Did the COVID-19 pandemic enhance your food safety measures in the following aspects?" ($n = 325$).

the pandemic, official records emphasized the importance of maintaining personal hygiene and handling food according to proper food safety measures (FAO, 2020; FDA, 2021).

During the COVID-19 pandemic, the majority of the participants reported using cleaning and disinfecting agents more frequently and eating less from restaurants (88.3 and 80.9%, respectively) (Figure 4). Similar findings were reported among university students in Jordan as 78.0% of them reported dining out less frequently during the pandemic (Osaili et al., 2021). Maintaining proper personal hygiene, frequent cleaning, and disinfection of contaminated surfaces are all core actions that limit the transmission of the COVID-19 virus (FDA, 2021). However, despite the increased level of concern among participants, the level of practice was lower than expected. Less than half of them reported disinfecting food delivery packages before eating or storing and wiping food packages after shopping with disinfectant during the COVID-19 pandemic (44.9 and 46.5%, respectively). This was higher than observation made among consumers in Indonesia and Malaysia (40.0%) (Soon et al., 2021), but lower than that in the MENA region (65.0%) (Cheikh Ismail et al., 2021).

Logistic regression of reported enhanced food safety measures during the COVID-19 pandemic

Figure 5 shows the query statements and responses concerning enhanced food safety measures in the domains of cross-contamination, personal hygiene, temperature control, and cleaning and sanitation. Most participants agreed that the pandemic has had a positive impact on enhancing the measures taken to decrease food contamination, maintain personal hygiene at a high level, control the temperature of food during storage, cooking, and holding, and clean and sanitize food and food contact surfaces (74.5–85.8%). A recent meta-analysis of 48 studies on knowledge, attitude, and practice during the COVID-19 pandemic indicated that the overall scores of the three components were 78.9, 79.8, and 74.1%, respectively (Saadatjoo et al., 2021).

Moreover, binary logistic regression was conducted to further investigate the relationship between those who reported enhanced food safety measures with their scores in the corresponding domain as shown in Table 10. Binary logistic regression revealed a positive relationship between the following enhanced food safety-related practices and their corresponding specific food safety domain score: decreasing food contamination; maintaining personal hygiene at a high level; as well as controlling the temperature of food during storage, cooking, and holding ($p < 0.001$, $p < 0.001$, and $p = 0.005$, respectively). These results indicated that people who reported enhanced food safety measures had better knowledge scores in the matching domain as opposed to people not reporting enhancing food safety measures. However, no significant association was observed for enhanced cleaning and sanitation of food and food contact surfaces. This could be explained as people might be adherent to good cleaning and sanitation practices as a general practice in their day-to-day life. Nonetheless, it is important to note that, while COVID-19 has increased the level of concern regarding food safety and enhanced the measures taken to maintain it, greater emphasis should be put on the fact that this group is at an increased risk of foodborne diseases in general and all their acquired practices should be maintained regardless of a pandemic.

TABLE 10 Binary logistic regression of enhanced food safety measures during the COVID-19 pandemic of the study population and the aligned food safety domain score ($n = 325$).

Enhanced food safety practice	OR (CI)	*p*
Decreasing food contamination * *Cross contamination domain score*	1.68 (1.258–2.258)	<0.001
Maintaining personal hygiene at a high level * *Personal hygiene domain score*	1.495 (1.319–1.694)	<0.001
Controlling the temperature of food during storage, cooking, and holding * *Temperature control domain score*	1.247 (1.070–1.453)	0.005
Cleaning and sanitation of food and food contact surfaces * *Cleaning and sanitation domain score*	1.205 (0.766–1.894)	0.420

Significance set at <0.05.

Strengths and limitations

The tool used to collect data in the study was a questionnaire. As a questionnaire involves no observation and self-response, the data recorded could be prone to bias from the respondent. Although the study did not collect personal information, to look good, the respondents may have answered all questions correctly/positively (social desirability bias). Evidence suggests that reported food safety practices vs. observed food safety practices are usually not in tandem (Redmond and Griffith, 2003). Furthermore, using an online survey limited the reach of the survey to only those who have internet access. The population with no or poor internet access was left out. This could lead to less generalizability of the results. On the other hand, given the precautions taken due to the pandemic and the vulnerability of the studied group (pregnant women), this design allowed data collection from a larger number of participants with no associated risks of contact. Other strengths of this research include being the first study to assess food safety knowledge among pregnant women in Jordan. In addition, this study identified gaps in knowledge amongst this group, thereby opening scopes for improvement. This can be done by developing proactive and efficient interventional strategies which involve health care professionals, family doctors, public health professionals, nurses, and via pre-natal/pre-marital counseling.

Conclusion

Data from this study revealed that food safety-associated education for pregnant women needs a more robust reinforcement. This role can be taken up by health care providers and local health authorities. Pregnant women in Jordan have suboptimal food safety awareness levels, particularly in cross-contamination, safe food consumption, and temperature control domains. Knowing did not necessarily translate to having better food safety practices. Moreover, while the COVID-19 pandemic remains to be a threat, pregnant women must be well-educated about the nature of the virus, and its prevention strategies to avoid being infected. This includes providing pregnant women with educational sessions and preparing informative, easy-to-understand material regarding food safety principles and application at home with an emphasis on weaknesses described in our study. Nonetheless, education on food safety is recommended for all groups and should focus on the "five keys to safer food" guide by the WHO (Fontannaz-Aujoulat et al., 2019). Future studies should involve participants from other Jordanian/Middle East regions. The most appropriate method for a successful interventional program (e.g., via counseling/brochure distribution/ lecture format) and at which stage (pre-marital/pre-conceptual/first or second trimester/school or university) for this segment of the population can also be studied.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Jordan University of Science and Technology (JUST) [Number: 100/136/2020]. The patients/participants provided their written informed consent to participate in this study.

Author contributions

TO, AA-N, AA, and LC conceptualized and designed the project. SA, NO, AS, LE, and HA prepared the original protocol. MM, SS, TO, AA-N, and LC did data management and analysis. SA, TO, AA-N, NO, AS, LE, HA, MM, SS, RA, AA, and LC collaborated in the overall implementation and data collection of the project and read the report and made suggestions on its content. SA, MM, and SS wrote the original report with input from all co-authors. All authors approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.996302/full#supplementary-material

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The governance of quality and safety in Tanzania's informal milk markets

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Despite significant economic and social transformation in Tanzania, 95% of the milk produced in Tanzania is marketed informally. Most of this is commercialized raw (unpasteurized) and distributed and sold through informal traders and vendors to low-income consumers, making it an important source of nutrition and livelihoods. While Tanzania's official dairy policy promotes pasteurization and formal industry, in practice the regulatory environment is relatively permissive of informal raw milk trade. We draw on original data from a survey with over 200 informal market actors, and insights from key informant interviews, to examine the context, perceptions and practices that affect quality and safety in the informal milk market in Tanzania. Our insights contribute to the potential for a more realistic and effective engagement with the informal sector, in Tanzania and beyond. Our results show that all informal market actors are concerned with milk quality and safety and take measures to mitigate risk. Loyalty and repeated interactions between buyers and sellers contribute to ensuring milk quality and safety in the absence of formal mechanisms such as testing. Despite this there is room for improvement. Informal actors expressed interest in training and finance to upgrade their premises and equipment and would also like to see improved communication with policymakers. Any future policy interventions should build on the indigenous practices being used by informal actors that already contribute to risk management. Efforts to better understand the informal sector and address the broader challenge of the lack of voice and representation of the informal sector in policy making in Tanzania are needed.

KEYWORDS

informal markets, milk, governance, nutrition, food safety, Tanzania

Introduction

By most metrics, Tanzania has experienced significant economic and social transformation in the last two decades, moving it from low-income to lower-middle-income country status in 2020 (World Bank, 2022). Between 2002 and 2012 it had one of Africa's fastest-rising economies, with an average annual GDP growth of 6.5% (Diao et al., 2020), though growth has slowed significantly since the COVID pandemic (World Bank, 2022). Economic growth has resulted in poverty reduction, as well as improvements in housing, education, and access to water (Arndt et al., 2017). These changes have occurred

in parallel with a doubling of population from 25 to 50 million between 1990 and 2015. Rapid urbanization has also taken place, at an annual rate of 5% in the last 20 years (Arndt et al., 2017).

Against the backdrop of these socio-economic shifts, food systems in Tanzania have also undergone significant transformations, from production to consumption. In rural areas, there is evidence of the so-called "silent revolution" observed elsewhere in Africa – the increased dynamism of and investments in the middle of the food supply chain (e.g., aggregation, wholesaling, and processing) which is bringing cash and markets deeper and deeper into the countryside (AGRA, 2019). At the same time, urbanization and rural to urban migration, and rising incomes, have been changing dietary patterns – such as the rising consumption of unhealthy processed foods – especially as a result of rising incomes (Cockx et al., 2018; Sarfo et al., 2021).

However, these transformations are uneven. Incomes have increased on average, but poverty remains widespread, particularly in rural areas (Cockx et al., 2018). Inequality has widened and hunger and malnutrition remain critical challenges; Tanzania has one of the highest chronic malnutrition rates in Africa (World Food Programme, 2022). While the agro-food economy has shown significant change, the modernization of production and retail have been very slow, with supermarkets struggling to gain market share and remain profitable (Allafrica.com, 2021). Despite government policy, the food economy–from production to marketing and retail–continues to be largely informal (Sarfo et al., 2021).

Food safety is an increasingly important aspect of food system change. One assumption is that, as incomes rise and supply chains become more complex, consumers will become more aware of food safety issues, triggering other market actors, including regulators, to move from rudimentary methods like visual inspection to regulatory standards and third-party guarantees (Ortega and Tschirley, 2017). However, this modernization agenda is unlikely to be relevant to most people and trade where informal food markets/systems continue to dominate. Evidence suggests traditional approaches to food safety persist even in the context of income rises and modernization of retail (see e.g., Wertheim-Heck et al., 2014 for Vietnam and Blackmore et al., 2021 for Kenya).

Tanzania's dairy sector exemplifies some of the unevenness of food systems transformations that are common in many low and lower-middle-income countries. Milk production has grown considerably over the past decade[1], but milk yields remain low due to the lack of improved breeds and specialized feed (CSIRO, 2022). Demand for milk has risen sharply in recent years due to population and economic growth, widening the gap between demand and local supply (See footnote 1).

Although the milk processing industry is growing, with the number of dairy processors increasing from 22 in 2001 to 83 in 2017 (Lunogela and Gray, 2020), most of the milk produced in the country is consumed directly by cattle-rearing households or traded informally, meaning that it is unprocessed (i.e., not pasteurized) and typically distributed and sold through small-scale traders and vendors, often street-based, to low-income consumers (NIRAS, 2010). The informal sector therefore remains dominant in Tanzania–with 95% of the milk produced being marketed informally. This resembles the situation in other lower-middle-income countries: raw milk accounts for up to 80% of milk sold in Kenya, and over 95% in Assam, where it is typically consumed in the form of traditionally processed products (Blackmore et al., 2020). The dominance of informal market channels poses challenges for milk quality and safety. Given milk's perishability, the general lack of refrigeration throughout the supply chain, use of non-sterile plastic containers (Gwandu et al., 2018) and the absence of pasteurization or heat treatment create conditions for public health risks (Häsler et al., 2019). The total bacterial count and *Escherichia coli* load, two commonly used hygiene indicators, have been repeatedly found to be high in milk samples in the informal sector in Tanzania (Kilango et al., 2012; Schoder et al., 2013). While boiling–a common practice among consumers–makes milk generally safer, there is still a risk of exposure to pathogenic bacteria due to possible re-contamination, depending on storage practices. Moreover, other chemical hazards such as aflatoxins or antimicrobial residues—which have been found to be common (Gwandu et al., 2018)–cannot be removed *via* heat treatment.

As modernization and food safety become a growing priority of countries in Africa, governments throughout the continent are revisiting their approaches to monitoring and regulating milk quality and safety. Despite their contribution to food security and livelihoods, informal markets are often marginalized from policy and harassed by regulators (Skinner, 2019). Much of this adversarial approach is justified by real or imagined concerns about food safety (Resnick, 2017; Mwango et al., 2019). These negative perceptions and discourses are often based on unfounded perceptions that modern food systems are better, although evidence suggests that the safety performance of informal markets is often no worse than that of modern supply chains (Roesel and Grace, 2015; Skinner, 2019). Urban food governance in Africa–defined not just as the actions of Government, but as decision-making of all involved stakeholders and institutions – has therefore focussed "too much on what should be rather than helping understand what is" (Smit, 2016, p. 80; Wegerif and Kissoly, 2022).

The Tanzanian government has promoted the dairy industry in policy, but has remained pragmatic with regard to regulation and enforcement. This means that, while official support

1 ILRI (International Livestock Research Institute) and GAIN (Global Alliance for Improved Nutrition) (2019). *Food Safety Landscape Analysis for the Dairy Value Chain in Tanzania*.

focuses on the modernization of the dairy sector, including the formalization of the industry and the sale of pasteurized milk, the regulatory approach to informal milk trade is still relatively permissive (Mbwambo et al., 2017). Regulations are interpreted differently by different government actors, and those that could feasibly be used to criminalize informal milk traders are not enforced. Yet, as seen in other low- and middle-income countries, there may be a growing push for stringent formalization of the sector on the grounds of food safety (Blackmore et al., 2021).

This paper explores the tensions between the official vision of modernity in food systems and the reality of informal trade through the lens of milk quality and safety in Tanzania. We show that actors in the informal dairy market in Tanzania–from producers to traders and consumers–place significant importance on food safety and quality, and take measures to ensure them, and that they do so despite rather than due to government support. The consequences of policies governing informal milk trade can have enormous impacts on the services informal markets currently offer to the country in terms of livelihoods, economy and health. Evidence like the one presented in this article can help guide policy-making so it is based on an adequate understanding of the operations and needs of the sector.

In this paper we examine how milk quality and safety are understood, managed and contested in informal markets in Tanzania, in a context of a rapidly transforming food system. We draw on primary data obtained from a survey with over 200 informal market actors in Arusha, and insights from key informant interviews, to better understand how milk trading chains operate, the policy environment in which those chains are situated, the incentives and capacities of sector stakeholders to ensure milk quality and safety, and the effectiveness of policy interventions–such as training–to improve milk safety.

Methods

We used mixed methods and both qualitative and quantitative data sources, including a survey among market actors in the informal sector, and interviews with key sector stakeholders. The survey sample size was 208: 20 producers, 24 intermediaries, 82 vendors (retailers), and 82 consumers Intermediaries were defined as those who purchase milk from producers and transport and sells it to vendors. Transport is typically *via* a motorbike or small van. Vendors are typically immobile, with fixed premises from which they sell milk to consumers. In a few cases vendors may be mobile, without premises, selling milk to consumers from the roadside in urban or peri-urban centers. The survey took place in and around Arusha city, including the districts of Arusha, Meru and Monduli in September 2019. These areas were chosen based on a high prevalence of milk trade, and where previous efforts to improve milk safety through training and certification of informal milk vendors had been piloted. The survey was intended to be illustrative rather than statistically representative and aimed to obtain a diverse range of opinions and perspectives from market actors about market linkages and milk quality and safety. Questionnaires combined closed and open questions and sought qualitative and quantitative insights. The list of survey questions in available as Supplementary material. Sampling was convenient: after initial participants were identified with the assistance of local guides, we used snowballing to recruit further respondents. Surveys were conducted in Swahili, and we obtained prior and informed consent from respondents and assured them of their anonymity. Data were collected using CSPro software. For each question the responses were tabulated and disaggregated by actor (i.e., producer, wholesaler, vendor, or consumer). As the data was assumed to be illustrative rather than representative, no further statistical analysis was performed.

In addition to the survey, we carried out 15 semi-structured interviews with key stakeholders including government officials (Tanzanian Dairy Board, Business Registrations and Licensing Agency BRELA, Tanzania Bureau of Standards, representatives from local government); private sector (processors, processor representative organization and business development service providers); and donors. These stakeholders were chosen based on their engagement in the sector and their contribution to policies and decision-making processes relevant to the sector. These interviews were used to capture a range of opinions on how the sector is working, specifically in relation to the capacities and incentives of key sector stakeholders to work with informal market actors to improve milk safety and quality. The interview guides can be made available upon request. Interviews were transcribed and coded according to themes, and then analyzed by extracting the relevant text.

Results

Policy, regulation and enforcement

Though the contribution of trade in unpasteurized milk to poverty alleviation and nutrition was acknowledged by government officials during interviews, the government's agenda and vision are of a formal, industrialized dairy sector where pasteurized milk is the norm. The National Livestock Policy of 2006, which outlines Tanzania's broad dairy policy framework, has an overall ambition to modernize and increase the productivity of the dairy sector. This policy has an emphasis on smallholder farmers, partly because of the considerable potential of smallholder dairying to reduce poverty (Ministry of Livestock Development, 2006). The National Livestock Policy is operationalized through several strategies and initiatives, including the Tanzanian Livestock Modernization Initiative (2015), the Livestock Sector Development Programme (2011), and the Agricultural Sector Development Programme (2017). These policies emphasize the need to: improve the genetic

potential of the dairy herd; strengthen technical support services and promote use of appropriate technologies; promote investments in production, processing and marketing; and promote dairy organizations and strengthen the Tanzania Dairy Board (Nell et al., 2014).

The Tanzania Livestock Master Plan of 2017 (Michael et al., 2018), which expresses the consensus of institutions and experts in the sector, prioritizes marketing and processing to support the construction of ultra-heat treatment (UHT) and powder milk processing plants. The Plan promotes the production and consumption of processed milk and dairy products; the introduction of quality-based standards and price premiums to encourage increases in the supply of high-quality milk; strengthening of the enforcement of milk and milk products quality standards; and most notably, the formalization of milk trade through the training and licensing of milk traders.

The legality of raw milk trade in Tanzania remains somewhat uncertain, even within government. According to several government representatives, the sale of raw milk is legal when a number of registration and licensing requirements, and safety standards are satisfied by those who are selling such milk. A representative from a government agency stated that: "*the sale of raw milk is legal as long as the milk passes platform tests and the traders are registered with [Tanzania Dairy Board] TDB and licensed to trade by the local government*." Another stated that "*the sale of raw milk is legal. For sale of milk from fixed premises or mobile locations without packaging, a daily levy or a business permit is required*." However, the representative of a food safety government agency declared that "*sale of raw milk is illegal but sales happen and no action is taken.*" These differing perspectives may reflect differing interpretations of the key regulations governing the sector. The Dairy Industry Regulations ban sale of milk that has not been *"pasteurized, sterilized or subjected to such treatment to render it safe for human consumption"* (Dairy Industry Regulations, 2007). It is unclear whether the government considers boiling to be a treatment which would render milk safe for human consumption. If it does, then a number of informal actors–such as milk bars–which sell boiled milk to consumers – are likely in compliance with this aspect of the regulations, while others–informal shops or "*dukas*" may not be as they are selling predominantly raw milk, which the consumer then boils at home. Regardless, we found no evidence to suggest the government is interpreting these regulations in a way that promotes criminalization of informal milk traders.

The Tanzania Dairy Board (TDB) is the main body in charge of regulating and coordinating the development of the dairy industry (See footnote 1). To operate legally, milk producers, collectors, traders, transporters, processors, and traders must register with the Tanzania Dairy Board to obtain a registration certificate–as stated in the Dairy Industry (Registration of Industry Stakeholders). Regulations of 2007, which fall under the Dairy Industry Act of 2004. For milk traders to register, they must possess suitable milk handling equipment, adhere to hygienic milk handling and possess basic platform milk testing facilities, and undergo a medical examination. In addition to registration with the TBD, vendors must obtain other licenses to operate. Transporters must obtain a milk transport permit from Ministry of Livestock and Fisheries Development, Local Government Authorities and Ministry of Health (Urassa, 2014). Additionally, according to the Tanzania Dairy Board small dairy traders must obtain a general trading license issued by the local government (Blackmore et al., 2020).

TABLE 1 Number (and percentage) of surveyed milk traders who have the required licenses.

	Intermediaries	Vendors
TDB certificate (23 and 52 responses respectively)	3 (13%)	1 (2%)
Local government permit for business (23 and 79 responses respectively)	11 (48%)	51 (65%)
Medical certificate (23 and 79 responses respectively)	4 (17%)	23 (29%)

Our interviews suggest that the capacity of government to enforce dairy regulations is limited, especially due to the financial constraints of the Tanzania Dairy Board. Ninety per cent of the Board's revenue is generated from its own fees *via* registration, permits, and certificates. The TDB is understaffed and lacks assets to effectively execute its roles and responsibilities–a challenge exacerbated by the country's size and geography. In addition, it is much more difficult to register and tax the large number of informal actors that far outnumber the formal processors who–being easier to monitor and register–contribute the bulk of TDB fees. This becomes a self-reinforcing cycle–the lower the capacity to enforce relevant regulations around registration, the fewer the opportunities for revenue generation.

As a result of the constraints outlined above, there has been a recent push for government authorities, including TDB, to focus on cess collection and enforcement of laws from businesses in the informal markets–including by devolving some enforcement roles to local government–rather than capacity building to increase revenues of government agencies. According to one government official, this comes with a risk of weakening the relationship between informal agents and government representatives, undermining trust and pushing players "underground," which can decrease milk quality and safety.

Licensing levels among surveyed intermediaries and vendors were reported to be low (Table 1). Only three out of 23 intermediaries had the mandatory TDB registration certificate, and all but one of 52 vendors said they were not registered with

TDB (although more than a third of vendors declined to answer, presumably out of fear of negative repercussions). Twelve of 23 intermediaries (52%) did not have the local government permit which allows them to conduct business, while the remaining 11 (48%) did have the permit. Sixty-five per cent of vendors had the required local government permit, whereas 35% did not. Nineteen out of 23 intermediaries (83%) did not have the legally required medical clearance certificate, and similarly 56 out of 79 vendors (who answered the question) (71%) did not have the medical certificate either.

From the government's perspective, low licensing levels are linked to its own low capacity to enforce the relevant legislation. According to a government official, *"TDB is not able to access the traders for registration and inspection for compliance."* Another government stakeholder acknowledged that "*licenses are expensive for majority of the small traders,*" while a local government stakeholder stated that "*many of the licenses are individually affordable, but there are too many and consolidation is needed to reduce the number of licenses required.*" In interviews, vendors and private sector processors attributed low licensing levels to the requirements being onerous. They complained that there are too many licenses, which are too expensive (either individually or in combination), and that dairy actors don't know the requirements. A representative from an international development organization explained that *"[informal traders] are not keen to formalize because they do not want to remit the fees required by the multiple regulatory agencies which amounts to huge sums of money. The time taken to obtain licenses is also disincentivizing for them.*" Penalisation of those who don't comply with licensing is rare, and this has prompted calls from the formal sector for stricter enforcement.

The Tanzanian government was perceived by our interviewees as relatively supportive of the informal sector–meaning there is little harassment, confiscation of milk etc, and that trade is allowed to continue without interruption. This was partly seen as the result of an electoral calculus, as the ruling party did not want to alienate potential voters. There was also a recognition among government officials of the importance of the informal sector for income generation and food security of low-income households. However, there are differences in approach and attitude to the informal sector between government agencies. For example, some interviewees regard TDB's approach as "friendly"–to enable rather than harass–while the attitude among trade officers and public health officers is seen as more punitive.

What vendors perceive, is somewhat different, however. They perceive a policy environment defined by government indifference, with little impact on their daily life. In the survey, about a quarter of intermediaries defined the government's attitude toward them as "lack of support," and a similar proportion as "lack of harassment," with about a fifth not knowing what the attitude is. About two thirds of vendors said that there was either no conflict or no support from the government, and a quarter stated that there was no conflict if they are licensed. Few traders reported any practical impact of the government's attitude toward them. Some intermediaries and vendors stated that government presence manifests itself as inspections, especially by the Ministry of Health.

Interviews with regulators reveal that informal traders are not well represented in policy discussions in Tanzania, in part because they are not well organized into official associations, and as a result cannot be represented on, for example, the Annual Council of the Tanzania Dairy Board. In addition, many traders are reluctant to have a greater voice with government because they may not comply with all necessary regulatory requirements and fear being exposed.

In terms of changing the relationship with government, intermediaries suggest a closer relationship with government officials, who would then provide support through better communication on requirements, tax exemption, capital provision and input provision. Vendors would like to see a more consistent regulatory environment; more equipment being provided (lactometers and milk cans); subsidized inputs for dairy production; and links to processors to secure a market. Suggested changes by consumers to improve the relationship with government include promotion of practices to enhance quality, regulation of the market and increased campaigns to promote milk consumption.

Perceptions and practices of milk quality and safety

Milk quality and safety was found to be important for all actors in the supply chain, but perceptions and approaches to managing quality and safety vary. Consumers prefer to purchase unpasteurized milk (96% of those we sampled bought such milk, but this may be driven by our purposively sampled consumers purchasing milk from informal vendors in informal settlements) and believe the health risks to be manageable. Consumers who purchased raw milk cited freshness most frequently (26% of mentions), followed by availability (19% of mentions) and safety (14%), convenience of buying (13%), taste (11%) as the key reasons they prefer raw/unpackaged to unpasteurized milk. When thinking about which milk to buy the most important factors consumers consider (they could give up to three responses) is the safety of the milk (27% of all responses), followed by freshness (18%) and the cleanliness of the retail outlet (16%) (see Table 2 below). In interviews, key sector donors highlighted the availability and affordability of unpasteurized milk as a driver of its popularity. Milk is most commonly delivered to the consumers' door by mobile vendors in peri-urban and urban areas, or is collected by consumers at the farm gate in rural areas. During fieldwork we found that unpasteurized milk costs, on average, about half as much as the

TABLE 2 Most important factors considered by consumers when buying milk (157 responses).

	Number (and %) of mentions
Safety	42 (27%)
Freshness	28 (18%)
Nature of retail outlet/cleanliness	25 (16%)
Taste	18 (11%)
Convenience (of buying)	15 (10%)
Availability	12 (8%)
Price	9 (6%)
Nutrition value	3 (2%)
Packaging	3 (2%)
Fat content	2 (1%)

TABLE 3 Number (and percentage) of value chain actors reporting approximate volumes of milk spoiled each week.

	Producers (18 responses)	Intermediaries (23 responses)	Vendors (79 responses)
0%	15 (83%)	6 (26%)	22 (28%)
1–10%	3 (16%)	9 (39%)	45 (57%)
11–20%		2 (9%)	6 (8%)
21–30%		1 (4%)	4 (5%)
31–40%			
41–50%	1 (1%)	3 (13%)	1 (1%)
Above 50%		2 (9%)	1 (1%)

same volume of pasteurized milk. In addition, there are negative, perceptions among low-income consumers regarding processed milk, for example that additives are used to prolong shelf life.

Consumers overwhelmingly consume milk boiled, as part of chai (sweet tea). More than 80 percent of consumers state that drinking milk has not produced any instances of illness in their households. However, 18 per cent of respondents did link intake of milk to symptoms including diarrhea, vomiting, and "inflammation" and to specific diseases, such as brucellosis. Seventy-nine percent (or 10 people) of those who have fallen ill from milk consumption were able to link their illness to specific milk consumed, and 57 percent of these (8 consumers) changed vendor as a result. Lacking formal methods or guarantees that milk is safe and/or of high quality, consumers rely on visual checks of premises or milk.

When consumers were asked how they check the safety and quality of milk once at the vendor's premises, about three-quarters of consumers stated that they look at the milk before they buy it to ensure its quality and safety; others look at the milk after they boil it (6 percent) or taste it (5 percent). Over 70 percent of consumers associated milk safety with the cleanliness of the vendor, the premises and/or the containers, followed by 16 percent who mentioned trust in the vendor's milk or absence of problems with the milk purchased from that vendor in the past. Consumers tend to buy from a small number of vendors (80%), rather than shop around (20% of surveyed consumers). Only a fifth of consumers had noticed the display of legal licenses in vendors' premises, and except one respondent, all others did not associate those licenses with milk health and safety.

The survey reveals that concerns of quality and safety are not exclusive to consumers, but instead common among all actors in the supply chain. Almost all producers, intermediaries, and vendors (100 per cent (20 producers), 96 per cent (23 intermediaries) and 92 per cent (76 vendors), respectively) believe that quality is the most important characteristic customers look for when buying milk from them. Vendors mentioned milk safety as the second most important characteristic, while the cleanliness of storage containers, whether milk has been adulterated and the cleanliness of premises were also mentioned. Trading relationships are relatively stable throughout the value chain–only 4% of intermediaries (1 intermediary) and 6% (5 vendors) of vendors change their suppliers regularly–but when they do, this is due to poor quality or insufficient volumes of milk supplied.

To test for quality and safety, most surveyed intermediaries (71 per cent, or 17 respondents) and vendors (57 per cent or 46 respondents) use sight alone rather than formal testing methods, and only about a fifth of them, or slightly less in the case of vendors, use a lactometer. Despite relying mostly on visual cues, milk spoilage rates were reported by each actor to be relatively low (Table 3). Some degree of spoilage is expected as milk, a perishable product, deteriorates over time in its journey along the chain from producer to intermediary to vendor. However, vendors stated that the most common challenge faced in running their business is spoiled milk.

Traders reported facing challenges in obtaining and maintaining milk quality. A third of intermediaries (8 out of 24) and half of vendors (40 out of 81) stated that they are not always able to obtain the right quality milk due to adulteration and a lack of testing equipment. Even where safe milk or milk of high quality is obtained from farmers, 62.5% of intermediaries and many vendors (40%, or 32 out of 82 respondents) stated that they face difficulties in maintaining the quality and safety of milk. Lack of cold storage was the most cited reason (47% or 8 out of 17 intermediaries). Fluctuations in electricity supply, presumably also affecting the ability to keep milk cold for those who do have access to refrigeration, was also mentioned.

Measures to ensure and improve milk quality and safety in the informal sector

Informal actors reported taking measures to ensure milk quality and safety. Producers most commonly clean containers regularly (23%); keep milking areas clean (20% of responses)

and; ensure cattle are healthy and treated for disease (20%). Washing hands was also mentioned as an important approach (14%), as was using special containers (6%). Similarly, intermediaries and vendors typically clean their containers regularly (35% of responses for intermediaries and 41% of vendors), followed by washing hands (15% of responses for intermediaries and 14% for vendors). Keeping premises clean was also an approach used by vendors (14% of all responses).

To improve the quality and safety of the milk they sell, the majority of vendors (60%), intermediaries (75%), and producers (75%) said they needed training, and others cited the need for more money to invest in testing equipment or containers. The survey showed that most value chain actors—78% of vendors, 71% of intermediaries and 63% of producers–expect the government to help them meet these needs *via* training and provision of assets. In interviews, other sector stakeholder agreed on the need for training to improve on milk quality and safety in the informal sector. An informant from the processing industry argued that dairy sector stakeholders should make efforts to "*educate all raw milk value chain actors in appropriate milk handling approaches to ensure quality and safety.*" A representative from a farmer organization explained that "*enhancing training among producers with little or no knowledge*" can help to overcome the challenge the sector faces. A government stakeholder acknowledged the constraints in capacities–knowledge, financial and physical assets–in the informal sector, emphasizing the "*inappropriate milk handling equipment which is usually not clean*" and the lack of knowledge in handling practices. Other government stakeholders agreed on the need for training and the provisioning of appropriate equipment to improve milk safety in the informal sector.

Despite wide agreement on the need for training, actual opportunities and access to training for informal traders appear to be uncommon. According to the survey, between 2004 and 2019 only 12 of the 82 surveyed vendors and four of the 24 surveyed intermediaries had participated in training. This includes traders who were trained by several organizations including government extension workers, a private sector agency, a non-governmental organization (NGO), ILRI, TDB or by a fellow trader.

Discussion

Our results on the Tanzanian informal milk market point to some of the broader tensions arising between regulation and reality as food systems transform partially and unevenly. While changes to food systems are happening in Tanzania, and some aspects of "modernization" are emerging in response to urbanization, poverty alleviation and population growth, we have found no evidence of a clear or predictable shift toward impersonal or standardized measures to ensure safety in the milk market. Our work highlights four broad issues relevant to the governance of quality and safety in informal markets.

First, there is a disconnect between official visions of food systems modernization and the stubborn persistence of–and broad preference for–informal milk trade. Tanzania's policy environment of promoting commercialization, mechanization and standardization, which involves pasteurization, is arguably disconnected from the cultural and socio-economic realities of Tanzania's milk trade and consumption (Wegerif and Martucci, 2018). Our research shows that this persistence is explained by strong consumer demand, driven by preference and price, as well as higher perceived benefits by other market actors. Consumer preference for unpasteurized milk in many African countries is widely documented due to perceptions of this milk's freshness, availability, price and taste (Wegerif and Martucci, 2018; Blackmore et al., 2020). For producers, sale into the informal market in Tanzania–as opposed to industrial processors in the formal sector–often results in better terms including higher prices (Twine, 2016) and cash payments (Baker et al., 2013; Wegerif and Martucci, 2018). This is consistent with data on the popularity of informal milk markets in Kenya, Uganda and Mali, where the vast majority of milk is produced by smallholders and sold through informal markets (Roesel and Grace, 2015). As has been argued for Latin America, the informal sector is not a residual part of the economy to which people arrive by exclusion, but rather a "voluntary entrepreneurial small firm sector" that people actively seek because they see it as beneficial (Maloney, 2004; Perry et al., 2007).

Second, low compliance with official sanitary or licensing regulations does not mean there is a vacuum or lack of food safety. A system exists to manage quality and safety based on the practices and interpersonal relations of consumers and actors in the informal market. Our research demonstrates that quality matters to all supply chain trading partners and consumers and is a driver of trading decisions throughout the chain; these insights are consistent with evidence from other countries in sub-Saharan Africa showing that most consumers in informal markets care about food safety and respond to food safety scares by stopping or reducing purchases (Roesel and Grace, 2015). This is contrary to many widely held perceptions about the informal economy, particularly within government–that the informal food economy poses significant risks to food safety–while governments ignore their contribution to food security (Skinner and Haysom, 2016; Resnick, 2017).

Research in Ghana found that consumers prioritize cleanliness of both the vending site and the vendor when making purchasing decisions (Rheinländer, 2006). Vendors and consumers are also highly concerned with neatness, which includes aspects of cleanliness, order, aesthetic appearance as well as neat manners during social interactions (Rheinländer, 2006). Similarly, in Tanzania, consumers rely on visual checks of premises, equipment, or milk to assess safety and will remain loyal to vendors as long as milk remains safe. The

importance of loyalty between consumer trust of vendors has also been demonstrated elsewhere (Wertheim-Heck et al., 2014). Moreover, our results show that producers, intermediaries and vendors are concerned about milk safety and take measures to ensure food safety such as cleaning their containers regularly, washing their hands and keeping their premises clean. This is consistent with evidence from Kenya (Blackmore et al., 2021) and South Africa (Campbell, 2011), where street vendors were found to have adequate information regarding food safety principles and to ensure safe practices in food preparation.

For consumers in East Africa, the predominant way of consuming milk–boiled, as part of *chai* (sweet tea)–is a deliberate and effective measure to reduce health risk from germs (Grace et al., 2008), meaning that a potential hazard (e.g., pathogens or harmful substances) does not translate into a significant risk to human health (Roesel and Grace, 2014). Our research confirmed this approach by consumers in Tanzania of boiling unpackaged milk before consumption.

Third, while the informal sector takes many proactive approaches to managing milk safety and quality, there is room for and improvement. Our study has shown that, for the most part producers, vendors and consumers rely on sensory milk quality attributes to assess quality and safety of milk. These measures are an important way to mitigate risk (Roesel and Grace, 2015), but they may be insufficient. There is scope to build on the existing interests and efforts already being made by informal actors to manage health and safety to upgrade the sector. This could be achieved without criminalizing informal actors and compromising food security and livelihoods.

Research in East Africa shows that small-scale and informal traders have limited knowledge in hygienic handling of milk (Cherono et al., 2012), and this lack of knowledge has been linked to poor safety outcomes (Kilango et al., 2012). Other studies confirm a lack of food safety and hygiene knowledge as well as insufficient public services (e.g., clean water) to facilitate safe handling of food in low- and middle-income countries, such as Nigeria (Dipeolu et al., 2007; Chukuezi, 2010). The informal market actors we surveyed from across the supply chain and key informants acknowledge that there is room for improvement in milk safety and quality. Our research shows there is demand by the sector for interventions or support that enhances their ability to sell higher quality and safe milk, particularly training and access to finance for investment in equipment. While training is widely perceived as needed to improve on milk safety in informal markets (Monney et al., 2013; Ledo et al., 2021), the evidence on the impact of training on safety outcomes is mixed (Omore et al., 2005; Monney et al., 2013; Lapar et al., 2014; Alonso et al., 2018; Lindahl et al., 2018; Ledo et al., 2021). This is because while improved knowledge and awareness can lead to different practices, the pathways of change that lead to different outcomes are not always clear or guaranteed.

Fourthly, the informal sector operates in spite of, rather than due to government action or support–but there is scope for change. The current regulatory environment in Tanzania for trade in raw milk is ambiguous. Government agencies interpret regulations differently, and it is unclear whether boiling is officially considered a suitable form of treatment for milk. In addition, the government lacks capacity to enforce regulations that do exist around licensing and registration. In practice this means that informal milk trade exists and persists without government interference. This sets it apart from the adversarial relationships between regulators and informal actors found in several other low- and middle-income countries, whose contexts are defined by harassment, forced relocations, confiscation of goods, and physical abuse (Patel et al., 2014; Resnick, 2017; Grace et al., 2019; Young and Crush, 2019; Blackmore et al., 2021).

The importance of the sector for livelihoods and nutrition, and votes, is likely influencing the decision not to criminalize informal traders, though some of the government's tolerance may also stem from its lack of capacity to support or enhance food safety or informal livelihoods. Public extension services have played a significant role in improving the skills of small businesses engaged in milk marketing, but this capacity has declined in recent years, thereby adversely affecting its quality assurance services for marketed milk. The decline in capacity of government-led extension and technical assistance services in Africa is well documented, as is the general constraints on the private sector stepping in to fill that gap, especially for the low-income majority (Christoplos, 2010; Salami et al., 2010). The government's hands-off approach to managing the sector may change in the future, to be more in line with neighboring countries such as Kenya (Blackmore et al., 2021)–that may come in the form of support for upgrading or possibly greater scrutiny and criminalization. Either way, a failure to base food safety policy on evidence may jeopardize the poor who dominate and rely on informal markets and value chains (Grace et al., 2007; Roesel and Grace, 2014).

Conclusions and policy implications

Milk sold in the informal sector–unpasteurized and unpackaged–is an important source of affordable and accessible protein for low-income consumers in Tanzania. It is cheaper relative to packaged milk from the formal sector, is accessible and available in small quantities, and consumers perceive it to be of high quality (Galiè et al., 2021). Our study demonstrates that quality and safety are key factors driving trading decisions throughout the chain in Tanzania's dominant informal milk sector and shows scope for using those as drivers of upgrading the sector. Most producers, intermediaries, and vendors believe that quality is the most important characteristic buyers look for when buying milk, and for

consumers safety is one of the most important factors in determining which milk they buy (packed or unpacked), and where they buy it. We also found agreement on the need to improve milk quality and safety, as acknowledged by vendors themselves and supported by literature (Cherono et al., 2012 and Kilango et al., 2012). The sector seems to offer much potential for enhanced contribution to livelihoods, food security and the economy, though it already plays a central role in these.

The Tanzanian government has a pragmatic approach to governing informal milk trade, unlike countries such as Kenya that are more repressive (Blackmore et al., 2021). Government authorities are willing to allow for trade in unpasteurized milk so long as traders are registered, have the relevant permits, and standards are met. Though there is a simultaneous push to increase pasteurization levels in the country, current regulations are sufficiently ambiguous to allow trade in unpasteurized milk to continue. In doing so the government avoids alienating its voter base and ensures the livelihoods and food security of low-income actors. However, registration and licensing levels remain low–we found only 2% of vendors had TDB certificates–and the government faces challenges of a vicious cycle of a lack financial capacity to monitor compliance with standards and licensing and registration regulations and in turn a lack of revenue generation from licensing and registration.

Informal actors made it clear that they would like greater support to upgrade their milk testing and handling in a way that ensures its safety and quality, and they felt that this support should come from government. While training schemes in Tanzania that focus on improving the safety of milk in informal market positively impacted those who participated, these schemes have not been scaled or their effects sustained overtime. Our research showed that this is likely due to the lack of incentives and barriers such as the time taken to participate, the business losses incurred in that time, and direct costs and the lack of consumer "pull."

Our research offers a more detailed understanding of the priorities, needs and concerns of informal market actors. In the absence of this understanding, initiatives and interventions to enhance food safety in the informal sector are unlikely to be well designed, scaled or sustained. Any policy interventions need to begin with genuine efforts to understand the informal sector, including its strengths. As part of these efforts to better understand the informal sector, the broader challenge of the lack of voice and representation of the informal sector in policy making in Tanzania should be urgently addressed.

The challenge to researchers and policy makers is to recognize and enable the positive norms, values and practices around which socially embedded economic actors govern urban food markets (Wegerif and Kissoly, 2022), some of which have been documented in this research. Interventions should build on the indigenous practices being used by the entire chain of informal actors that already contribute to risk management, as well addressing informal actors' needs for finance to invest in equipment, and capacity building, to improve on milk quality and safety.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by ILRI Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EB developed research tools, conducted data analysis, and drafted and adapted the manuscript based on feedback from other experts in the field and the co-authors. AG provided strategic oversight to the research and helped draft the first manuscript and revise it after initial reviews. CK lead primary research in the field and conducted some preliminary data analysis. WV, SA, and DG contributed to conception and design of the research. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.971961/full#supplementary-material

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Burden of foodborne disease due to bacterial hazards associated with beef, dairy, poultry meat, and vegetables in Ethiopia and Burkina Faso, 2017

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Foodborne disease is a significant global health problem, with low- and middle-income countries disproportionately affected. Given that most fresh animal and vegetable foods in LMICs are bought in informal food systems, much the burden of foodborne disease in LMIC is also linked to informal markets. Developing estimates of the national burden of foodborne disease and attribution to specific food products will inform decision-makers about the size of the problem and motivate action to mitigate risks and prevent illness. This study provides estimates for the burden of foodborne disease caused by selected hazards in two African countries (Burkina Faso and Ethiopia) and attribution to specific foods. Country-specific estimates of the burden of disease in 2010 for *Campylobacter* spp., enterotoxigenic *Escherichia coli* (ETEC), Shiga-toxin producing *E. coli* and non-typhoidal *Salmonella enterica* were obtained from WHO and updated to 2017 using data from the Global Burden of Disease study. Attribution data obtained from WHO were complemented with a dedicated Structured Expert Judgement study to estimate the burden attributable to specific foods. Monte Carlo simulation methods were used to propagate uncertainty. The burden of foodborne disease in the two countries in 2010 was largely similar to the burden in the region except for higher mortality and disability-adjusted life years (DALYs) due to *Salmonella* in Burkina Faso. In both countries, *Campylobacter* caused the largest number of cases, while *Salmonella* caused the largest number of deaths and DALYs. In Burkina Faso, the burden of *Campylobacter* and ETEC increased from 2010 to 2017, while the burden of *Salmonella* decreased. In Ethiopia, the burden of all hazards decreased. Mortality decreased relative to incidence in both countries. In both countries, the burden of poultry meat (in DALYs)

was larger than the burden of vegetables. In Ethiopia, the burdens of beef and dairy were similar, and somewhat lower than the burden of vegetables. The burden of foodborne disease by the selected pathogens and foods in both countries was substantial. Uncertainty distributions around the estimates spanned several orders of magnitude. This reflects data limitations, as well as variability in the transmission and burden of foodborne disease associated with the pathogens considered.

KEYWORDS

foodborne disease, Africa, *Campylobacter*, *Salmonella*, STEC, ETEC

Introduction

Food safety is linked, directly or indirectly, to the achievement of many of the Sustainable Development Goals (SDGs), especially those on ending hunger and poverty, and promoting good health and wellbeing. According to the World Health Organization (WHO), 420,000 deaths occurred globally among 600 million cases of foodborne disease (FBD) in 2010 and 230,000 of these deaths were due to foodborne hazards which cause diarrheal disease. In 2010, seven diarrheal disease hazards [Norovirus, *Campylobacter* spp. (CAMP), enteropathogenic *Escherichia coli* (EPEC), *enterotoxigenic E. coli* (ETEC), non-typhoidal *Salmonella enterica* (NTS), *Shigella* spp., and *Vibrio cholerae*] were identified to each cause an estimated global FBD burden of 1–3 million disability-adjusted life years (DALYs), as estimated for 2010 (Havelaar et al., 2015). Africa was estimated to have the highest FBD burden of all regions analyzed (2,460 DALYs per 100,000 population compared to a global average of 477 DALYs per 100,000 population). The World Bank has estimated that the economic cost of FBD in low- and middle-income countries (LMIC) amounts to $95.2 billion per year (Jaffee et al., 2019), with the annual cost of treating FBD being estimated at $15 billion. Due to data limitations, both assessments only capture part of the total burden and cost of FBD in LMIC. For example, there is limited data on the incidence, severity and long-term health outcomes associated with several foodborne pathogens. Costs are often limited to those related to medical treatment and lost productivity. Important public health and economic benefits could be achieved by improving food safety in LMIC.

The objectives of this study are to update estimates of the burden of FBD for the pathogens selected by two projects in Ethiopia and Burkina Faso—TARTARE (TARTARE, 2018) and Pull-Push (International Livestock Research Institute, 2018) and estimate the burden associated with consuming food products selected for intervention in these projects. Further details on the objectives of these projects can be found in Sapp et al. (2022).

Materials and methods

WHO data

Disease burden data were extracted from the 2010 WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) database (Devleesschauwer et al., 2015) for Ethiopia and Burkina Faso. While all calculations were done at the country level, FERG only presented results on a global scale and for subregions. The subregions were based on the official grouping of WHO Member States. FERG further subdivided each region into subregions based on child and adult mortality as described by Ezzati et al. (2002): very low child and adult mortality (stratum A), low child mortality and very low adult mortality (stratum B), low child mortality and high adult mortality (stratum C), high child and adult mortality (stratum D), and high child mortality and very high adult mortality (stratum E). Burkina Faso was assigned to the Africa Region (AFR), stratum D (AFRD) and Ethiopia to AFR, stratum E (AFRE).

Disease burden estimates are expressed as incidence, mortality, and DALY at the population level and per 100,000 population. Monte Carlo samples of the uncertainty distributions of the foodborne disease burden of the pathogens selected by the projects (CAMP, ETEC, STEC, and NTS) were abstracted from the FERG database for the total population, children under 5 years of age, and children over 5 years of age and adults. Details on FERG methods can be found in Devleesschauwer et al. (2015). FERG used 10,000 draws to reach convergence. While FERG reports medians as point estimates for the uncertainty distribution, means are reported here due to their probabilistic properties: in contrast to medians, the sum of means is the same as the mean of sums. We note that at the country level, mean and median disease burdens are very similar for all hazards in both countries (data not shown).

Updating burden estimates to 2017

To provide a clearer picture of the current burden of disease associated with the selected pathogens, estimates were updated from 2010 to 2017. Since the WHO does not provide data to assess trends in diarrheal disease burden by specific pathogens, data from the Global Burden of Disease (GBD) study (Institute of Health Metrics and Evaluation, Seattle, WA, Kyu et al., 2018) were used. Even though there are methodological differences between the FERG and GBD estimates, it was assumed that trends in burden per 100,000 of specific pathogens in GBD could be used to update the FERG estimates. Estimates of mortality, of life lost (YLL), years lived with disability (YLD), and disability-adjusted life years (DALY) for three pathogens (CAMP, ETEC, NTS) were downloaded from the GBD Compare website (https://vizhub.healthdata.org/gbd-compare/, accessed September 17, 2019) for 2010 and 2017, and for the three population groups separately. GBD data are prevalence-based, therefore, it was assumed that the change in incidence was proportional to the change in YLD. GBD does not provide data for the burden of STEC. Furthermore, the currently documented burden of STEC in Africa is very low. Therefore, STEC was excluded from further calculations.

Estimates at the population level were converted to burden per 100,000 by dividing by population numbers in both years (Supplementary material S1). Uncertainty distributions were derived for each pathogen of interest. A request to IHME to provide sample data from the uncertainty distributions was denied. The GBD website provides means and 95% uncertainty intervals for the uncertainty distributions of the variables of interest. Therefore, 10,000 samples from the uncertainty distributions of burden per 100,000 in 2010 and 2017 were reconstructed by fitting Gamma distributions to the available percentiles and means. For all metrics, the GBD burden per 100,000 in 2017 ($\rho_{2017,GBD}$) divided by the GBD burden per 100,000 in 2010 ($\rho_{2010,GBD}$) was used as a multiplier to the FERG burden per 100,000 in 2010 ($\rho_{2010,FERG}$) to calculate the FERG burden per 100,000 in 2017 ($\rho_{2017,FERG}$):

$$\rho_{2017,FERG} = \frac{\rho_{2017,GBD}}{\rho_{2010,GBD}} \times \rho_{2010,FERG}$$

The burden estimates per 100,000 were applied to 2017 population data (pop_{2017}) to provide absolute burden estimates ($\beta_{2017,FERG}$):

$$\beta_{2017,FERG} = \rho_{2017,FERG} \times pop_{2017}$$

2017 estimates for the whole population are presented for all ages and children under 5 years of age. Note that the burden for children under 5 years is correlated with the burden for older children and adults. Given the dependence between the two age groups, the distribution of burden for older children and adults cannot simply be derived by subtraction at the Monte Carlo sample level of the burden for children under 5 from the distribution the burden for all ages. Since we don't have information about this dependence, we refrain from presenting estimates for older children and adults.

Attribution

Estimates of the proportion of FBD incidence associated with CAMP and NTS were attributed to beef, dairy, poultry meat and vegetables based on FERG attribution results for the AFRD and AFRE subregions (Hoffmann et al., 2017) as described by Li et al. (2019). Attribution to food types and food products (see Sapp et al., 2022 for details) were based on a structured expert judgment study organized specifically for this study according to Cooke's Classical Model (Cooke, 1992; Hald et al., 2016). This study also included food group attribution for ETEC as these have not been provided by FERG, who focused their food group attribution on zoonotic pathogens. Experts participating in this study had a diversity of backgrounds with experience in the target countries in one or more of the following domains: diarrheal diseases, zoonoses, microbial food safety, water and sanitation or veterinary public health. We assume, in agreement with FERG, that attribution of mortality and DALY is proportional to the incidence of disease and used the same attribution estimates for all burden metrics.

Monte Carlo simulations (10,000 samples) were used to generate estimates of the uncertainty distributions of the disease burden per food group, type or food product for all hazards individually for three population groups.

All data extraction, manipulation, tables, plots, and statistical testing were done in the R statistical software version 4.1.0 (R Core Team, 2021).

Results

Foodborne disease burden estimates for 2010

Global, sub-regional and country-level data for the standardized FBD burden in 2010 (DALYs per 100,000 population) by the four hazards included in this study are presented in Table 1. The burden per 100,000 of CAMP in AFRD and AFRE was approximately twice as high as the global average, and about the same level in the target countries as in their subregions. The burden per 100,000 of ETEC in AFRD and AFRE was more than three times as high as the global average, and about the same level in the target countries as in their subregions. According to FERG, the burden per 100,000 of STEC at the global level was more than two orders of magnitude lower than the burden of the other three hazards included in this study. It was more than two-fold lower in AFRE than

TABLE 1 Foodborne disability-adjusted life years (DALY) per 100,000 population for four bacterial hazards for the global population, subregions AFRD and AFRE, Burkina Faso and Ethiopia in 2010 according to WHO FERG (Havelaar et al., 2015).

Hazard	Global	AFRD%	Burkina Faso	AFRE*	Ethiopia
Campylobacter spp.	31 (22–46)^	71 (35–119)	75 (37–126)	70 (33–117)	69 (32–114)
Enterotoxigenic *E. coli*	30 (17–51)	107 (26–245)	113 (28–260)	105 (17–240)	102 (17–235)
Shiga-toxin producing *E. coli*	0.2 (0.09–0.5)	NA$	NA	0.08 (0.02–0.2)	0.005 (0.0006–0.02)
Non-typhoidal *S. enterica*	59 (36–91)	338 (94–612)	633 (173–1,180)	193 (44–336)	115 (25–200)

%Subregion AFRD (African region mortality stratum D) includes Algeria; Angola; Benin; Burkina Faso; Cameroon; Cape Verde; Chad; Comoros; Equatorial Guinea; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Liberia; Madagascar; Mali; Mauritania; Mauritius; Niger; Nigeria; Sao Tome and Principe; Senegal; Seychelles; Sierra Leone; Togo.

*Subregion AFRE (African region mortality stratum E) includes Botswana; Burundi; Central African Republic; Congo; Côte d'Ivoire; Democratic Republic of the Congo; Eritrea; Ethiopia; Kenya; Lesotho; Malawi; Mozambique; Namibia; Rwanda; South Africa; Swaziland; Uganda; United Republic of Tanzania; Zambia; Zimbabwe.

^Median (95% uncertainty interval).

$Not applicable; not a target pathogen in the Pull Push project.

the global average and more than 10 times lower in Ethiopia. Because the currently documented burden of STEC in Africa is very low, this hazard was excluded from further calculations. The burden of NTS in AFRD was more than five times higher than the global average, and more than three times higher in AFRE. It was two times higher in Burkina Faso (more than 10 times higher than the global average) than in AFRD, while it was lower than the AFRE estimate in Ethiopia at approximately twice the global average.

Different metrics for the burden of FBD by the selected hazards in the target countries in 2010 are presented in Table 2. In both countries, the incidence per 100,000 of CAMP was ∼3 times higher than that of ETEC and NTS. Mortality per 100,000 of CAMP and ETEC were similar in both countries. In both countries, the mortality per 100,000 of NTS and ETEC was higher than the mortality per 100,000 of CAMP. In Burkina Faso, the mortality per 100,000 of NTS was more than five times higher than that of ETEC, and more than 10 times higher than that of CAMP. While the mortality per 100,000 of CAMP and ETEC were ∼10 times higher in children under 5 than in older children and adults, the mortality per 100,000 for NTS was only slightly higher in children under 5 [11.9 (3.24–21.4) per 100,000] than in older children and adults [9.25 (2.41–17.1) per 100,000], see Supplementary materials S2, S4. As noted above, this high mortality per 100,000 of NTS results in a high DALY burden per 100,000 for NTS in Burkina Faso.

Updated foodborne disease burden estimates for 2017

The estimated relative changes from 2010 to 2017 in incidence, mortality, and DALY per 100,000 for the three hazards in both countries and per age group based on GBD estimates (Devleesschauwer et al., 2015) are presented in Table 3 by age group. For the total population in Burkina Faso, there was an increase of 4–10% in the burden of CAMP and ETEC for all metrics. At the same time, there was a decrease of 5–20% in the burden of NTS. For children under 5 in Burkina Faso, the increase in the burden of CAMP and ETEC was more substantial (10–20%) than for the total population, while the burden of NTS increased (Supplementary material S2). In Ethiopia, for all metrics and age groups, the FBD burden per 100,000 decreased in 2017 compared to 2010. Mortality and DALY per 100,000 decreased more than incidence per 100,000. The uncertainties in the changes in burden per 100,000 in Ethiopia in the GBD estimates were substantially smaller than in Burkina Faso.

Updated foodborne disease burden estimates for 2017 were calculated for all hazards, countries and age groups by applying the relative change in GBD burden metrics per 100,000 between 2010 and 2017 to the corresponding FERG estimates. These were then applied to 2017 population data to calculate absolute burden estimates for 2017 (Table 4, detailed results in Supplementary materials S2, S3). Despite decreases in burden per 100,000 for several hazards and age groups, the ordering of hazards and age groups in 2017 was the same as in 2010. All burden estimates were higher in 2017 than in 2010, because the impact of increases in population size outweighed that of a lower burden per 100,000 in both countries. A graphical representation of the uncertainty in the data is shown in Figures 1, 2. Note that the support of the uncertainty distributions spanned several orders of magnitude.

In 2017, children under 5 bore a major share of the total burden of these three pathogens, as measured by DALYs, despite accounting for only 18% of the population of Burkina Faso and 16% in Ethiopia. As shown in Figures 1, 2 the burden for children under 5 per 100,000 was always greater than for the total population. Age distributions differed markedly between pathogens with children under 5 bearing 73% of the burden of CAMP, 73% of ETEC and 30% of NTS in Burkina Faso and 62% of the burden of CAMP, 63% of the burden of ETEC and 49% of the burden of NTS in Ethiopia (see data in Supplementary materials S2–S4).

TABLE 2 Foodborne disease burden for three bacterial hazards in Burkina Faso and Ethiopia in 2010 (all ages) according to WHO FERG (Havelaar et al., 2015).

Hazard	Incidence (×1,000)	Incidence per 100,000	Mortality	Mortality per 100,000	DALY (×1,000)	DALY per 100,000
A. Burkina Faso						
Campylobacter spp.	466 (49–1,440)^	2,990 (313–9,230)	130 (61–214)	0.84 (0.39–1.38)	12.0 (5.75–19.6)	76.9 (37.0–126)
Enterotoxigenic *E. coli*	180 (29–448)	1,150 (183–2,870)	230 (53–481)	1.48 (0.34–3.09)	19.0 (4.3–40.4)	122 (27.7–260)
Non-typhoidal *S. enterica*	188 (24–533)	1,210 (156–3,420)	1,510 (400–2,770)	9.73 (2.58–17.8)	101 (26.8–183)	648 (173–1,180)
B. Ethiopia						
Campylobacter spp.	2,380 (270–7,310)^	2,720 (310–8,340)	671 (306–1,100)	0.77 (0.35–1.26)	61.5 (28.1–100)	70.2 (32.1–114)
Enterotoxigenic *E. coli*	934 (122–2,346)	1,070 (140–2,680)	1,190 (180–2,500)	1.36 (0.21–2.85)	97.4 (16.8–235)	111 (17–235)
Non-typhoidal *S. enterica*	932 (87–2,750)	1,060 (100–3,140)	1,340 (300–2,300)	1.53 (0.34–2.63)	101 (22–175)	115 (22–175)

^Mean (95% uncertainty interval).

TABLE 3 Relative change for 2017 compared to 2010 of disease burden per 100,000 for three bacterial hazards in Burkina Faso and Ethiopia according to the Global Burden of Disease study (Kyu et al., 2018).

Hazard	Age group	Incidence per 100,000	Mortality per 100,000	DALY per 100,000
A. Burkina Faso				
Campylobacter spp.	All ages	+7%^ (+5, +9%)	+4% (−1, +11%)	+6% (+1, +14%)
	Under five	+18% (+17, +19%)	+14% (+8, +23%)	+14% (+8, +26%)
Enterotoxigenic *E. coli*	All ages	+8% (−4, +31%)	+10% (−16, +88%)	+9% (−15, +79%)
	Under five	+13% (−1, +44%)	+16% (−10, +93%)	+14% (−9, +79%)
Non-typhoidal *S. enterica*	All ages	−5% (−6, −3%)	−19% (−24, −17%)	−20% (−24, −18%)
	Under five	−8% (−14, −4%)	−20% (−30, −13%)	−20% (−30, −13%)
B. Ethiopia				
Campylobacter spp.	All ages	−3%^ (−5, 0%)	−14% (−30, +9%)	−17% (−18, −15%)
	Under five	−8% (−8, −8%)	−12% (−13, −11%)	−12% (−13, −12%)
Enterotoxigenic *E. coli*	All ages	−6% (−7, −5%)	−20% (−13, −18%)	−23% (−24, −22%)
	Under five	−16% (−17, −15%)	−22% (−25, −20%)	−22% (−25, −20%)
Non-typhoidal *S. enterica*	All ages	−2% (−3, −1%)	−13% (−18, −10%)	−18% (−18, −17%)
	Under five	−10% (−11, −8%)	−16% (−19, −11%)	−16% (−18, −13%)

^Mean burden 2017/mean burden 2010 (95% uncertainty interval).

TABLE 4 Foodborne disease burden for three bacterial hazards in Burkina Faso and Ethiopia in 2017 (all ages) according to WHO FERG (Havelaar et al., 2015), updated according to the Global Burden of Disease study (Kyu et al., 2018).

Hazard	Incidence (×1,000)	Incidence per 100,000	Mortality	Mortality per 100,000	DALY (×1,000)	DALY per 100,000
A. Burkina Faso						
Campylobacter spp.	616 (64–1,900)^	3,210 (335–6,600)	166 (78–271)	0.83 (0.39–1.37)	15.5 (7.5–25.6)	81.1 (38.8–133)
Enterotoxigenic *E. coli*	239 (37–596)	1,250 (196–3,106)	310 (65–725)	1.61 (0.34–3.78)	25.4 (5.2–59.2)	132 (27.2–308)
Non-typhoidal *S. enterica*	221 (28–626)	1,150 (148–3,260)	1,500 (393–2,750)	7.81 (2.05–14.3)	98.6 (26.0–180)	513 (135–936)
B. Ethiopia						
Campylobacter spp.	2,800 (310–8,600)^	2,640 (300–8,100)	704 (320–1,160)	0.66 (0.30–1.09)	62.6 (28.6–102)	58.8 (26.9–95.9)
Enterotoxigenic *E. coli*	1,070 (140–2,680)	1,000 (130–2,510)	1,170 (180–2,440)	1.09 (0.16–2.30)	91.3 (13.8–193)	85.8 (13.0–181)
Non-typhoidal *S. enterica*	1,110 (100–3,290)	1,050 (100–3,090)	1,420 (320–2,450)	1.33 (0.30–2.30)	101 (22–175)	95.1 (20.8–165)

^Mean (95% uncertainty interval).

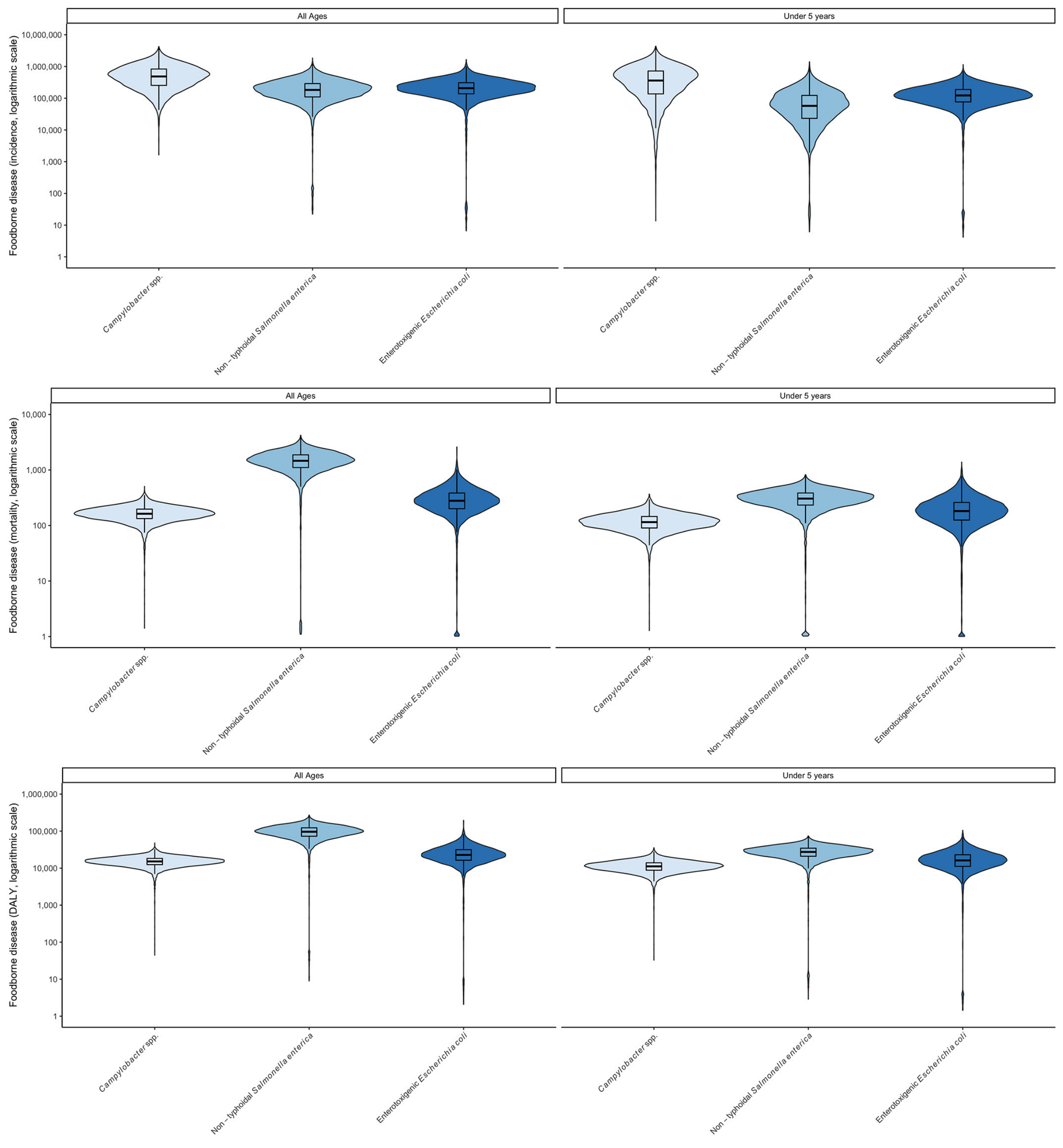

FIGURE 1
Uncertainty in foodborne disease burden for three bacterial hazards and age groups in Burkina Faso in 2017 (all ages) according to WHO FERG (1), updated according to the Global Burden of Disease study (8). **(Top)** incidence; **(Middle)** mortality, **(Bottom)** DALYs. Violin plots display two mirror images of the probability density function of a distribution, smoothed by a kernel density estimator. Embedded in the violin plot is a boxplot, showing lines for the median, boxes for the interquartile range and whiskers for the 95% uncertainty intervals. Values beyond the 95% UI have been omitted.

Attribution of disease burden to specific foods

Attribution estimates are described in detail in Sapp et al. (2022). In this paper, we summarize the results for the four food groups of interest in the TARTARE and Pull Push projects: beef (Ethiopia), dairy (Ethiopia), poultry meat (Burkina Faso and Ethiopia) and vegetables (Burkina Faso and Ethiopia). The burden attributed to these different food groups was further attributed to specific food types and food products as defined in

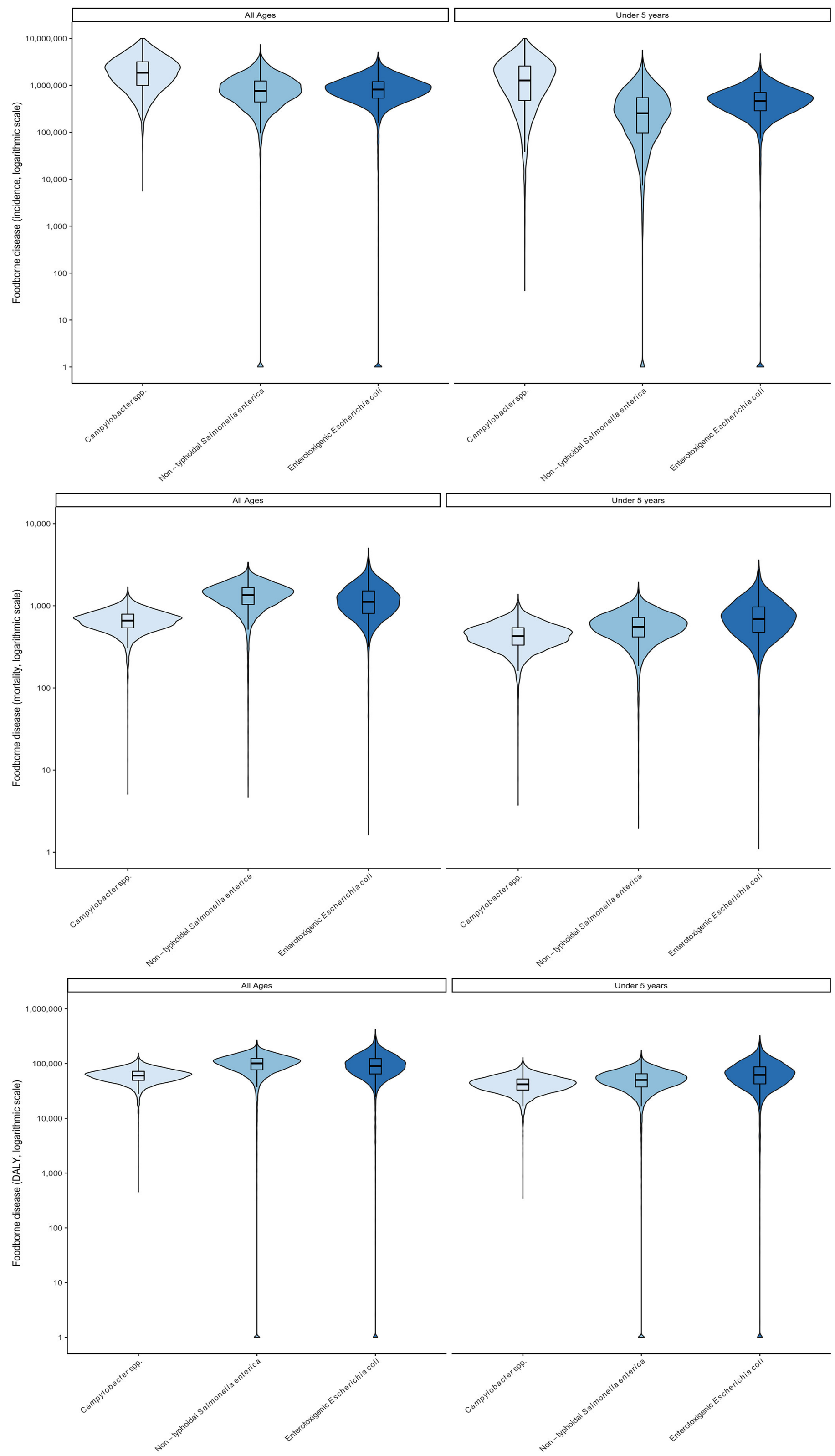

FIGURE 2
Uncertainty in foodborne disease burden for three bacterial hazards and age groups in Ethiopia in 2017 (all ages) according to WHO FERG (1), updated according to the Global Burden of Disease study (8). **(Top)** incidence; **(Middle)** mortality, **(Bottom)** DALYs. See Figure 1 for explanation of violin plots.

TABLE 5 Disease burden associated with specific food consumption in Burkina Faso in 2017 (total population).

Hazard	Incidence (×1,000)	Incidence per 100,000	Mortality	Mortality per 100,000	DALY (×1,000)	DALY per 100,000
A. Poultry meat						
Campylobacter spp.	328 (30–1,070)	1,710 (158–5,590)	89 (30–165)	0.46 (0.16–0.86)	8.3 (2.9–15.3)	43.3 (15.12–80.0)
Non-typhoidal *S. enterica*	76.8 (3.4–244)	400 (17–1,270)	522 (32–1,140)	2.7 (0.2–5.9)	34.3 (2.1–74.6)	179 (11–389)
B. Vegetables						
Enterotoxigenic *E. coli*	44.7 (1.6–447)	287 (10.3–1,060)	58.3 (2.3–206)	0.37 (0.0–1.3)	4.8 (0.2–16.8)	30.5 (1.2–108)
Non-typhoidal *S. enterica*	15.2 (0–63.0)	79.0 (0–328)	104 (0–361)	0.54 (0–1.88)	6.8 (0–23.6)	35.4 (0–123)

^Mean (95% uncertainty interval).

TABLE 6 Disease burden associated with specific food consumption in Ethiopia in 2017 (total population).

Hazard	Incidence (×1,000)	Incidence per 100,000	Mortality	Mortality per 100,000	DALY (×1,000)	DALY per 100,000
A. Beef						
Campylobacter spp.	317 (0–1,540)	297 (0–1,450)	79.6 (0–282)	0.07 (0–0.26)	7.1 (0–24.9)	6.7 (0–23.4)
Non-typhoidal *S. enterica*	83.2 (0–334)	78.2 (0–314)	107 (0–327)	0.10 (0–0.30)	7.6 (0–23.5)	7.1 (0–22.0)
B. Dairy						
Campylobacter spp.	424 (8.9–1,640)	399 (8.3–1,540)	106 (3.8–270)	0.10 (0–0.25)	9.4 (0.30–24.0)	8.9 (0.30–22.5)
Non-typhoidal *S. enterica*	78.1 (0–320)	73.4 (0–301)	101 (0–329)	0.09 (0–0.30)	7.1 (0–23.4)	6.7 (0–22.0)
C. Poultry meat						
Campylobacter spp.	1,420 (141–4,730)	1,340 (132–4,450)	358 (110–688)	0.33 (0.10–0.65)	31.8 (9.8–61.0)	29.9 (9.2–57.3)
Non-typhoidal *S. enterica*	383 (8.6–1,210)	360 (8–1,135)	492 (19.8–1,380)	0.46 (0–0.97)	34.9 (1.4–74.4)	32.8 (1.3–70.0)
D. Vegetables						
Enterotoxigenic *E. coli*	202 (4.9–775)	190 (4.6–729)	221 (6.3–777)	0.21 (0–0.73)	17.3 (0.5–61.1)	16.3 (0–57.4)
Non-typhoidal *S. enterica*	79.0 (0–336)	74.2 (0–316)	101 (0–334)	0.09 (0–0.31)	7.2 (0–23.8)	6.8 (0–22.3)

^Mean (95% uncertainty interval).

Sapp et al. (2022). We present estimates for the total population only. Full results are available in Supplementary materials S2, S3 as means, medians and 95% uncertainty intervals.

In Burkina Faso, the disease burden associated with poultry meat consumption for CAMP and NTS together was estimated to be 42,600 DALYs in 2017 (Table 5A). Approximately 400,000 persons or 1 out of 50 in the total population fell ill from consuming poultry meat in 2017 by the two hazards and, of these, ~600 or 1 out of 30,000 in the total population died. Incidence of CAMP associated with poultry meat was ~4 times higher than NTS, but mortality and DALY burden of NTS was ~4 times higher than CAMP. The estimated disease burden of NTS and ETEC associated with vegetables was lower than that associated with poultry meat, resulting in ~60,000 cases, 160 deaths and 14,000 DALYs in 2017 (Table 5B). The incidence of ETEC-associated disease was ~3 times higher than NTS, but mortality was two times lower. The DALY burden of the two hazards was comparable.

In Ethiopia, the disease burden associated with CAMP and NTS from beef consumption was ~15,000 DALYs in 2017. An estimated 400,000 persons, or 1 out of 250 of the total population, fell ill; of these, ~190, or 1 out of 2 million, died (Table 6A). The burden of dairy products was slightly higher with 500,000 cases, 200 deaths and 17,000 DALYs than the burden of beef consumption (Table 6B). As in Burkina Faso, the burden of poultry meat was highest among the food groups, with 1.8 million cases, 850 deaths and 65,000 DALYs. Incidence of CAMP associated with poultry meat was ~4 times higher than NTS, but mortality and DALY burden per 100,000 were similar for both pathogens (Table 6C). While the incidence of illness associated with vegetables in Ethiopia was similar to beef and dairy, with ~400,000 cases, there were more deaths (330) and DALYs (24,000), with about 2/3 attributed to ETEC-associated disease (Table 6D).

Attribution of disease burden (DALYs per 100,000) to food types and food products is visualized in Figures 3–8. Clearly,

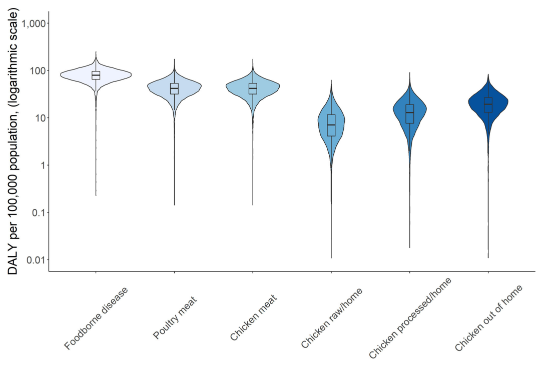

FIGURE 3
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of *Campylobacter* spp. to food types and food products in Burkina Faso in 2017. See Figure 1 for explanation of violin plots.

uncertainty increases with every step in the attribution process. Note that the burden of poultry meat and chicken meat is the same as chicken is the only poultry species consumed in both countries.

Discussion

While the WHO estimates of the global burden of FBD have increased awareness of food safety as a global health problem, their use for national food safety decision-making is limited because they have only been published on a subregional basis. Furthermore, the reference year for these estimates is 2010, and decision-makers would be better informed by more recent data. Working closely with the governments of two countries in Africa, we obtained permission to extract and publish national estimates from the FERG database. This interaction was formalized in a data request by the University of Florida and a communication plan between the project management of the TARTARE project at the Ohio State University (Columbus, OH), and of the Pull-Push project at the International Livestock Research Institute (Nairobi, Kenya).

The GBD study is the only available data source for annual updates of the burden of three of the four hazards of interest in this study (i.e., CAMP, ETEC, and NTS). Directly using the GBD estimates was not possible because those estimates are prevalence- and outcome-based while the FERG estimates are incidence- and hazard-based. These methods only provide equivalent results under the assumption of a stable epidemiologic situation, which clearly is not the case for foodborne diseases. A detailed discussion of these methods and their differences is provided by Mangen et al. (2013) and Devleesschauwer et al. (2015). Briefly, the incidence- and hazard-based approach attributes the current and future burden of acute disease and sequelae resulting from current exposure to a hazard (e.g., diarrheal disease and Guillain-Barré syndrome due to exposure to *Campylobacter* spp.) to the year of exposure while the prevalence- and outcome-based approach attributes disease burden to different outcome categories (e.g., diarrheal disease and neurological disease) based on the prevalence of disease in the reference year. The prevalence- and outcome-based method is preferred for the GBD study because it allows straightforward corrections for comorbidities. For chronic diseases or diseases with a long incubation time, the disease burden reflects past exposure. However, in food safety, it is preferable to estimate the impact of infectious disease control programs by using the current burden, resulting in a preference for the incidence- and hazard-based approach. This preference is further supported by the general use of incidence estimates rather than prevalence estimates in infectious disease epidemiology. Notwithstanding these differences in methodology, we suggest that the relative change in the burden of diarrheal disease in GBD estimates can be used to update the FERG estimates because the duration of diarrheal diseases is short, and any changes reflect changes in the current year. The GBD estimates also reflect impacts of non-food related factors such as access to and effectiveness of health care systems, which may explain the larger decrease (or lower increase) of mortality compared to incidence in both countries.

The FERG data, combined with new attribution estimates generated for this study, suggest a considerable burden is associated with CAMP, ETEC and NTS in both countries. In contrast, the burden of STEC in Ethiopia was very small, but these results should be interpreted carefully because the FERG estimates of the burden of STEC for Africa were based on very limited data (Mangen et al., 2013). Importantly, STEC estimates for Africa were derived from data from a single surveillance system in South Africa that had substantial under-reporting biases, including only one case of Hemolytic Uremic Syndrome being reported for the entire continent (Majowicz et al., 2014). Nevertheless, even in countries with better data, such as the US (Hoffmann et al., 2012) and the Netherlands (Havelaar et al., 2012), the population burden of STEC is several orders of magnitude lower than the burden of CAMP and STEC. In contrast, the individual burden of STEC is much higher, largely due to the risk of Hemolytic Uremic Syndrome and End-Stage Renal Disease in children under 5 years of age, which drives much of the concern about these infections.

Due to data limitations, the uncertainty in the estimates of the disease burden is considerable and spans several orders of magnitude. This statistical uncertainty needs to be considered when using these results for further decision-making, e.g., by including the full uncertainty distributions in cost-benefit calculations. There are additional, unquantified sources of uncertainty in our analysis, including those previously documented by FERG. As mentioned earlier, GBD and FERG use different approaches to estimate the burden of disease.

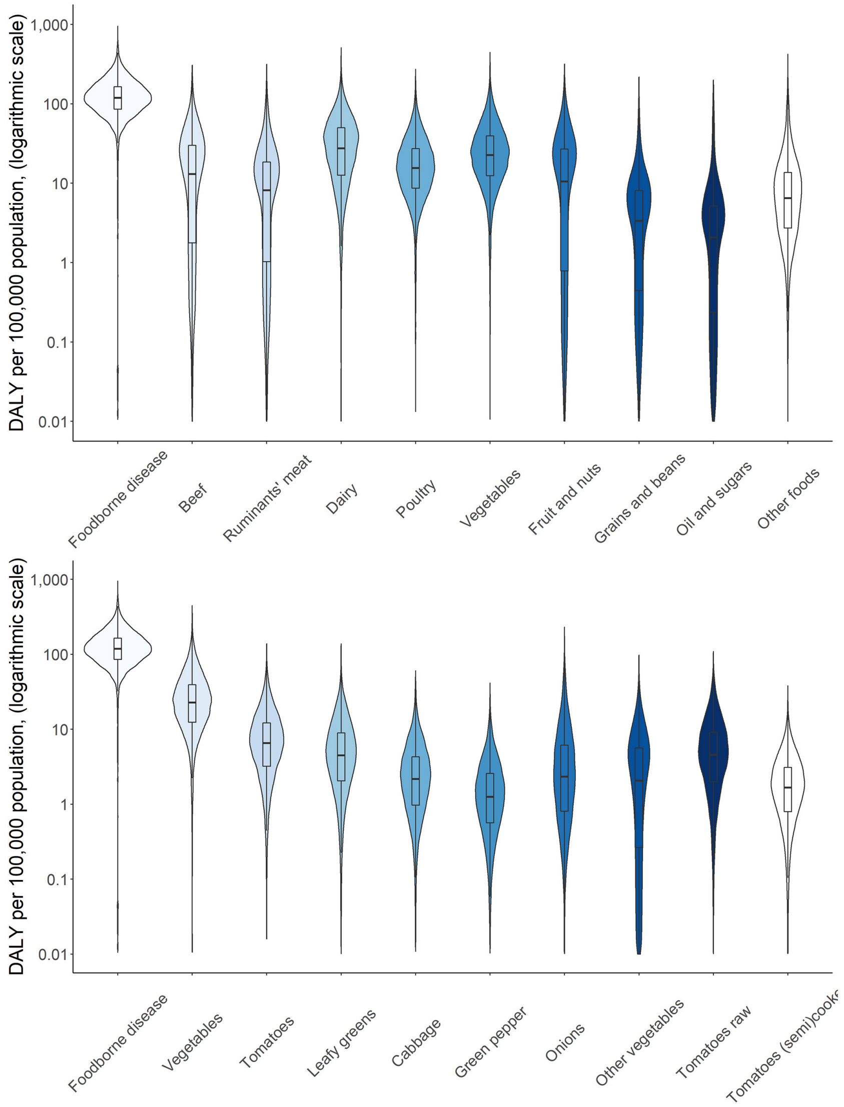

FIGURE 4
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of enterotoxigenic *E. coli* to food group, food types and food products in Burkina Faso in 2017. See Figure 1 for explanation of violin plots.

We assumed that the relative change in GBD burden metrics is representative of the relative change in FERG estimates. In addition, there are uncertainties introduced from the assumption that attribution results at the subregional level from FERG are applicable to the country level and that mortality and DALY attribution is proportional to incidence attribution. Both assumptions reflect the best current data, and the general problem that data-driven attribution is currently not possible given data gaps and the inability to combine data from different study types (e.g., outbreaks, case-control studies, and microbial typing) in one consistent theoretical framework. These problems occur in high-income countries and low- and middle-income countries alike and expert judgment studies are frequently applied at international and national levels to provide best estimates of the (uncertainty in) foodborne disease attribution (Butler et al., 2015; Beshearse et al., 2021).

Conclusions

Foodborne disease is a global public health problem that disproportionally affects low- and middle-income countries. To support evidence-based food safety decision making, detailed, up-to-date insight on the burden of foodborne disease is

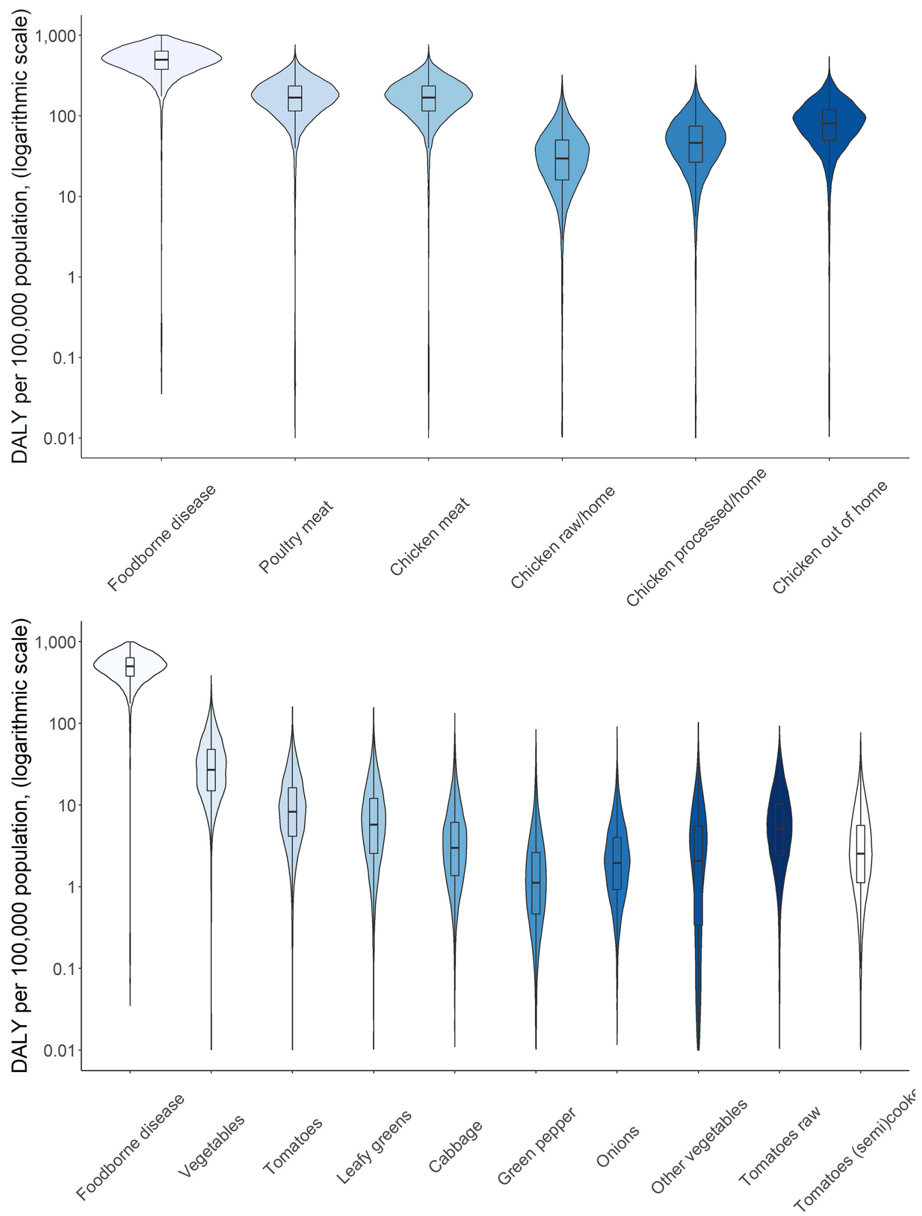

FIGURE 5
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of non-typhoidal *Salmonella enterica* to food types and food products in Burkina Faso in 2017. See Figure 1 for explanation of violin plots.

required at the national level. The WHO has presented estimates of the global burden of foodborne disease at subregional level for the reference year 2010. We demonstrate how the WHO data can be used in tandem with estimates from the Global Burden of Disease study published by the Institute for Health Metrics and Evaluation to update foodborne disease burden estimates for two countries in Africa (Burkina Faso and Ethiopia) to 2017. Moreover, we demonstrate how the total burden of foodborne disease by specific microbial pathogens can be attributed to specific food using data from structured expert elicitation studies. Our results demonstrate a considerable burden of foodborne disease associated with three pathogenic bacteria (*Campylobacter* spp., enterotoxigenic *E. coli* and non-typhoidal *Salmonella enterica*). The burden of Shiga-toxin producing *E. coli* was several orders of magnitude lower but should be interpreted with caution since data on the incidence of this pathogen in Africa is very limited. *Campylobacter* spp. caused the largest number of illnesses in both countries,

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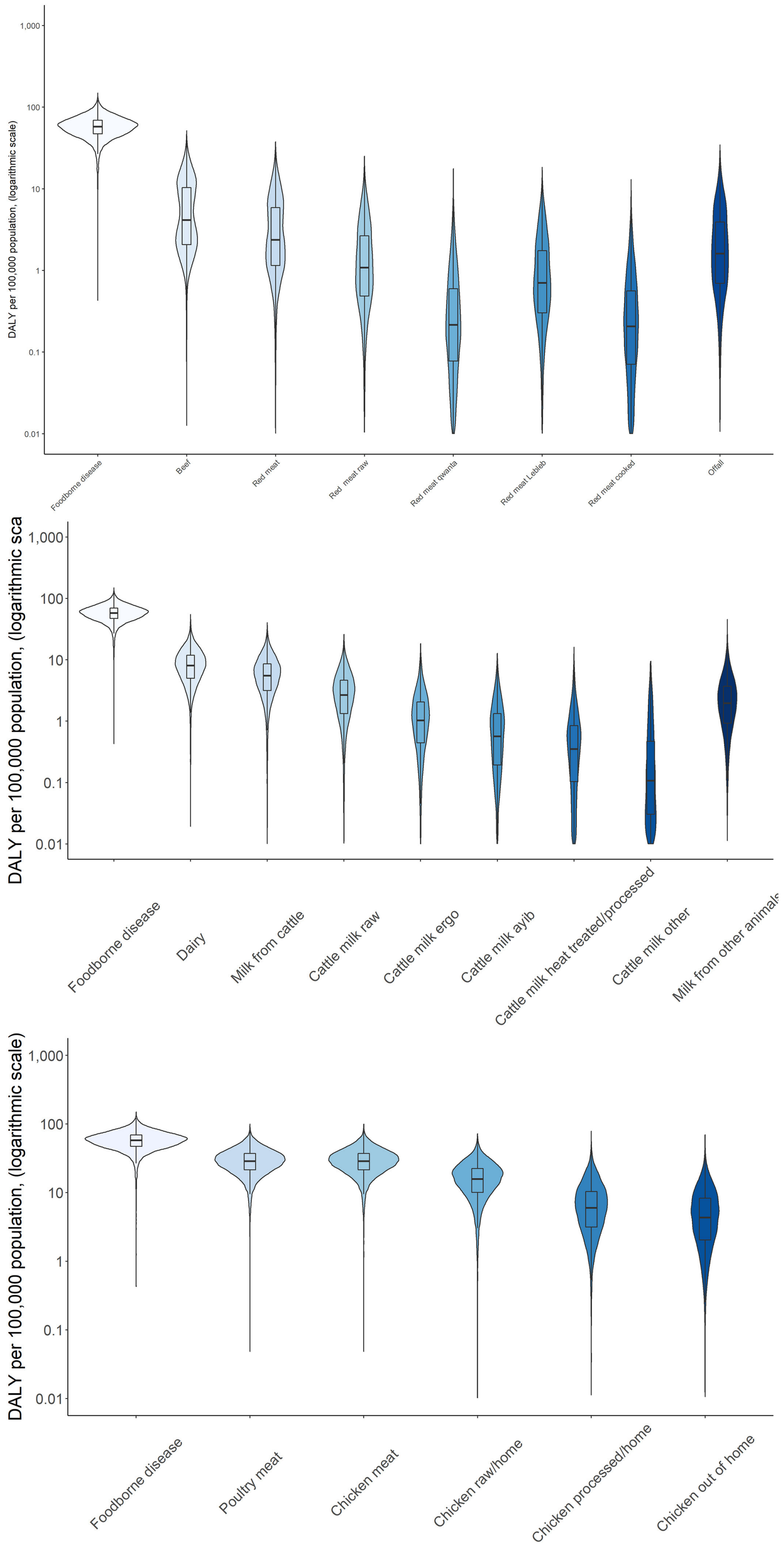

FIGURE 6
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of *Campylobacter* spp. to food types and food products in Burkina Faso in 2017. See Figure 1 for explanation of violin plots.

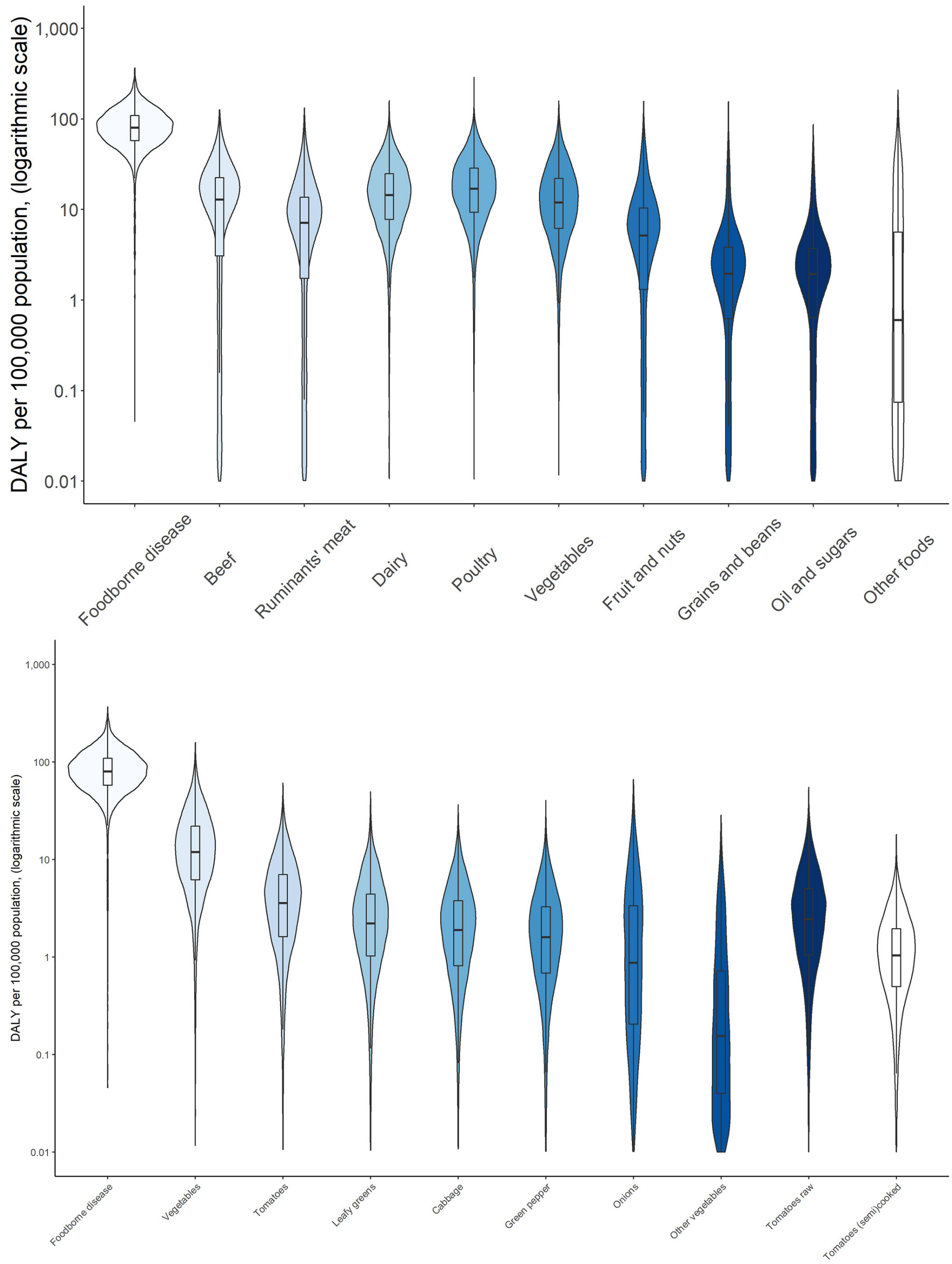

FIGURE 7
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of enterotoxigenic *E. coli* to food group, food types and food products in Ethiopia in 2017. See Figure 1 for explanation of violin plots.

while non-typhoidal *Salmonella enterica* caused the largest number of deaths and disability-adjusted life years. Children under 5 years of age bore ¾ of the burden of *Campylobacter* spp. and enterotoxigenic *E. coli* in Burkina Faso and 2/3 of this burden in Ethiopia. The burden of non-typhoidal *Salmonella enterica* was more uniformly distributed over age groups. In both countries, the burden (in disability-adjusted life years) of consuming poultry meat was higher than the burden of consuming vegetables. In Ethiopia, estimates were also generated for the burden of consuming beef and dairy, which were similar and somewhat lower than the burden of consuming vegetables.

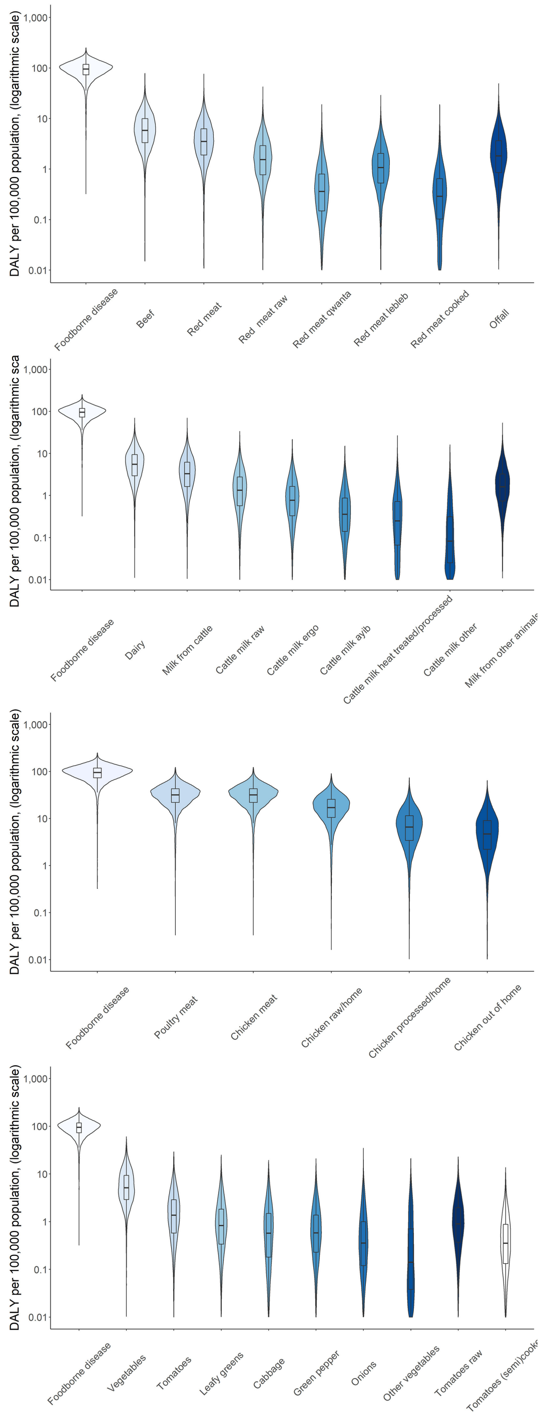

FIGURE 8
Attribution of disease burden (Disability-Adjusted Life Years per 100,000 population) of non-typhoidal *Salmonella enterica* to food types and food products in Burkina Faso in 2017. See Figure 1 for explanation of violin plots.

Data availability statement

The original contributions presented in the study are included in the article and Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AH: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, and writing—original. AS: data curation, formal analysis, investigation, software, validation, visualization, and writing—original. MA: data curation, investigation, and writing—review and editing. GN: formal analysis, methodology, software, visualization, and writing—review and editing. KM: conceptualization, supervision, validation, and writing—review and editing. BD: formal analysis, software, validation, and writing—review and editing. DG: funding acquisition, validation, and writing—review and editing. TK-J: project administration, resources, validation, and writing—review and editing. BK: funding acquisition, project administration, validation, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1024560/full#supplementary-material

SUPPLEMENTARY MATERIAL S1
Population size in Burkina Faso and Ethiopia, 2010 and 2017.

SUPPLEMENTARY MATERIAL S2
Detailed statistics of foodborne disease burden in Burkina Faso, 2010 and 2017.

SUPPLEMENTARY MATERIAL S3
Detailed statistics of foodborne disease burden in Ethiopia, 2010 and 2017.

SUPPLEMENTARY MATERIAL S4
Foodborne disease burden for three bacterial hazards in Burkina Faso and Ethiopia in 2010 (children under 5 years of age) according to WHO FERG (1).

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Antibiotic use, knowledge, and practices of milk vendors in India's informal dairy value chain

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Background: Milk vendors play an important role in India's dairy value chain; however, their food safety practices are poorly understood. From a milk safety perspective, vendor behavior is significant because it has the potential to affect both consumer and producer behavior. This study describes the types of milk vendors in two Indian states, in an attempt to investigate vendors' hygienic knowledge and practices toward safety and antimicrobial resistance (AMR).

Methods: A cross-sectional study was conducted in the states of Assam and Haryana, India. In selected villages, all the milk vendors identified at the time of visit were interviewed. A questionnaire was used to assess the knowledge and practices on antibiotics, milk safety and hygiene. The milk samples were tested for presence of antibiotic resistant bacteria using antibiotic susceptibility testing.

Results: In total, 244 milk vendors were interviewed during the survey. Out of these, 146 (59.8%) of the vendors traded raw milk, while 40.2% traded pasteurized milk. Vendors were categorized depending on whom they supplied milk to. Five categories were identified: (a) those who sold at grocery shops; (b) those who sold on roadside (roadside vendors); (c) those who sold from door to door; (d) those who sold to sweet makers/tea stalls, and (e) those who sold from own home/other entity. The level of training among vendors on milk hygiene was non-existent and the knowledge related to antibiotics was low. Most of them [210/244 (86.07%)] agreed that boiled milk is always safer than raw milk but almost half [119 (48.77%)] of them admitted that sometimes they drink milk without boiling it. Most vendors believed that they could identify whether milk is safe or not for consumption just by its appearance and smell. Out of 124 milk samples collected from surveyed milk vendors and tested for the presence of antibiotic-resistant bacteria, 80 (64.52%) were tested positive.

Conclusion: This study highlights the low levels of knowledge regarding food safety among milk vendors. It shows the predominance of informal milk

vendors in the surveyed states and prevalence of AMR bacteria in milk traded by them. Training may be a beneficial strategy for addressing the issue.

KEYWORDS

milk vendors, AMR, informal milk supply chain, food safety, milk hygiene, raw milk, antibiotics

Introduction

India is the world's leading milk producer, currently producing more milk than the European Union or the United Sates (FAO., 2021), with ever-increasing domestic milk consumption (Douphrate et al., 2013; Milk Production in India., 2022). Unlike other countries, where large-scale commercial units dominate dairy production (Douphrate et al., 2013), India's milk production is driven by numerous small dairy holdings (Kumar et al., 2011; Sudhanthiramani et al., 2015). Up to 80% of Indian milk is produced by rural dairy farmers and handled through an unorganized sector with only 20% handled through a regulated, organized industry (Dhanashekar et al., 2012; Sharma et al., 2020).

Milk is responsible for the spread of several foodborne bacterial pathogens from animals to humans in India (Mutua et al., 2020). Antimicrobial-resistant (AMR) bacteria have earlier been found prevalent in milk across India (Kuralayanapalya et al., 2019), which is a significant public health concern given the morbidity and mortality related to AMR infections in humans (Mutua et al., 2020; Sharma et al., 2020). Across India, milk vendors allow consumers to access this highly perishable commodity, transporting it from source to distribution (Sudhanthiramani et al., 2015). A number of laws in India regulate the safety of milk and milk-based products (Mutua et al., 2020). However, it is unclear how successfully these laws are being implemented on the ground mainly in regard to informal dairy sector. Given that animal-sourced foods, including milk, are estimated to be responsible for 70% of India's 100 million yearly cases of foodborne illnesses (FBD) (Smeets Kristkova, 2017), and the way in which vendors handle food has been associated with milk contamination and cross-contamination of other foods (Alimi, 2016), the handling procedures among milk vendors warrant closer examination.

Milk vendors have been found to play a key role in the etiology of foodborne zoonoses (Grwambi, 2020). In India, the infrastructure facilities for milk collection, storage, transportation, and processing raw milk are inadequate (Rajendran and Mohanty, 2004). Given the highly perishable nature of raw milk, handling of raw milk for hours without cooling allows pathogens in milk to multiply, increasing the risk of FBD for the consumer (Roesel Kristina, 2014). In India, research at the dairy-farm level shows poor adoption of hygiene practices in milk handling, while adoption of milk safety practices at vendors' level is poorly understood. Given the lack of studies performed across India to assess the knowledge and attitude of milk vendors related to milk safety, antibiotics, or AMR, we selected this important informal dairy value chain actor for this cross-sectional study. The study explores the hygienic procedures followed by the milk vendors and their awareness level related to basics of safe milk handling, antibiotics, and AMR in two milk producing states of India-Haryana and Assam. The states were chosen based on their strong disparity in regard to dairy production system, processing infrastructure and presence of vegetarian population. Haryana, a state with good dairy production system coupled with better processing infrastructure and predominance of vegetarian population might perform differently in the given areas of this study objectives than in Assam where dairy production, processing and consumption are inferior to Haryana (Kumar et al., 2021).

Methods

Sampling design

A cross-sectional study was conducted in the states of Assam and Haryana, India, from October-November 2017, as a follow up on a previous study done in April 2016—March 2017 where farmers were interviewed (Kumar et al., 2021). The farmer survey was based on a multi-stage selection process. In brief, the district selection was guided by Animal Husbandry and Veterinary Department officials regarding the district's potential for dairy development (low, medium, and high). Accordingly, the three districts Kamrup (metropolitan), Golaghat, and Baksa were selected for Assam and Karnal, Kaithal and Bhiwani for Haryana. Figure 1 shows a map of the two states with the three respective districts per state where the study was conducted.

From each district, two community development blocks (CDBs), one urban and one rural, had been randomly selected. In case of Assam, the rural CDBs were close to urban (peri-urban) CDBs as the informal milk vendors were more predominant in urban and peri-urban areas. In each CDB, four villages were selected randomly from the list of villages having dairy production and presence of milk vendors. In case of Kamrup Metropolitan district, villages were defined as

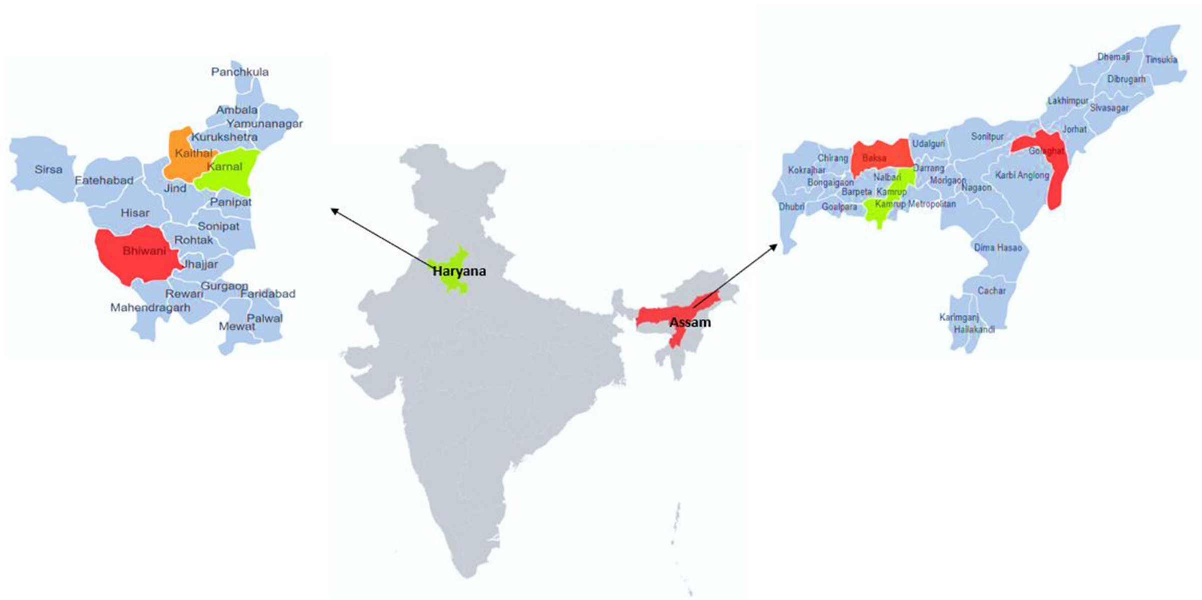

FIGURE 1
Map of India showing the Haryana and Assam states, with the three districts within each state included in a study on knowledge on food safety and antimicrobial resistance among milk vendors.

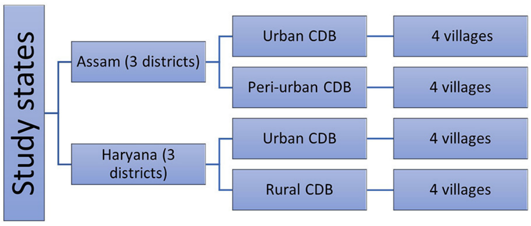

FIGURE 2
Sampling design for each of the study states specifying the number of districts and villages from each community development block (CDB) in the district.

clusters of milk producers, as they were located only in certain clusters, irrespective of the village boundaries. Other villages were defined to be a cluster based on the official boundaries. Figure 2 gives the scheme that outlines the sampling design used for each state.

The same villages were visited for the current vendor survey, and all milk vendors identified at the time of the visit were contacted for the interview. All vendors who agreed to participate were then questioned. A questionnaire was used to assess their knowledge and practices on antibiotics, milk safety and hygiene. If they had milk available, a sample of the milk was purchased at the local going rate for fresh milk.

Data collection

Knowledge on antibiotics and their usage, milk safety, hygienic measures regarding handling of milk was assessed through a pre-tested vendor questionnaire (Supplementary material). The questions were asked by enumerators in local language and the responses were filled in the questionnaire written in English at the time of the interview.

Analysis of milk samples

Milk samples were purchased from vendors and stored in sterile containers maintaining a cold chain, during transportation to the laboratory, and kept at 4°C until further analysis at the National Institute of Veterinary of Epidemiology and Disease Informatics (NIVEDI). The samples were initially inoculated on mannitol salt broth and buffered peptone water and incubated at 37°C for 18–24 h to isolate presumptive pathogenic bacteria. Following their incubation at 37°C for 18–36 h, the culture broth was inoculated on *Staphylococcus* Agar No. 110 (Hi-media, Maharashtra, India) to grow staphylococci, and MacConkey agar (Hi-media, Maharashtra, India) to grow *E. coli*, *Shigella* spp., and *Klebsiella* spp. Brain Heart Infusion agar (Hi-media, Maharashtra, India) was used for purification and maintenance of cultures. The isolated bacteria's were screened for resistant bacteria using antibiotic disc diffusion testing as per the Kirby Bauer. (2022)

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method and following the Clinical Laboratory Standard Institute guidelines (Testing, 2013). Gram-positive bacteria were tested against cefoxitin (30 μg), methicillin (5 μg) and oxacillin (1 μg) for screening of methicillin resistance. Gram-negative bacteria were tested against cefoxitin (30 μg), cefotetan (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), imipenem (10 μg) and meropenem (10 μg) was used for screening of beta-lactamases (extended spectrum beta-lactamase, metallo-beta-lactamase, AmpC beta-lactamase). For the purpose of this paper on vendor behavior, any sample that had either a gram-positive or gram-negative bacteria isolated as per above that showed resistance to at least one antibiotic was classified as being positive for AMR, and used as a binary variable in the analyses.

Data analysis

Data was entered in Microsoft Excel and analyzed using STATA 14.0 (STATACorp Ltd., College Station, TX, USA). Firstly, descriptive analyses of all vendors were carried out, followed by ANOVA (analysis of variance) to test for association between different variables for all vendors. Secondly, a descriptive analysis for the vendor milk samples that were analyzed in NIVEDI, Bangalore, for the presence of antibiotic resistant bacteria was carried out and it was combined with laboratory results corresponding to these milk samples. For these vendor samples, univariable analysis was conducted to check for associations between categorical variables like age, gender, education, milk sold, type of vendor and the presence of AMR bacteria in milk samples.

Results

Vendor demographics

A total of 244 milk vendors were interviewed, 122 in Assam and 122 in Haryana, of these 220 were males (114 in Assam and 106 in Haryana) and 24 were females (8 in Assam and 16 in Haryana). Most, 47.5% (116/244), of the surveyed vendors from Assam and as well as from Haryana were in the age group of 31–45 years.

Only 3.1% (7/226) [18 non-respondents (NR)] had received specific milk hygiene training. In terms of general education levels, 5.7% (14/244) had not received any formal education (1 in Assam and 13 in Haryana), 9.4% (23/244) had received primary education (17 in Assam and 6 in Haryana), 41.8% (102/244) had studied up to 5–10 class (44 in Assam and 58 in Haryana), 29.5% (72/244) had reached higher secondary (41 in Assam and 31 in Haryana) and 13.5% (33/244) were graduates or above (19 in Assam and 14 in Haryana).

Most, 64.8% (158/244), milk vendors sold 2–50 ltrs. of milk/day. And 10.2% (25/244) sold ≤5 ltrs./day. Most of the vendors selling <50 ltrs. milk/day were shop owners. Less than half (40.2%, 98/244), vendors sold pasteurized milk while the remaining 59.8% (146/244) traded raw milk. Five categories of milk vendors were identified; vendors who sell milk at a grocery shop (37.3%, 91/244), vendors supplying milk from door to door (16%, 39/244), the vendors who sell on a roadside (6.6%, 16/244), vendors who supply milk to sweet makers/tea stall (17.6%, 43/244), and the ones selling from home/other entity (22.5%, 55/244).

The results from the ANOVA analysis (Table 1) showed that 34.4% (84/244) of the vendors checked milk for dirt, checking of milk was significantly associated with the category of vendor ($p < 0.01$) with shop owners more likely to check the milk for dirt as compared to other vendors. The quantity of milk sold (ltr/day) differed significantly between vendor categories ($p < 0.01$). Most, 86.1% (210/244) vendors agreed that boiled milk is always safe but almost half, 48.8% (119/244) of them admitted that they sometimes drink milk without boiling.

Knowledge and attitude toward antibiotics

When vendors were asked if they had ever heard of antibiotics, 34.8% (85/244, confidence interval (CI) 28–41%) vendors answered yes and 65.2% (159/244, CI 58–71%) no. It may be noted here that there was no distinction made between antibiotic for animal or human usage in this question. The majority of those answering what antibiotic is, answered that they kill germs, or they cure diseases. Figure 3 gives various responses of vendors who agreed to know about antibiotics. Out of 85 vendors who had heard about antibiotics, 28.2% (24/85, CI 19–39%), believed that antibiotics stop all diseases. Some (44.7%, 38/85, CI 33.9–55.8%) believed that all diseases need to be treated. Most vendors (55.2%, 47/85, CI 44.1–66%) agreed that more an antibiotic costs, the better it is. Some vendors (20%, 17/85, CI 12–30%) also agreed that the pharmacist is as good as the doctor to decide which antibiotic to use and 40% (34/85, CI 29.5–51.1%) of them agreed that increasing the amount of antibiotic makes it more effective. Only some of them, 45.8% (39/85, CI 35–57%) agreed that if antibiotics are used too often, they become ineffective. Out of 244, only one vendor knew about "withdrawal period" and could describe it correctly.

Presence of antibiotic resistance bacteria

Total 124 milk samples were tested in NIVEDI, Bangalore, for the presence of antibiotic resistant bacteria. Out of these tested samples, 85.5% (106/124, CI 78–91%) were raw milk samples and 14.5% (18/124, 8–21%) were pasteurized milk

TABLE 1 Distribution of demographics, knowledge and practice among categories of milk vendors in Assam and Haryana, India.

	Shop owner (*N* = 91)	Roadside vendor (*N* = 16)	Door-door vendor (*N* = 39)	Supplier to sweet maker/teashop (*N* = 43)	Others* (*N* = 55)	*P*-value
Gender						0.98
Male	82 (90.1%)	15 (93.7%)	35 (89.7%)	38 (88.4%)	50 (90.9%)	
Female	9 (9.89%)	1 (6.25%)	4 (10.3%)	5 (11.6%)	5 (9.1%)	
Milk sold (ltr./day)						<0.01
2–50 ltr	69 (75.8%)[a]	8 (50%)[a,b]	26 (66.7%)[a]	19 (44.2%)[b]	36 (65.5%)[a]	
51–100 ltr	12 (13.1%)	4 (25%)	8 (20.5%)	6 (13.9%)	7 (12.7%)	
101–300 ltr	9 (10%)	2 (12.5%)	5 (12.8%)	8 (18.6%)	6 (10.9%)	
>300 ltr	1 (1.1%)	2 (12.5%)	0	10 (23.2%)	6 (10.9%)	
Training on hygiene						0.01
Yes	0[a]	0[a,b]	0[a,b]	2 (4.6%)[b]	5 (9.1%)[b]	
Check milk before buying it						<0.01
Yes	57 (62.6%)	3 (18.7%)[a]	6 (15.4%)[a]	9 (20.9%)[a]	9 (16.4%)[a]	
Boiled milk is always safe						0.10
Yes	72 (79.1%)[a]	15 (93.7%)[a]	34 (87.2%)[a]	37 (86.1%)[a]	52 (94.6%)[a]	
I can tell by the look and smell of milk if it safe to drink						0.01
Yes	25 (27.5%)[a]	8 (50%)[a,b]	20 (51.3%)[a,c]	17 (39.5%)[a,d]	29 (52.7%)[b,c,d]	
Drink milk without boiling						<0.01
Yes	64 (70.3%)[a]	1 (6.3%)[b]	6 (15.4%)[b]	15 (34.9%)[b,c]	33 (60 %)[a,c]	

[a,b,c,d]Categories with same superscript are not significantly different.
*Others include selling from home/other entity.

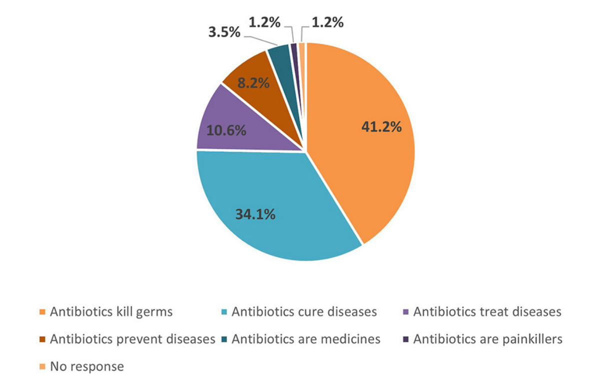

FIGURE 3
The various definitions of antibiotics given by milk vendors who agreed to know about antibiotics in Haryana and Assam, India.

samples. In total, 64.5% (80/124, CI 55–72%) out of these samples tested positive for the presence of antibiotic resistant bacteria (either *Staphylococcus* or gram-negative bacteria). Out of the samples that tested positive, 83.7% (67/80, CI 73–91%) were from the raw milk vendors and 16.2% (13/80, CI 8–26%) were from the pasteurized milk vendors. The percentage of positive samples from each vendor type is presented in Figure 4. The highest proportion of milk samples with antibiotic resistant bacteria was from the vendors in the other category (selling from home/other entity) where 23/31 (74.19%) were found positive, and similar proportions were found among shop owners, 25/36 (69.44%) and door-to-door vendors, 17/24 (65.38%). Half (50%, 12/24) of the milk samples from suppliers to sweet makers/teashops and 42.86% (3/7) of milk samples from roadside vendors were positive. All six milk vendors who had attended milk hygiene training had resistant bacteria present in their milk. Of the vendors whose milk tested positive for antibiotic-resistant bacteria, 43.75% (35/80, CI 32–55%) believed they could tell if milk is safe to drink just by its look and smell. Only 8.75% (7/80, CI 3–17%) of these 80 vendors kept the milk chilled after purchase; 30% (24/80, CI 20–41%) agreed to know about antibiotics and 70% (56/80, CI 58–79%) answered negative. Only one of them knew about "withdrawal period."

Univariable analyses did not show any significant association ($p > 0.05$) between age, gender, education level, amount of milk traded or type of trader with the presence of AMR in vendor milk samples.

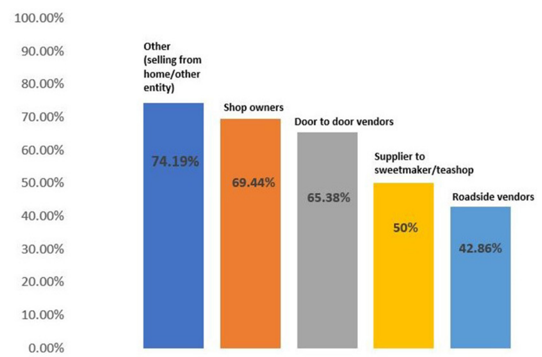

FIGURE 4
Percentage of milk samples with detected antibiotic-resistant bacteria out of the total milk samples collected from different milk vendor categories in Assam and Haryana, India.

Discussion

The results of this study show that milk vendors in Assam and Haryana are predominantly male, are poorly educated, only few vendors receive training on milk hygiene, trade in low daily volumes of milk, and form a very heterogenous group with five major types of vendors identified based on how they sell the milk. Such informal dealers may not appear to pose a serious threat to public health, however, almost sixty percent of these vendors traded exclusively in raw milk. Raw milk may pose a direct risk to the consumer by exposing them to foodborne pathogens through consumption, and indirectly through cross-contamination (Oliver et al., 2005). In this regard, proper boiling of milk before consumption reduce the risk to great extent. The results of this study revealed poor handling and storage of raw milk by the vendors, only 8.2% (12/146, CI 0.04–0.13) of raw milk vendors kept the milk chilled between purchasing from farm and selling, leaving the remaining 91.8% (134/146, CI 0.86–0.95) in breach of regulations of the Food Safety and Standards Authority of India (FSSAI., 2018) which stipulates that all raw milk must be stored at 4–5°C (FSSAI., 2018). Similar results were found in a study among dairy value chain actors in Assam (Lindahl et al., 2019) where milk traders reported never storing milk but delivering it as soon as possible. Considering that at the time this study was conducted, the average daytime weather conditions for the study sites in Assam and Haryana were slight hot and humid with temperature varying from 25 to 35°C (Ministry of Earth Sciences, 2017), a lack of cold chain allows for microbial growth and milk spoilage (Lingathurai and Vellathurai, 2013).

Indeed, milk vendors themselves are also at risk of foodborne disease given that we found that 48.8% (119/244, CI 0.42–0.55) of milk vendors agreed that they sometimes drink raw milk themselves. The majority of the sellers here were shop owners who might be referring to drinking tetra pack milk or pasteurized milk, which is acceptable to consume without boiling, but there were others who concurred on consuming raw milk without boiling. This is in line with earlier findings from other countries where milk vendors and their families consume more milk than the general population, possibly as they consume unsold products (Kirino et al., 2016). Most of the vendors in our study believed that they could recognize if milk is safe to consume by just its sight and smell. This is also in line with the results of a previous study conducted in Assam (Lindahl et al., 2019) where 99.4% of the milk traders agreed on a statement saying, "you can tell if milk is safe by sight or taste." This highlights that attitudes and practices around milk consumption merit further investigation among key members within these households responsible for food preparation, especially those responsible for preparing food for infants and the elderly.

This study found a high prevalence of antibiotic resistant bacteria in raw milk samples, similar to other studies in India, where raw milk samples were found positive for the presence of antibiotic resistant bacteria (Kumar and Gupta, 2018; Kumar et al., 2021). This is indeed a matter of concern. The simplistic, binary focus of the diagnostic method used in our study give results which show milk samples to be positive or negative for the presence of one or more bacteria resistant to one or more antibiotic, as a first step in scoping out AMR prevalence among vendors of different categories. While other studies focus on identifying specific AMR bacteria, such as a study in southern India showing that raw milk samples collected from local milk vendors had a 39.09% prevalence for the presence of antibiotic resistant *Staphylococcus aureus* (Sudhanthiramani et al., 2015). For this study, it was decided to focus primarily on the presence or absence of antibiotic resistant bacteria rather than on identifying specific pathogens, given that the cross-cutting measures needed to reduce the risk of AMR bacteria moving along the informal dairy value chain will be overarching, regardless of what specific bacteria or antibiotic resistance involved.

Similar to the research carried out among small-scale dairy farmers in the same study sites in Assam and Haryana, vendors in this study, demonstrate little or no knowledge of the concept of milk withdrawal period, which is similar to farmers, and we have earlier reported the presence of antibiotic residues in the milk from these two states (Kumar et al., 2021), highlighting the lack of appropriate measures taken to prevent such hazards entering the dairy supply chain. If vendors, and indeed farmers, are unaware of such concepts as milk withdrawal periods, then reducing the risk of contamination of milk is very challenging. Our study also showed that 65.2% (CI 0.58–0.71) of all milk vendors did not know what an antibiotic is, and 34.8% (CI 0.28–0.41) of those who said they did know could not give a clear definition of an antibiotic. A previous study on the prevailing practices in the use of antibiotics by dairy

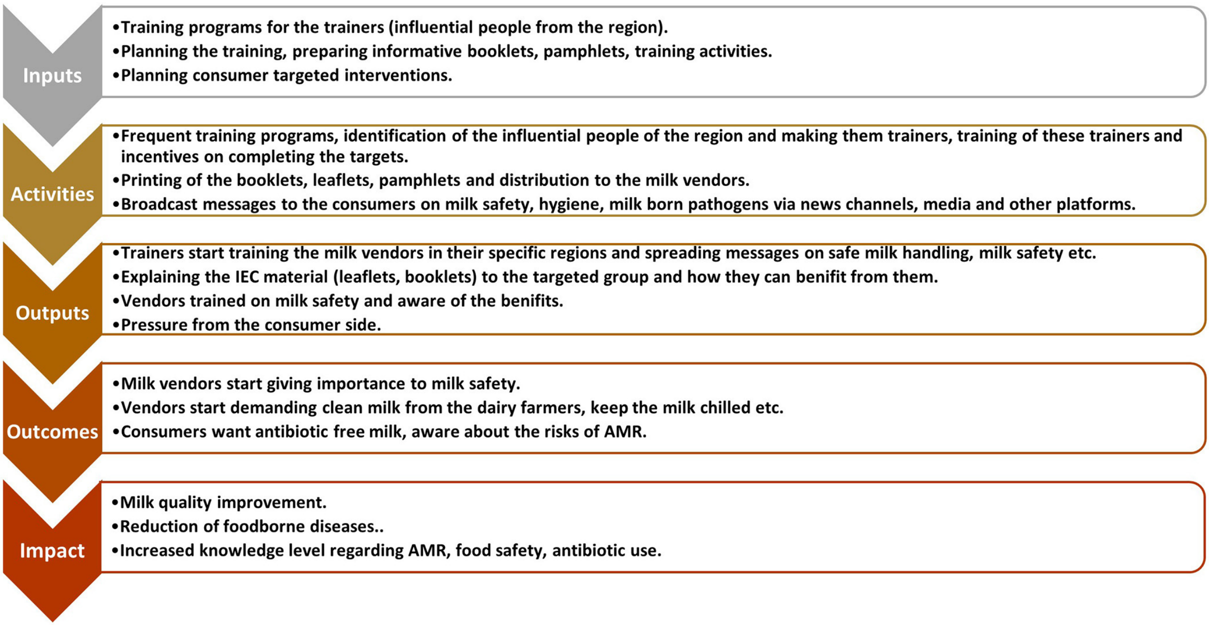

FIGURE 5
A hypothetical theory of change targeting different actors along the dairy value chain to improve milk safety.

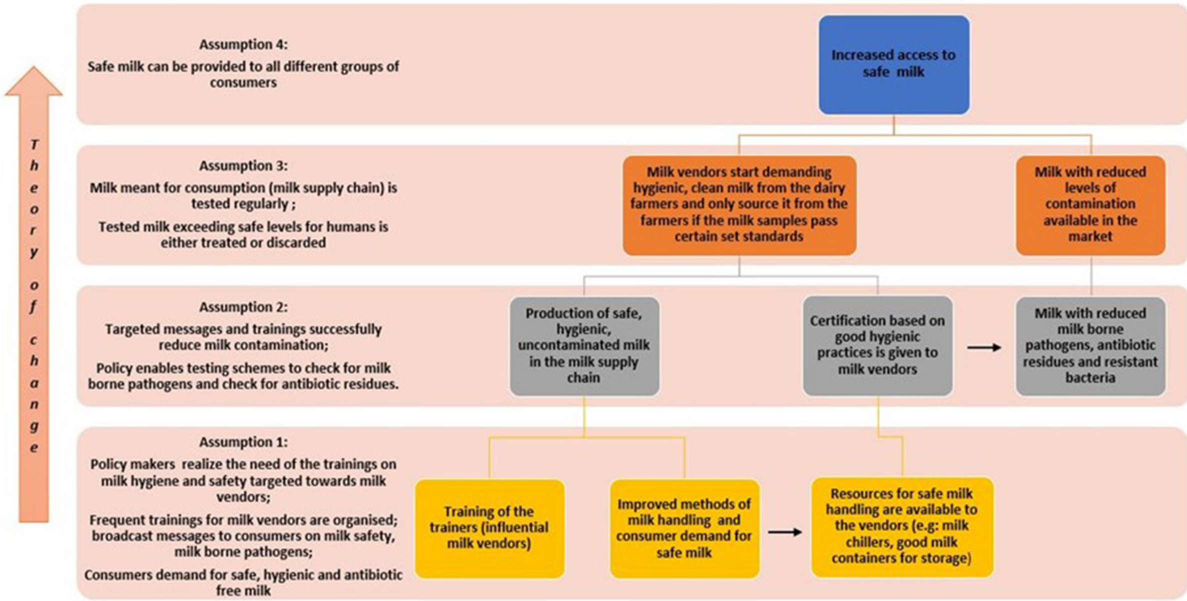

FIGURE 6
A theory of change for how milk vendor intervention can affect milk safety, including assumptions 1–4 listed on the left, actions, and outcomes flow from the bottom of the diagram upwards.

farmers in Haryana (Kumar and Gupta, 2018) revealed that the smallholder dairy farmers (7.14%) procured antibiotics from the milk vendors for the treatment of their animals. The fact that milk vendors have so limited awareness of antibiotics while yet being found to be giving antibiotics to dairy farmers raises serious concerns.

The level of formal training received by the milk vendors in our study was very low, only 6 vendors had ever received any training on milk hygiene or safety. The vendors attended training from either a private milk cooperative or by the local veterinary department. This poor level of training is not unique to our study cohort, rather it is very much reflected throughout other parts of India as highlighted by other KAP studies along the informal dairy value chain (Mutua et al., 2020; Sharma et al., 2020). We believed that increased levels of milk hygiene training could impact on food safety practices among vendors as seen in other studies where milk traders and dairy farmers training interventions proved to increase hygiene standards (Lindahl et al., 2018, 2019).

While training may be a beneficial strategy for addressing farmers' and vendors' lack of knowledge regarding food safety practices, antibiotic usage, and the possible harm to public health, it is just one part of a larger solution. Farmers need to be incentivized to produce safe milk, vendors need to demand certain standards when purchasing milk and follow certain hygienic standard, and the consumer needs to also demand safer milk and indeed be willing to pay for a safe product.

A Theory of Change (ToC) approach has helped bring about this shift in mentality among the stakeholders in the informal dairy value chain in other LMICs (Johnson et al., 2015). A ToC approach improves our understanding of how change occurs and outlines what needs to be done to achieve desired outcomes, it requires a number of activities by different entities, to address different objectives to be undertaken, rather than relying on a single intervention (Mutua et al., 2020). Figure 5 is an example of how a ToC approach could be taken, targeting different actors, including consumers, to mitigate milk-borne pathogens in these two states of India.

ToC suggests how and why a program works and is a critical reflection on program's strategy, context, and outcomes. It was developed because it is hard to evaluate complex social change programs. The ToC explains the assumptions behind the change process that are written down and tested (Lam et al., 2021). Figure 6 explains the assumptions for the suggested ToC above.

Limitations of the study

Many vendors failed to fully answer the questionnaire, making statistical analysis of many variables impossible. Some vendors refused to commit to specific responses; for example, when asked about the type of container used to store milk, interestingly, 26 vendors responded that they did not know what kind of container, open or closed, they used. It is unlikely that a vendor does not know if the container has a lid or not. Also, issues surrounding how the questionnaire was delivered come into play. Did some vendors feel obliged to give a "right answer?" This highlights the challenges involved when carrying out research among economically invested stakeholders who may fear financial repercussion if they do not give the expected answer, they feel the interviewer is looking for. Future studies should include other forms of research techniques beyond the use of a single questionnaire, such as the use of focus groups, to help ease out such complexities.

Conclusion

This study highlights the unsafe ways in which milk vendors handle milk, specifically raw milk. Increased bacterial load might result from the unhygienic ways the informal vendors handle the milk. Many milk samples that were tested for the presence of antibiotic-resistant bacteria were found positive, indicating transmission of AMR along the dairy values chain. There exists a vast knowledge gap when it comes to awareness related to milk hygiene and antibiotics or antimicrobial resistance. The knowledge and attitude of the surveyed vendors related to milk safety; antibiotics were found to be poor, and only a very few vendors had attended any training on hygiene and food safety. Caution must be taken not to demonize the informal nature of this value chain; while risk to human health exist, small-scale food production and processing is an essential pathway out of poverty, existing food safety regulation is often ineffective at the ground level. However, conditions on the ground must be taken into consideration when attempting to implement change. Most of the vendors in this study did not comply with the national milk safety regulation, yet they cannot be blamed if they are unaware of the existence of this legal framework. The policymakers must focus on how these informal vendors can be made aware on the basic safety measures to be followed while handling milk. Also, a monitoring and evaluation mechanism needs to be set up to investigate if the regulations are adhered to at the ground level. The ToC approach could be a useful way of bringing about a change in mindsets of farmers, vendors, and consumers, englobing multiple stakeholders along the value chain with the aim of bringing about sustainable, lasting improvements.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval for the study was granted by the Institutional Research Ethics Committee (IREC) of the International Livestock Research Institute (ILRI) on 21 September 2015 (No: ILRI-IREC 2015-12). The patients/participants provided

their written informed consent to participate in this study.

Author contributions

JL, BS, SB, NG, and RD: conceptualization, investigation, and resources. JL, BS, SB, and NG: methodology and validation. GS and EL: formal analysis and writing—original draft preparation. GS, JL, and EL: visualization. TD, BS, and JL: laboratory data interpretation. JL and ÅL: supervision. JL and RD: project administration. JL and DG: funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1058384/full#supplementary-material

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Food safety knowledge, needed and trusted information of pork consumers in different retail types in Northern Vietnam

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Introduction: Food safety is an important public health concern globally. Risk communication is one of crucial element to manage food safety. While current food safety studies have focused on contamination of hazards or risk factors, limited research exists on consumer concerns, knowledge and perception about the actual risk food poses to their health. This study aimed to assess and compare the knowledge and perceptions regarding food safety, and the information needed and trusted by pork consumers in Northern Vietnam.

Methods: A total of 225 consumers recruited from three different market types: modern urban, traditional urban and traditional rural, were interviewed using a questionnaire between November to December 2019.

Results: The majority of participants (81.8%) were female and consumers interviewed at modern urban retail were younger than those interviewed at traditional retail settings ($p < 0.01$). Sixty-five percent of participants across the three retail types agreed that microbes were the most common hazards which can make them sick, but the adverse health effect due to chemical hazards was ranked higher than that of biological hazards. Most participants often received food safety information that was about animal diseases (such as African swine fever most recently), chemical contamination and the unknown origin of food rather than about food poisoning and measures to prevent it. Food safety messages from television and professional experts were the most trusted sources and consumer preference was for information about the origin of food (traceability) and how to choose safe food. Participants were willing to receive food safety information daily to weekly. A lack of perception and awareness about animal welfare related to pig farming or slaughtering was reported by most respondents (84.3%).

Discussion: These findings provide insight on Vietnamese consumer knowledge gaps, information demand and communication channels for food safety, so that risk communicators and managers can implement better food safety awareness campaigns and communication to consumers.

KEYWORDS

risk communication, food safety, mass media, consumers, Vietnam

Introduction

Food safety is an important public health concern globally. The World Health Organization (WHO) estimated that over 600 million foodborne illness cases were recorded in 2010, leading to losses such as nearly 420.000 deaths and 33 million DALYs (Disability Adjusted Life Years). As foodborne illness is contributing to the global disease burden, particularly for young children and for people living in low-income subregions of the world (World Health Organization, 2015), action to improve food safety from farm to fork across all food commodities and all production systems is needed. A joint FAO/WHO Food Standards Program emphasized the importance of the risk analysis framework, in which risk communication is one of the crucial elements to manage food safety. Risk communication promotes the interactive exchange of information about risks among risk assessors, managers or policymakers, the media, interested groups and the community or consumers. Close interaction and timely communication about food safety risks help to improve consumer knowledge of food safety, the belief and trust in the safety of the food supply and the food management system and the quality of food for human consumption (Codex Alimentarius Commission, 2019). In addition, food safety communication campaigns (e.g., World food safety day) and messages (e.g., WHO 5 keys to Food Safety), plus regular training and certification help to increase the knowledge of food producers, and thus support actors to comply with suggested food safety practices (Cohen et al., 2001; Roberts, 2008; Kassa et al., 2010).

In Vietnam, food retail markets can be classified into traditional and modern retail channels. Traditional retail channels include traditional markets and wet markets, where many types of food are sold and at a permanent location in close proximity to residential areas (Nga, 2014). Modern retail markets can be divided into several types, including supermarkets, convenience stores and boutique food shops, which are equipped with better infrastructure to store and display food to sell and predominantly located in urban areas. The food retail landscape in Vietnam is largely dominated by traditional retail due to market accessibility, availability, convenience, package-size flexibility and competitive prices, especially for fresh produce and dried food (Lapar and Toan, 2010; USDA, 2020). In spite of such advantages, traditional markets and wet markets often lack mechanisms for food safety control, such as assessment of food quality and product traceability (Nga, 2015; Dang-Xuan et al., 2016). In contrast, modern retail channels with an extensive store network, committed to providing high-quality products with known origin source are gradually gaining the trust of customers, especially middle-income consumers in big cities (Unger, 2020).

Nowadays, consumer perception is greatly influenced by mass media (television, radio, newspapers, Facebook, Twitter). In Vietnam, pork constitutes nearly 70% of total meat consumption and this percentage has been rising steadily, linked to population growth, improved living standards and a shift in diet favoring animal-based proteins (Ruengjirachuporn, 2017; OECD, 2022). However, media reports about swine disease outbreaks have impacted pork consumption in Vietnam. In 2019, the African swine fever outbreak in Vietnam, which rapidly spread and devastated pig farms across almost all provinces of the country over 5 months (DAH, 2019), resulted in a dramatic decrease in pork consumption (USDA, 2019; Nguyen-Thi, 2021). This reduction was due in part to misinformation about the health risk posed to people such that people were scared of disease transmission from sick pigs to themselves, along with the doubling of the pork price due to the reduced supply of pigs (Chau, 2020; Nguyen-Thi, 2021). Further, information on health risks associated with meat of unknown origin, contaminated meat and poor-quality meat that appear frequently in the mass media, has raised consumer concern about the quality and safety of pork. Despite these adverse impacts, as yet risk communication on food safety issues has not been integrated into the risk-based food safety management system in Vietnam (The World Bank, 2016; Nguyen-Viet et al., 2017).

While food safety research has focused on potential risk factors, limited research exists on consumer concern and consumer knowledge and perception about the risk food poses to human health. Microbial pathogens are reported to be responsible for the great majority of foodborne diseases (Havelaar et al., 2015). This is in the line with recent findings from Ngo et al. (2021) for Northern Vietnam, in which a high *Salmonella* contamination in pork has been found across all retail types, included traditional retails, modern retails, canteen and street food services. However recent studies in Vietnam indicated that people are more concerned about chemical rather than microbial hazards (Nguyen-Viet et al., 2017; Ha et al., 2019). A food safety report from the General Statistics Office of Vietnam revealed that within 10 years from 2010 to 2020, the proportion of food poisoning cases (i.e., cases of illness due to eating contaminated food) found to involve chemical hazards was 4.2%, which was notably less than the 38.7% caused by biological hazards (38.7%) (GSO, 2020). This mismatch between Vietnamese consumer perception and actual causes of illness indicates that accurate information is not being conveyed effectively to the public. If not managed appropriately, even accurate information on food safety risks can result in changes to consumer behavior that have unintended adverse consequences for both human nutrition and producer livelihoods (Hoffmann et al., 2019). Therefore, this study aimed to assess and compare the knowledge and perceptions regarding pork safety and risk communication of consumers at urban and rural traditional retail outlets, and modern retail outlets in Northern Vietnam. A better understanding of consumers' knowledge and perception will inform the government, policymakers and food safety risk communicators about the information consumers want and the

avenues to convey it leading to more targeted and effective communication strategies.

Materials and methods

Ethical approval

The Institutional Review Board of Hanoi University of Public Health reviewed and approved the methods for this survey (Decision number: 110/2018/YTCC-HD3). Consumers over 18 years old were briefed on the purpose of the survey and asked to give written informed consent if they agreed to participate. Participants were also informed that their answers would remain anonymous, they could withdraw at any time, and that the information collected would be kept confidential. As an incentive, each participant was given the gift of a cloth apron (valued at $9USD) upon completing the questionnaire.

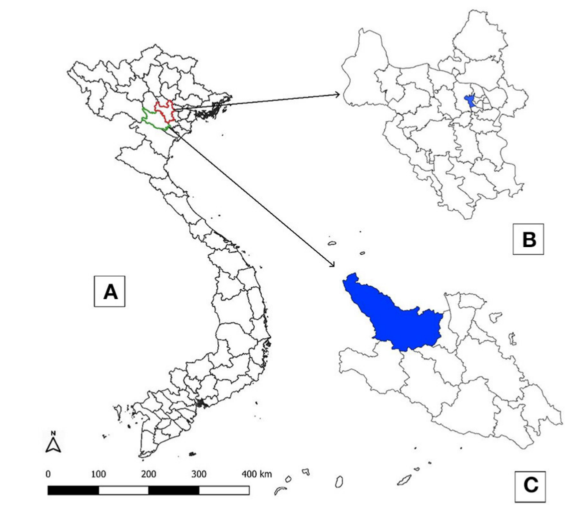

FIGURE 1
Map of study sites in Da Bac district, Hoa Binh province and Cau Giay district, Hanoi city, Vietnam. **(A)** Map of Vietnam with Hoa Binh province highlighted in green and Hanoi city highlighted in red. **(B)** Hanoi city with Cau Giay district highlighted in blue. **(C)** Hoa Binh province with Da Bac district highlighted in blue.

Study design and area

This cross-sectional study was conducted from November to December 2019 in Cau Giay district, Hanoi and Da Bac district, Hoa Binh province, which represented urban and rural areas of Northern Vietnam, respectively (Figure 1). Cau Giay district, one of the 12 urban districts among the 30 districts in Hanoi, is where over 293,000 people reside with a population density of 23,516 people/km^2, and an average annual income per person in 2019 of 102 million VND (approximate 4,430 $US) (Hanoi Portal, 2019). It was selected as it is a built-up central area in the capital city of Hanoi which whilst still largely dominated by traditional retail, has an established and increasing number of supermarkets and convenience stores (USDA, 2017, 2019). In contrast, Da Bac district, one of 10 districts in Hoa Binh province, is a rural district where over 55,000 people reside with the population density of 80 people/km^2 and an average annual income per person in 2019 of 59.58 million VND (approximate 2,503 $US). Traditional markets are the only retail option in Da Bac district (Hoa Binh statistic office, 2019).

Sample size and target groups

The sample size for this survey was calculated for a two-independent proportions comparison using a 95% confidence level and 80% power with an assumed difference of 25% in consumer food safety knowledge among the three retail types (rural and urban traditional markets and urban modern markets). With the inclusion of a target group of consumers per market (cluster), the sample size calculation was adjusted using the intra-cluster coefficient of 0.1 and equal proportions among the three retail types. Thus, the minimum number of consumers required for interviews in each group was 72. Ten to fifteen markets each for urban traditional and for urban modern in Cau Giay district and five markets for rural traditional in Da Bac district were convenience sampled from the respective sampling frame that listed all markets by retail type in each study area. On average, five consumers per urban market and fifteen consumers per rural market were interviewed in the study. The actual number of consumers interviewed from rural traditional, urban traditional and urban modern retails was 76, 76, and 73, respectively.

Questionnaire and data collection

A structured questionnaire was first developed in English and then translated into Vietnamese by experienced, bilingual research team members. The questionnaire was pretested with five consumers in Gia Lam district, Hanoi and subsequently the wording of questions was refined to clarify meaning. The questionnaire consisted of 25 questions across four sections: (i) general demographic information, (ii) food and pork safety knowledge and perception (iii) information that the consumer wants to know about food and pork safety, and (iv) concern about animal and pig welfare. It was administered during a face-to-face interview that took approximately 20 min to complete. The interviews were conducted in Vietnamese. All interviewers attended a training session prior to administration of the questionnaire in the field.

Participants were selected through convenience sampling by directly approaching potential consumers who were observed purchasing pork. These consumers were politely approached by a research team member, given a brief explanation of the research and invited to participate in an interview. For consumers that were eligible and willing to participate in the survey, the interview was then conducted on the spot either within the market or outside the market entrance.

Data management and analysis

Data was collected using Kobotoolbox (version 1.27.3-3, www.koboToolbox.org), then extracted into spread sheets (Microsoft Corporation, 2018) and cleaned before analysis. Data analysis was performed using R3.4.4 (R Core Team, 2018). Descriptive statistics were used such as percentages for categorical data and means, standard deviations and ranges for quantitative data. Chi-square or Fisher exact tests (where appropriate) were used to compare frequencies between groups. Multiple groups comparisons were done for ranking questions by using Kruskal-Wallis test. For significant results from Kruskal-Wallis test, *post*-hoc analysis (kruskalmc function) was conducted to identify significant difference between tested groups using "pgirmess" package in R. Statistically significance was set at $p \leq 0.05$.

Results

Demographic information of respondents

There were 225 participants in this survey, including 76 from rural traditional markets, 76 from urban traditional markets, and 73 from urban modern markets (Table 1). Participants were evenly distributed across five age categories except for consumers from modern urban markets that were younger than consumers from other groups ($p < 0.01$). In all market types, female participants made up the majority. A larger percentage of male participants were interviewed in the modern urban markets compared to traditional urban markets ($p < 0.01$). Most participants had at least a high school level of education, with a higher percentage of participants with college/university education in the modern urban group than the others ($p < 0.01$). The mean distance of the markets from the participant's residence was 2.9 km. Consumers from rural markets often travel further than consumers in urban areas (ANOVA, $p < 0.01$).

Consumer's knowledge of pork safety hazards and constraints

Microbes were identified as the most common hazard that can make consumers sick related to eating pork or pork products, followed by chemical hazards and physical hazards

TABLE 1 General information on 225 pork consumers interviewed at three retail types in Northern Vietnam in 2019.

Information	Modern urban		Traditional urban		Traditional rural		Total	
	n	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Age group ($n = 223$)								
18–25	21	29.6^{a}	3	3.9^{b}	0	0.0^{b}	24	10.8
26–35	30	42.3^{a}	12	15.8^{b}	14	18.4^{b}	56	25.1
36–45	11	15.5^{a}	11	14.5^{a}	19	25.0^{a}	41	18.4
46–55	3	4.2^{a}	20	26.3^{b}	19	25.0^{b}	42	18.8
≥56	6	8.5^{a}	30	39.5^{b}	24	31.6^{b}	60	26.9
Gender ($n = 225$)								
Male	23	31.5^{a}	5	6.6^{b}	13	$17.1^{a,b}$	41	18.2
Female	50	68.5^{a}	71	93.4^{b}	63	$82.9^{a,b}$	184	81.8
Education ($n = 225$)								
Primary school or less	0	0.0^{a}	6	7.9^{b}	8	10.5^{b}	14	6.2
Secondary school	3	4.1^{a}	23	30.3^{b}	30	39.5^{b}	56	24.9
High school	26	35.6^{a}	35	46.1^{a}	33	43.4^{a}	94	41.8
Colleges/university or higher	44	60.3^{a}	12	15.8^{b}	5	6.6^{b}	61	27.1
Distance to market ($n = 201$)*	***Mean ± SD***							
	2.0^{b}	3.3	1.7^{b}	4.6	5.1^{a}	3.3	2.9	6.1

[a,b]Difference superscripts indicate statistically significance at $p < 0.01$, * using t-test. SD, standard deviation.

TABLE 2 Perception of hazards associated with pork, main problems with access to buying pork and its reasons reported by pork consumers at three retail types in Northern Vietnam in 2019.

	Modern urban		Traditional urban		Traditional rural		Overall	
	n	%	*n*	%	*n*	%	*n*	%
Hazards associated with pork ($n = 225$)								
Microbes	49	67.1	48	63.2	49	64.5	146	52.2
Chemical	36	49.3	31	40.8	28	36.8	95	33.8
Physical	4	5.5	2	2.6	3	3.9	9	3.2
Don't know	8	$11.0^{a,b}$	16	21.1^{a}	5	6.6^{b}	29	10.4
Difficulty accessing pork to buy during last month ($n = 224$)								
Yes	45	61.6^{a}	62	81.6^{b}	59	$77.6^{a,b}$	166	73.8
No	27	37.0	14	18.4	17	22.4	58	25.8
Missing	1	1.4	0	0	0	0	1	0.4
Main problem with access to buying pork ($n = 166$)								
High price	42	93.3	59	95.2	49	83.1	150	90.4
Concern about disease in pigs	3	6.7	2	3.2	9	15.3	14	8.4
No pork in market	0	0.0	1	1.6	0	0.0	1	0.6
Market where pork is sold is too far from home	0	0.0	0	0.0	1	1.7	1	0.6
Reasons for the main problem ($n = 166$)								
African Swine Fever (ASF)/Pig diseases	32	71.1	48	77.4	37	62.7	117	70.5
Low income	7	15.6	14	22.6	15	25.4	36	21.7
Increase in export to China	10	22.2^{a}	9	$14.5^{a,b}$	3	5.1^{b}	22	13.3
Lack of pork retailers	6	13.3^{a}	2	$3.2^{a,b}$	1	1.7^{b}	9	5.4
Insufficient pigs/pork supply to meet demand	3	6.7	2	3.2	4	6.8	9	5.4
Other (specify)	0	0.0	2	3.2	1	1.7	3	1.8
Don't know	4	8.9	9	14.5	2	3.4	15	9.0

[a,b]Difference in proportion between groups statistically significant at $p < 0.05$ when superscripts differ.

(Table 2). There was no significant difference of these hazards among the 3 retail types. However, more of the participants from the traditional urban markets did not know what type of hazard could cause illness related to eating pork than at the traditional rural markets with 21.1 and 6.6%, respectively ($p < 0.01$).

Regarding the question asking respondents whether they had any difficulty accessing pork to buy during the last month, 73.8% (166/225) of them reported that they had, with a higher proportion reporting difficulty from the traditional urban market (81.6%, 62/76) than traditional rural (77.6%, 59/76) and modern urban markets (61.6%, 45/73) ($p < 0.01$). Among the listed difficult problems with accessing pork, high price was the most reported one (150/166, 90.4%), and this percentage was similar among all market types. Another reported problem was the concern of consumers about disease in the pigs (8.4%, 14/166, Table 2). Respondents then were asked for the most common reason leading to the high price problem. It is revealed that the African swine fever (ASF) outbreak was the most frequently reported reason (117/166, 70.5%), followed by reduced/low income (21.7%), and an increase in pig export to China (13.3%). Lack of pork retailers and increase in pig export to China were mentioned more by consumers from modern markets than traditional rural ones ($p < 0.05$, Table 2).

In addition to inquiring if pork had been difficult to access, consumers were asked if they were worried about the safety of eating the pork that they purchased in the last month. To which, 71.1% (160/225) of consumers answered yes. Consumers in traditional rural markets (82.9%, 63/76) were more likely to worry about the safety of purchased pork than those in modern urban markets (58.9%, 43/73) ($p < 0.01$).

Consumer's knowledge and attitude on food safety risk and trust of different retail types

Consumers were asked to rank different risks, included smoking cigarette, riding motorbike without helmet, chemicals in food, microbial contamination in food, alcohol abuse and air pollution, which are common factors in the Vietnamese context to compare their perception about the risks to their health (on a scale from 1-being less, to 10-being the most harmful to their health). Overall, microbial contamination of food and alcohol abuse were of less concern compared to chemicals in foods and smoking cigarettes by all groups ($p < 0.05$), with no significant

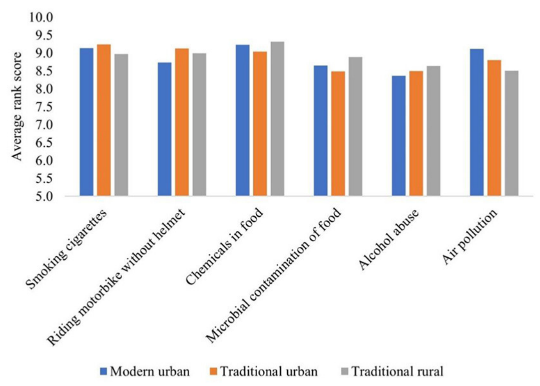

FIGURE 2
Ranking of different risks to their health on a scale from 1 to 10 (10 being the most harmful to their health) by pork consumers at three retail types in Northern Vietnam in 2019 ($n = 225$).

difference between retail types (Figure 2). Consumers were also asked to rank issues that worry them about eating pork or pork products from the most to the least source of worry. Pig diseases/ASF was in first rank, followed by reports of people getting sick from eating pork, media reports about unsafe pork product processing, and media footage of the killing of pigs to control disease, respectively. This rank order was the same across the 3 retail types, though notably consumers at traditional rural markets were more likely to worry about reports of people getting sick from eating pork than those at traditional urban markets ($p < 0.05$, data not shown).

More than 80% of respondents indicated that they consider whether food is safe or not when deciding where to buy pork. Consumers had more trust in the safety of pork sourced from their own pigs, from pigs raised by neighbor/known people and from modern retail outlets such as supermarkets, boutique shops and convenience stores. Mobile vendor and street/wet markets were the least trusted, being significantly lower than all other sources ($p < 0.01$) (Figure 3). There was no difference in trust levels of consumers at the 3 retail types for pork sourced from their own pigs and from pigs raised by neighbor/known people. Traditional rural consumers trusted less in the safety of pork in convenience stores and more in supermarkets compared with other groups ($p < 0.01$, chi square). Modern urban consumers trusted less in traditional markets and street/wet markets, and more in boutique shops compared with other groups ($p < 0.01$). Traditional urban consumers trusted more in street/wet markets ($p < 0.05$).

Content and consumer demand of food safety information

The food safety information that most participants reported usually hearing about was animal diseases (such as ASF most recently), and this was reported by a significantly higher proportion of consumers at the traditional rural markets ($p < 0.01$). Chemical contamination and unknown origin of food were the second and third most common types of information received by participants (Figure 4). A significantly lower percentage of consumers in traditional rural markets reported hearing about unknown origin of food and food poisoning compared to urban consumers ($p < 0.01$). The survey found that when going to buy pork, consumers were interested to have information provided about the specific farm of origin, production/expired dates and the region that pig came from. The other information such as cooperative of pig producers, pig raised with/without antibiotic, breed and age of pig, and slaughterhouse where the pig was slaughtered were of less interest. Traditional rural consumers were more interested in information about the region that pig came from, type of feed fed to pig, age of pig and slaughterhouse where the pig was slaughtered. Modern urban consumers were more interested in production/expired date information.

When consumers were asked what information they would like to know about food safety in general, the two most common responses were the origin of food (food traceability information) and how to choose safe food, being reported respectively by 61.6 and 45.2% of modern urban consumers, 44.7 and 32.9% by traditional urban consumers, and 39.5 and 44.7% by traditional rural consumers.

Trust of source on food safety information by consumers

In general, professionals (medical staff, veterinarian, food safety staff) and television were the most trusted sources for information on food safety while social media and famous person were the least reliable (Figure 5), being similar among consumers across the 3 retail types. From a list of channel options by which to receive food safety information, the two most common options chosen by consumers from all three groups were television and mobile phone, with 75 and 31.1% respectively. The third most common option chosen by urban consumers (both modern and traditional ones) was online newspaper (41.1 and 21.1%), while consumers from traditional rural markets prefer community meetings for the distribution of information (19.7%). The option of community meetings was less mentioned by modern urban consumers than other groups (chi square, $p < 0.01$). Compared to the other two groups, modern urban consumers mentioned more about the options of receiving food safety information through phone and news sites, and much less about the option of community meetings (chi square, $p < 0.01$). On the other hand, traditional urban consumers reported a higher preference to receive information via radio (22.4%)

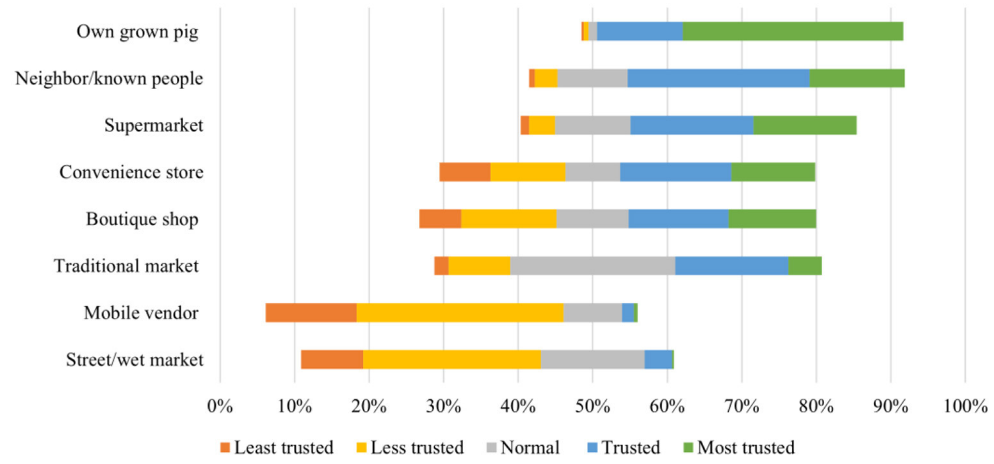

FIGURE 3
Trust of pork sources in relation to pork safety by pork consumers from traditional rural, traditional urban and modern urban markets in Northern Vietnam in 2019 ($n = 220$).

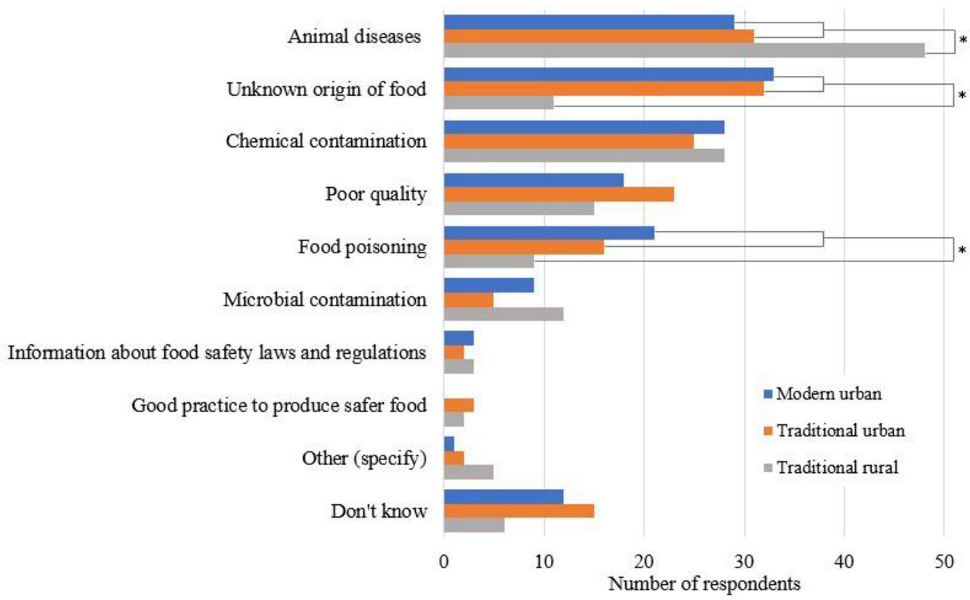

FIGURE 4
Information about food safety usually heard by pork consumers from traditional rural, traditional urban and modern urban markets in Northern Vietnam in 2019 ($n = 225$).

than the modern urban and traditional rural groups (1.4 and 3.9%, respectively) (chi square, $p < 0.01$). Consumers were then asked how often they would like to receive food safety information and across both the urban and rural markets, consumers said that they would like to receive food safety information daily or weekly, with 67.1 and 23.1% respectively.

Perception of consumer about animal welfare

The survey found that 84.3% (188/223) of participants had not heard of the term "animal welfare." Of the 35 participants that had heard the term, more than half (51.4%) were modern urban consumers, 25.7% were traditional urban and 22.9% were

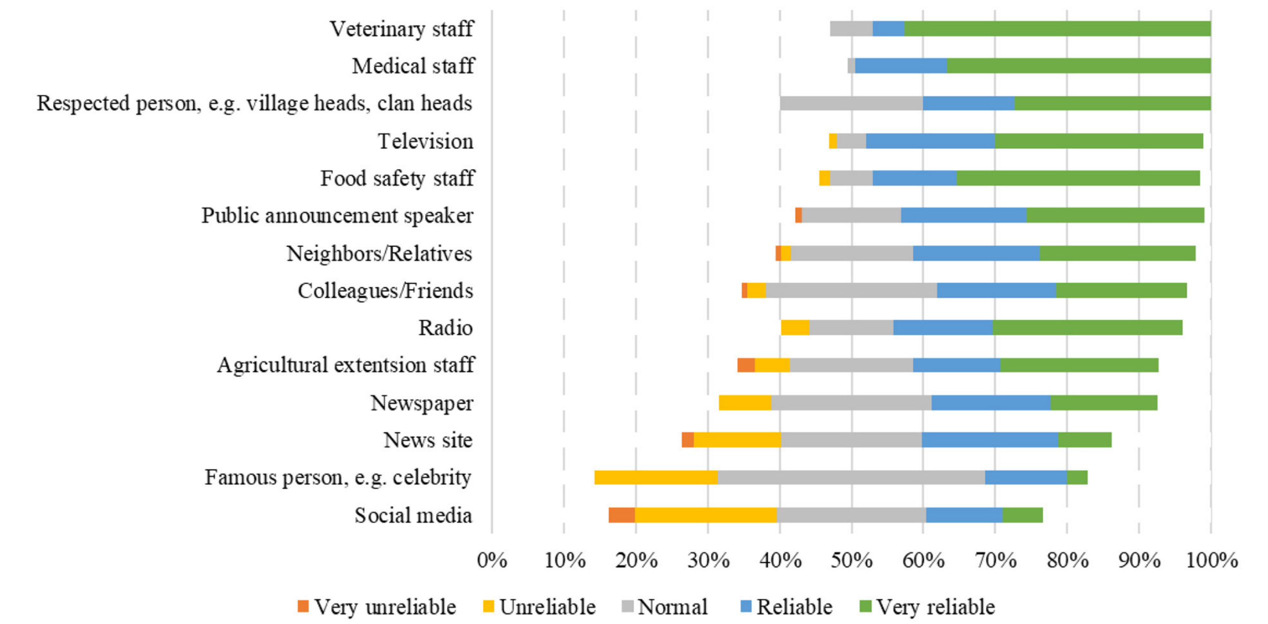

FIGURE 5
Trust among pork consumers of different information sources in relation to food safety information in Northern Vietnam in 2019 (*n* = 222).

traditional rural (statistically different between modern urban and traditional rural consumers, $p<0.05$). When participants were asked how pigs should be kept on the farm, the majority agreed that pigs should be kept in good hygienic conditions (175/218, 72.5%). In addition, 27.6% (62/218) thought the pigs should have enough space to move around and exhibit natural behavior such as nesting behavior for pregnant sows before giving birth (43/218, 19.1%). Less people were concerned that pigs be able to run around (17/218, 7.8%), of which the number was significantly higher among consumers from traditional rural markets ($p<0.01$). A small portion of consumers reported that pigs only need space to lay down (12/218, 5.5%).

In relation to slaughter, 43.7% (97/222) of participants said that they did not know how pigs should be slaughtered in terms of animal welfare. However, 34.2% of respondents said that pigs should be slaughtered with the least amount of suffering possible and for 40.2% (89/221) less suffering during slaughter was thought to improve the quality of pork, with this stated by a significantly higher proportion of consumers from traditional rural markets ($p=0.01$, data not shown).

Discussion

In general, consumers were concerned about the quality and safety of food, especially pork in this study. Similarly, a survey of food shoppers in rural and urban areas of Hanoi found that 95% of respondents expressed concern about the safety of food (Ha et al., 2021). These findings are further evidence of the growing public concern about food safety in Vietnam, indicated in previous studies (Nguyen-Viet et al., 2017; Unger, 2020).

However, there were gaps in knowledge and perception of consumers regarding pork safety and these varied between retail types. Microbial contamination was identified as the most common hazard that can make consumers sick, but participants were less concerned about the health impact to consumers of microbial hazards compared to chemicals in food and/or smoking cigarettes. This finding aligns with previous literature in both developed countries and Vietnam (Kher, 2013; Ha et al., 2019). It can be explained at least in part by the fact that gastrointestinal illness is common, everyone experiences it at some time, and most episodes resolve quickly without hospitalization, thus it is highly under reported (American Society for Microbiology, 2002). On the other hand, chemical hazards are less common but attract more media attention due to more serious health consequences (such as cancer) (Kher, 2013; Nguyen-Viet et al., 2017). In contrast to public perception, a recent study that assessed chemicals in 190 fresh pork samples (pooled in 18 samples) in two provinces of Vietnam found that arsenic, lead and cadmium were lower than the allowable level (Tuyet-Hanh et al., 2017), while another study collected 671 pork samples from different retail channels in Northern Vietnam found 58.1% of pork samples was contaminated with *Salmonella* and only 6.2% pork samples fulfilled the Vietnamese standard requirement for total bacteria count (Ngo et al., 2021). In addition, according to the food safety report from

General Statistics Office of Vietnam, from 2010 to 2020, the cause of foodborne outbreaks related to microbial and chemical hazards recorded were 38.7 and 4.2%, respectively (GSO, 2020). Therefore, more concrete evidence of chemical contamination and its health effect would be helpful to confirm the safety of pork in Vietnam. Such evidence could be used to shift consumers' focus to microbial contamination.

Additionally, significantly more of the participants from the traditional urban markets were unsure of what might make them sick in regard to eating pork than those from the traditional rural markets. A recent study found that urban consumers tend to have greater risk perceptions compared to consumers from rural areas because urban consumers were more aware of food safety concerns (Ha et al., 2021). As knowledge of potential food hazards are generally lower in areas where levels of educational attainment are low and we would generally expect that due to the rural-urban gap, rural populations would have lower levels of education (Hoffmann et al., 2019). The levels of educational attainment among participants were fairly equal between traditional urban and traditional rural markets. In this case, the higher perception among rural consumers compared to urban consumers could depend on more frequent dissemination of pork safety information at traditional rural markets. As a result, consumers in traditional rural markets were more worried about the safety of purchased pork and reports of people getting sick from eating pork.

The survey found that almost all participants had found it difficult to access pork in the last month (November-December 2019). The problem of accessing pork to buy was reported more by consumers at the traditional urban markets, which are supplied by smallholder farms, the sector in which most ASF outbreaks occurred due to a lack of capacity and biosecurity resources to prevent the disease (USDA, 2019). Government imposed restriction on the movement of live pigs and pig carcasses between provinces limited the source of pork for consumers in urban areas (MARD, 2018). These results were expected as following the 2019 ASF outbreak in Vietnam more than 5.9 million pigs were either culled or died from the disease despite significant government efforts to control the disease (Ngoc Que, 2020). Furthermore, during this time, the demand for live pig export from Vietnam to China increased (Bui and Gilleski, 2020). The higher demand and lack of pigs in the supply chain led to a substantial price increase for pork, and this is reflected by 90.4% of consumers in this survey reporting high prices as the main reason for difficulty with the purchase of pork.

Our findings indicated that pig diseases/ASF was a top concern of consumers when eating pork or pork products, concern likely influenced by mass media reporting about ASF which included in some instances disgusting images of culled pigs (no inspection, no control of carcass/infected pigs). From early in the ASF outbreak, many consumers changed their consumption behaviors and diets to select chicken, beef or fish instead of pork, although no evidence of human health risk from ASF was reported (USDA, 2019). In the present study, information about getting sick from eating pork was also ranked as a second concern by respondents. The vast majority of consumers also stated that they were worried about the safety of the pork that they were able to purchase in the last month. Once fears settled and risk in relation to ASF was clearly clarified, the demand for pork rose again, however many consumers still remain hesitant to re-introduce pork to their diets (USDA, 2019; Nguyen-Thi, 2021).

Risk communication allows authorities and experts to listen to and address people's concerns and needs so that the advice they provide is relevant, trusted and acceptable. Microbial contamination can be prevented and addressed by the application of sanitary measures throughout the production chain. This study suggested a need for proper and timely knowledge translation from experts to consumers with strong support from the authorities, in order to avoid misdirection of perceptions about risks that various hazards in food pose to consumers' health. This survey revealed consumer demand for information on food safety and pork safety specifically, and the most suitable communication channels and time interval by which to provide this. Food safety information points demanded by all groups included the origin of food (food traceability information) and how to choose safe food. Knowing consumer preference for pork labeling and traceability information gives insight into what value consumers place on certain areas of food safety and may aid in the formation of better risk communication strategies. Across both consumer groups, the majority rated pigs they raised themselves as their most trusted source of pork, followed by neighbor/known person producing pig or pork product. These results agree with previous literature which found that consumers felt more assured of the safety of pork that they themselves had produced or pork that had been produced by someone they personally knew and trusted (Ha et al., 2019). It was also consistent with their demands on pork safety information about the specific farm of origin, region that pig came from, and type of feeds given to pigs.

It is confirmed that the traditional media avenues of television and professionals working for the government are more trusted than social media. Rural consumers indicated that talking in the community/discussion with groups was a preferred way to receive information, a preference that likely reflects in part the differences in social constructs between urban and rural communities. In addition, although internet tools (e.g., social media, online newspaper) were quite popular, serious consideration is needed before application due to differences in their use by age group and geographic location. Although the way consumers would like to receive information varies greatly between the market types, the preference for frequency of information dissemination was consistent. Most consumers would like to receive information about food safety every day. This high demand emphasizes the strength of consumer concern about food safety, which may in part arise from

the substantial negative information about hazards related to food in the media which makes consumers perceive food-related risks to be more severe than other health-related issues (Nguyen-Viet et al., 2017).

Animal welfare which ensures animals' quality of life (both physical and psychological state) is an increasing concern worldwide [National Health and Medical Research Council, 2013]. Among consumers in developed countries, there is a high demand for animal products with declaration of animal welfare status (European Commission, 2005; Bozzo, 2019) but consumers from developing countries are as yet less engaged. It is important to gauge what the consumer understanding of animal welfare is and whether or not they care about it as consumer demand for better animal welfare could cause changes to the ways in which animals are managed in Asia, as it has in Europe for example (Sinclair et al., 2019). Pig management and slaughter are important not only from an animal welfare perspective, but also from a food safety perspective. In the present study, while few participants had heard of animal welfare, 72.5% of interview consumers stated that the pigs should be kept in good hygienic conditions, and 27.6% thought that pigs should have enough space to move around and lie down in their pens, and that pigs should be slaughtered with the least possible suffering. This perspective provides evidence of concern about pig welfare among consumers although they lack knowledge about the concept of animal welfare, and formalization of these concerns could be used to help demand changes in pig management practices along the pork value chain. A greater concern for pig welfare among rural consumers than urban consumers may be due to a more experience with the management and slaughter of livestock among rural consumers than urban consumers. Recent animal husbandry and veterinary laws of Vietnam have included consideration of animal welfare with the aim moving forward to align the development of the livestock sector and consumer's demand (National Assembly, 2015; National Assembly., 2018). Therefore, stronger future consumer demand in relation to animal welfare may lead to changes in pig management and slaughter practices in Vietnam.

This study had some limitations. First, the questionnaire was kept as short and concise as possible to maximize questionnaire completion. This meant other topics related to food safety, trusted information sources and animal welfare were not able to be covered, and as such further studies are needed. Second, it was obvious that some consumers, particularly consumers at the urban markets, were in a hurry and wanted to rush through the survey toward the end. This could have reduced the accuracy of the results and may partly explain the higher proportion of "don't know" responses from urban consumers compared to rural consumers. Lastly, there was fewer male participants as females are mainly responsible for purchasing food from markets. Therefore, the survey results may not reflect food safety knowledge and perceptions among Vietnamese men who eat pork.

Conclusions

This study assessed the food safety knowledge, needed and trusted information regarding pork and risk communication of consumers in Northern Vietnam. It highlighted some food safety knowledge gaps between current research findings and consumer knowledge. Our study has also identified differences among traditional and modern markets in regard to the information consumers would like to know about their pork and how to best get that information to them. But more importantly, this study showed that all consumers had strong concerns about the safety of the pork they eat. Promoting risk communication helps to develop law enforcement and disseminate scientific information to the community, build trust and let people have enough accurate information to choose, use and control food safely and profitably. This study informs researchers and policymakers about channels by which to reach target consumers, so that risk communicators and managers can implement better food safety awareness campaigns and communicate more effectively to consumers. Suggested educational materials to address gaps for consumers could be flyers, brochures, and posters provided by professionals working for the government in community meetings; radio broadcasts; or food safety channels in TV shows, depending on consumers' characteristics either modern or traditional, urban or rural settings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Hanoi University of Public Health Decision number: 110/2018/YTCC-HD3. The patients/participants provided their written informed consent to participate in this study.

Author contributions

J-AT, FU, SD-X, and HN-V conceptualized and designed the study. TT-H, SL, JD, LN-T, and HN conducted the field work, translated the raw data, performed the data processing, and validation. TT-H, SD-X, and J-AT analyzed the data and prepared the draft manuscript. J-AT, HN-V, FU, SL, JD, LN-T, and HN reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A review of the roles of men, women, and youth in ensuring food safety in the smallholder poultry value chain in Kenya

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Smallholder poultry production is a significant source of food and income for many rural households in Kenya, but poultry products can be contaminated with *Salmonella* and *Campylobacter* spp. Household members have different roles in poultry production, with women and youth more actively involved in the earlier steps of the value chain, such as poultry production and processing, particularly at the farm level. This literature review summarizes current knowledge and practices on the roles of women and youth in food safety in the smallholder poultry value chain in Kenya. Of the 19 articles identified, a majority referenced gender roles in the poultry value chain but few referenced youth or the roles of women and youth in ensuring food safety. Women and youth were found to be the primary smallholder poultry producers on-farm. Due to their direct involvement in poultry handling and production, women and youth may have higher potential risk of exposure to foodborne pathogens. Men, women, and youth were all found to participate in slaughtering and transportation of poultry products. It was also found that, although women may be the owners and caretakers of chickens, they may not have decision-making power on the use of income from the poultry, and poultry product sales. Therefore, women and youth may have limited decision-making power or access to resources, such as training, to increase food safety. Further research is needed to address the factors important to women and youth empowerment to ensure food safety in the smallholder poultry value chain and reduce the risks of foodborne disease (FBD) in Kenya.

KEYWORDS

youth, poultry, Kenya, food safety, value chains, gender

Introduction

In Kenya, poultry farming contributes to income, food security, and nutrition of many households, particularly in rural and peri-urban areas, and accounts for about 30% of the national agricultural GDP and about 7.8% of the total GDP (Kenya National Bureau of Statistics, 2014; Macharia et al., 2020). While important for livelihoods,

poultry products may be contaminated with foodborne pathogens and, as such, the handling and consumption of poultry can lead to foodborne disease (FBD). A systematic literature review of contamination of poultry with *Salmonella* and *Campylobacter* spp. in 27 African countries, including Kenya, estimated the prevalence to be 24.6% (95% CI: 18.0–32.7) and 13.1% (95% CI: 9.3–18.3), respectively (Thomas et al., 2020). The incidence of FBD due to poultry in the East African region has been estimated to be 96 disability adjusted life years (DALYs or the amount of years of health lost) per 100,000 people, which is at least double the burden in all non-African regions of the world (Li et al., 2019).

Gender roles have been associated with health risks in poultry production in informal markets in Africa. In livestock value chains such as poultry, men have been found to have greater injuries as a result of their occupational activities while women have greater exposure to foodborne pathogens (Grace et al., 2015). Further, men and women have different levels of decision-making ability and access to resources to ensure food safety. Women head more than 30% of households in Kenya, but frequently receive less income than males because of gender stereotypes in livestock value chains, and having multiple other responsibilities that minimize their ability to raise larger numbers of poultry (Winrock International, 2011). This statement was reinforced by a recent study in Burkina Faso with gender and poultry (Leight et al., 2021). In addition to gender, age also influences the roles and responsibilities of poultry production (Kryger et al., 2010). Poultry production offers significant economic opportunities for youth, which the United Nations defines as individuals between 15 and 24 years of age (United Nations, 1981), but they are often overlooked. Youth are both producers and consumers of poultry as well as a growing part of the population. For example, about 80% of Kenya's population is under 35 years of age with the median age in Kenya being 19 years old (Nkomo and Mwaura, 2018). To ensure food security (i.e., access to sufficient, safe, and nutritious food) for the growing population, young men and women need to be trained in safe poultry production practices in order to produce food that is safe to consume.

Smallholder poultry production is an important source of both protein and income for rural women in the world, particularly African countries. Previous literature on food safety interventions in the poultry value chain in Kenya focus on important challenges such as vaccination. Unfortunately, the majority of these articles do not take into consideration who is doing the particular task being studied (Ochieng, 2012). Training women on managing and marketing chickens can assist with reducing the risk of contamination of poultry products. Gendered data collection on who is conducting which activity in the value chain can aid in determining who to target interventions to so that resources can be used effectively and foodborne illness can be reduced (Grace et al., 2015). This review of literature emphasizes the connections between poultry production, gendered responsibilities, and the need to recognize who is most at risk for exposure to foodborne pathogens in the poultry value chain. The purpose of this literature review was to summarize current knowledge and identify future research priorities on how age and gender relate to ensuring food safety in smallholder poultry value chains in Kenya. Information from this literature review can be used to identify appropriate interventions for reducing FBD resulting from smallholder poultry value chains in these regions.

Methods

A literature review was conducted between April and August 2021. A total of 14 electronic data bases were searched for key literature in the fields of food safety, gender, and youth, poultry production, and smallholder poultry producers in Kenya (Table 1).

Titles and abstracts were reviewed by one of the authors to determine the relevance. Articles were included if they focused on either gender or youth, food safety interventions in the poultry value chain in Kenya, or a combination of those topics. The literature was synthesized, and results were categorized according to distinct steps in the poultry value chain: production, processing, transportation, retail and sales, and consumption. Interventions were categorized as farm management practices, government, and behavioral interventions (Figure 1, Table 2).

Results

Of the 19 articles identified in this review, 13 included the production of poultry, two included the processing of poultry, three included the transportation of poultry, five included the retail and sales of poultry, and four included the consumption of poultry (Figure 1, Table 2). The studies were conducted in Kenya (14), Ethiopia (4), and Tanzania (1) (Table 2). The results are presented by steps in the poultry value chain including production, processing, transportation, retail and sales, and consumption.

Production

Poultry production practices vary by region which can lead to differences in risks for exposure to pathogens. Women are primarily responsible for poultry rearing on farm. Of the women involved in poultry production in Kenya, the most common production practice was free-range poultry (35% women) followed by chicken kept in fences (34%) and then commercial poultry (30%) (Waithanji et al., 2020). Most traditional poultry farmers in rural areas use free-range production systems with <30 adult birds (Magothe et al., 2012; Odhiambo, 2020). During

TABLE 1 Databases and key words used for literature search.

Search category	Keywords	Databases searched
Roles and responsibilities of gender and youth in the poultry value chain in Kenya and East Africa	"gender AND poultry" "Kenya AND poultry" OR "gender AND youth AND East Africa" OR "gender AND food safety AND East Africa"	EBSCO host Gender Studies Database EBSCO host Women's Studies International ProQuest GenderWatch Web of Science CAB Direct International Livestock Research Institute (ILRI) International Food Policy Research Institute (IFPRI) World Bank Food and Agriculture Organization of the United Nations (FAO) United States Agency for International Development (USAID)
Food safety interventions used in the poultry value chain in Kenya and East Africa	"Kenya AND poultry" OR "food safety AND poultry" OR "food safety intervention" OR "education AND poultry".	PubMed Google Scholar Center for Agriculture and Bioscience International (CABI) WebMD

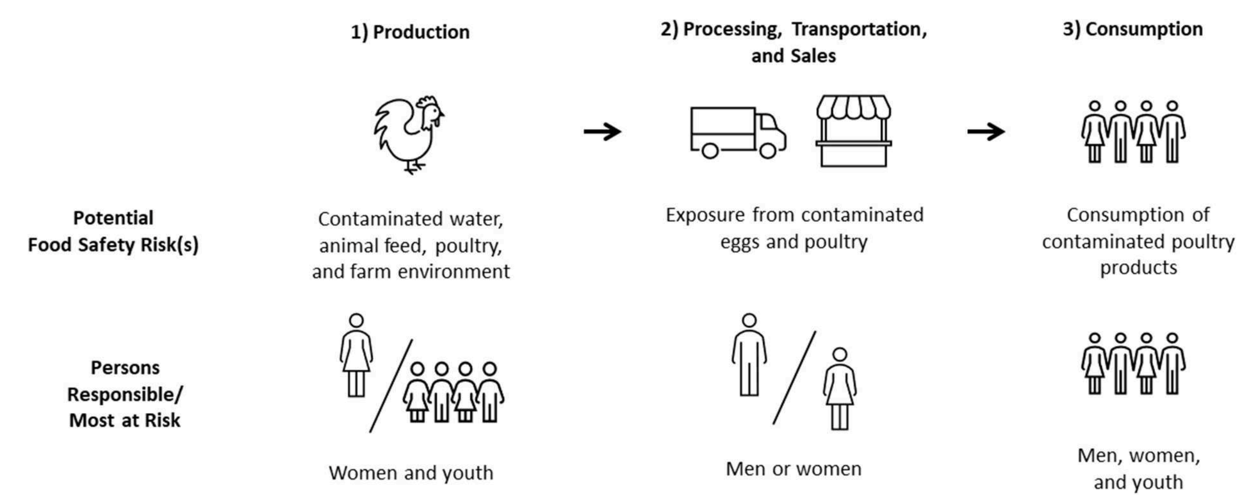

FIGURE 1
Responsibilities and risks for men, women, and youth in the poultry value chain in Kenya.

the rainy seasons, birds are placed in a confined space, and fed other feed sources such as kitchen leftovers. During the remainder of the year, birds scavenge for food such as grass, insects, earthworms, and seeds from around the farm (Magothe et al., 2012). The type of chicken produced can also vary by urbanicity. For example, in Kenya, indigenous chickens were more common in rural areas whereas layers and broilers were more common in peri-urban areas (Odhiambo, 2020).

Chickens in rural Kenyan households are primarily owned by women, although both women and youth participate equally in poultry production (Odhiambo, 2020). For example, in southern Kenya, ownership of smallholder chicken production assets is divided by gender with women being the primary owners of flocks of indigenous chicken for household consumption (39%) and men primarily owning the land (35–50%) (Mutua, 2018). In Western Kenya, women were reported to be the primary owners of poultry (63%) followed by youth (18%) (Okitoi et al., 2007). The number of chickens owned also differs by gender. A cross-sectional study of poultry keeping and management practices in Ethiopia concluded that female-headed households had statistically significantly less birds than male-headed households (Aklilu et al., 2008). Poultry can be shared between farms due to lack of feed for chickens. Sharing of poultry was reported to be more common in female-headed households (23%) than male-headed households (16%) (Aklilu et al., 2008).

TABLE 2 Overview of articles on gender, youth, and food safety in the poultry value chain in East Africa.

Categorization	Reference	Title	Study aim	Location
Gender	Aklilu et al., 2008	How resource poor households value and access poultry: Village poultry keeping in Tigray, Ethiopia	Examine socioeconomic factors for poultry keeping in rural homes.	Ethiopia
	Ochieng, 2012	Determinants of adoption of management interventions in indigenous chicken production in Kenya	Determine socioeconomic factors for adoption of management, feed, and vaccination interventions on smallholder farms.	Kenya
	Mutua, 2018	Challenges facing indigenous chicken production and adoption levels of biosecurity measures in selected areas of Makueni County, Kenya	Evaluate socioeconomic status, chicken production practices, and adoption of biosecurity measures on smallholder poultry farms.	Kenya
	Hailemichael et al., 2016	Analysis of village poultry value chain in Ethiopia: Implications for action research and development	Establish a baseline of poultry production practices, management, and marketing smallholder households.	Ethiopia
	Riang'a et al., 2017	Food beliefs and practices among the Kalenjin pregnant women in rural Uasin Gishu County, Kenya	Investigate the relationship between food consumption practices of women and birth outcomes.	Kenya
	Mulema et al., 2019	Can chickens empower women: Perceptions from chicken producers in peri-urban and rural Ethiopia (ILRI Project Report)	Describe livelihoods, what types of chickens are preferred, and empowerment.	Ethiopia
	Leight et al., 2020	The effects of poultry and unconditional cash transfers on livelihoods outcomes	Determine effectiveness of poultry and cash transfer interventions for poultry and livestock farms from rural households.	Ethiopia
	Waithanji et al., 2020	Insects as feed: Gendered knowledge attitudes and practices among poultry and Pond Fish farmers in Kenya	Determine knowledge, attitudes, and practices during poultry and Pond Fish farming.	Kenya
Youth	Okitoi et al., 2007	Gender issues in poultry production in rural households of Western Kenya	Determine roles of family members in poultry production in rural households.	Kenya
	Odhiambo, 2020	Hatching Hope: Gender and Youth Analysis Report	Determine roles of family members in poultry production in urban and peri-urban households.	Kenya
	MoALFC, 2021	Kenya County Climate Risk Profile: Narok County	Assess roles and responsibilities in agriculture value chains and how they relate to climate change.	Kenya
Food safety	Winrock International, 2010	Partnership for Safe Poultry in Kenya	Train women's groups on food safe poultry production practices as well as connecting them to buyers and financing.	Kenya
	Winrock International, 2011	Helping women feed their families in rural Kenya	Promote safe poultry production and create marketing systems that generate higher incomes and improve nutrition for smallholder families.	Kenya
	Nyaga, 2008	Poultry Sector Country Review	Determine roles and food safety risks of poultry value chain actors in farming, slaughtering, transporting, and marketing.	Kenya
	Kiambi et al., 2021	Understanding Antimicrobial Use Contexts in the Poultry Sector: Challenges for Small-Scale Layer Farms in Kenya	Discussion of growing antimicrobial resistance in poultry and other sectors.	Kenya
	Magothe et al., 2012	Indigenous chicken production in Kenya: I. Current status	Characterization of poultry management in rural households.	Kenya
	Ringo and Mwenda, 2018	Poultry Subsector in Tanzania: A Quick Scan	Describe the poultry value chain in Tanzania and identify future interventions.	Tanzania

Poultry sharing poses food safety and biosecurity concerns due to transmission of biological contaminants from one farm to another.

In countries identified in this study, women are primarily responsible for the care and keeping of chickens. For example, in Kenya, women are primarily involved in cleaning of poultry houses (75%), feeding (75%), and treating chickens for illness (60%) (Okitoi et al., 2007). Men were primarily responsible for construction of sheds for chickens (85%), although children also helped (10%) (Okitoi et al., 2007). Decisions regarding type of poultry raised (54.6%) and type of feed used (57%) were mainly made by women (Waithanji et al., 2020). Similarly, in Ethiopia, women were primarily responsible for the care and keeping of poultry on smallholder farms (64%) (Hailemichael et al., 2016). In Ethiopia, women and children were primarily responsible for the care and keeping of chickens including feeding, cleaning, and taking care of sick birds while men were found to be primarily responsible for making housing for poultry and, depending on the location, bringing chickens and eggs to the market (Mulema et al., 2019).

Women also spend more time engaged in daily activities around poultry production than men. For example, in Kenya, women spent 70.2% of their waking hours during the dry season on chores related to poultry whereas men only spent 39.9% of their waking time on these activities (Waithanji et al., 2020). Similar differences were seen during the wet season with women spending a greater percentage of their time focused on chores related to poultry then men (Waithanji et al., 2020). Due to these differences in time allocation for poultry production between men and women, poultry farming interventions need to consider women's time burden.

Farm management interventions vary in their adoption by gender. In 2008, a cross-sectional survey of 120 smallholder farm households in western Kenya was conducted to assess the relationship between household socio-demographic and poultry farm characteristics and the effectiveness of intervention packages that included feed supplements, vaccinations, and housing for chickens (Ochieng, 2012). Women were found to be less likely than men to adopt the full intervention package potentially due to women having limited access to resources such as land and finances (Ochieng, 2012). These limitations should be considered when interventions are being developed to meet the needs of women farmers.

There are multiple constraints faced by smallholder poultry farmers, especially women, that influence their ability to raise chickens and ensure food safety on the farm. Disease outbreaks and lack of access to biosecurity measures are two of these constraints (Wong et al., 2017). A program in Kenya was developed to train women's groups on safe poultry production practices as well as connect them to buyers and financing (Winrock International, 2011). This program promoted safe poultry production and marketing systems that generated higher incomes and improved nutrition for smallholder families. Technical training on safe poultry production practices was provided to 1,815 women poultry producers (Winrock International, 2010). As a result of this program, a reduction of chicken mortality of >15% was found indicating adoption of training practices (Winrock International, 2010). Other interventions such as poultry and cash transfers targeted to women in Ethiopia resulted in an increase in ownership of poultry from about 40 to 94%. The cash transfer package also resulted in an 8% increase in household poultry keeping from 54 to 62% (Leight et al., 2020). This demonstrates that it requires more than simply cash to increase poultry production amongst women smallholder farmers.

Smallholder poultry farmers, and particularly women, also lack access to veterinary services and appropriate extension services for improving farming methods and techniques (Wong et al., 2017). This is important, as studies show that there is a misuse of antimicrobial drugs for prevention and elimination of diseases at low-cost which is putting the poultry sector in Kenya at risk for the emergence and spread of antimicrobial resistance (Kiambi et al., 2021).

Development of food safety interventions needs to consider who is conducting the activity in the poultry value chain. Women are frequently engaged in other activities throughout the day which influences their ability to participate in trainings. Determinants of adoption are also important considerations since such factors may influence women's willingness to adopt new management practices, including food safety measures (Mutua, 2018). To increase safe poultry production, trainings are needed on food safety management practices in a format accessible to the target populations, and at a time when women and youth are available for trainings.

Processing

Poultry processing typically includes slaughter, scalding, defeathering, evisceration, cutting and, in some cases, deboning and/or grinding. Individuals engaged in poultry processing can be exposed to foodborne pathogens as well as other zoonotic diseases. In Kenya, women, men, and youth participate in slaughtering of poultry products (MoALFC, 2021). Chicken processing on smallholder farms typically includes the chicken being slaughtered, immersed in hot water, defeathered, and removal of internal organs. Smallholder farmers commonly lack access to hygienic slaughtering facilities, which can lead to cross-contamination of the carcasses and the surrounding environment. Slaughtering is commonly conducted at the home which could potentially expose individuals to foodborne pathogens through cross contamination (Nyaga, 2008). Wastes from processing are commonly buried on the home's property or fed to dogs. Burying wastes from processing could potentially cause environmental contamination including contaminating drinking water (Nyaga, 2008). To increase food safety practices

during processing and establish slaughterhouses, a poultry producer association was formed in Makueni county, Kenya. A small group of individuals delivered birds from rural households to the Makueni slaughterhouse facility. The birds were inspected, and acceptable birds were harvested and delivered to be sold (Nyaga, 2008). This producer association increased food safety knowledge and oversight of poultry processing in the community.

Transportation

Transportation of poultry may lead to increased bacterial contamination of both live birds and poultry products and potential exposure to these contaminants for the individuals transporting them. The role of women and youth in transportation of poultry varies by region in Kenya. In Kenya, women primarily transport chicken and eggs to the market. In Narok county, it was found that women, men, and youth all transported poultry (MoALFC, 2021). As in many agricultural enterprises, once poultry production becomes commercial, men are primarily involved and often take over from women (Odhiambo, 2020). Transporting chicken and eggs provides additional opportunities for youth to be involved in marketing of these products. However, unsanitary transportation vehicles and baskets may contaminate poultry products and birds. Additionally, densely packing and mixing of sick birds with other animal species creates a significant risk for the spread of diseases such as highly pathogenic avian influenza (HPAI) (Winrock International, 2010). Lack of feeding of birds before sales may decrease body mass, increase illnesses in birds, or result in the death of birds. Increasing food safety practices during transportation could increase profits earned by smallholder poultry producers.

Retail and sales

If products are not handled safely, retail and sales of poultry and poultry products can lead to increase bacterial contamination. Retail and sales of poultry and poultry products is an important source of income and livelihoods for women and youth in low- and middle-income countries (Randolph et al., 2007; Alders and Pym, 2009; Wong et al., 2017; Ngongolo et al., 2021; Wilson, 2021). Poultry production provides a way to empower youth and women in rural households. The individual who controls the income from poultry production determines who has the power to make decisions on how income is spent (Colverson et al., 2020). Empowerment includes increasing women and youth's ability to manage poultry production through training, access to capital, and increased decision-making power. Increased decision-making power could include the ability for individuals to decide when chickens or eggs are sold or eaten, and/or control of the resources generated from these sales. For example, management of chickens can empower women, because chickens can be assets used to increase women's income (Mulema et al., 2019).

Although women may be the owners and caretakers of chickens, they may not have decision-making power on the use of income from the poultry, and poultry product sales (Ringo and Mwenda, 2018). In western Kenya, women are primarily responsible for selling chickens (60%) and eggs (95%). Men primarily control the income generated while women control the access of food and gifts for guests (Okitoi et al., 2007). In the Kirinyaga, Kisii, and Nakuru counties in Kenya, men were found to be primarily in charge of selling poultry (Waithanji et al., 2020). Control of income may be dependent on who is the head of the household. In Ethiopia, the majority (90%) of the income from selling poultry was controlled by women in female-headed households. In contrast, in male-headed households, 57% of the income was jointly controlled with women controlling 30% of the remaining income and men controlling the remaining 13%. In contrast, women were primarily responsible for selling eggs (85%) and controlled 50% of the income from egg sales (Hailemichael et al., 2016).

Consumption

Storing poultry at proper temperatures and cooking poultry can prevent bacterial growth and inactivate bacterial pathogens. Women are primarily responsible for deciding when poultry and eggs produced by the household are consumed. In western Kenya, women were primarily responsible for distribution of chicken (60%) and eggs (100%) for consumption as well as giving gifts to visitors (60%) (Okitoi et al., 2007). In Ethiopia, it was found that women make the decisions of when poultry and eggs are consumed in the home (Mulema et al., 2019).

Division of animal meat according to age and gender is also a common cultural practice. Women and female youth frequently receive the lowest quantity and quality of meat. For example, a study in Ethiopia found the drumstick, breast, and gizzard are customarily eaten by men whereas the skin, neck, and wings are eaten by women and children. Since pregnant and lactating women and growing female youth require greater quantities of protein in their diet, the distribution of poultry meat is a concern for nutrition interventions (Aklilu et al., 2008). In the Luhya community in Kenya, women and children eat eggs while men and guests eat chicken meat.

There are also multiple myths associated with consumption of poultry products by pregnant women. In some parts of Kenya, pregnant women are forbidden to consume eggs for fear that the child may become fat. Similarly, chicken gizzards are not permitted to be eaten by pregnant women because they are believed to make women infertile (Kariuki et al., 2017). Among the Kalenjin community in Kenya, pregnant women are not

allowed to eat organs of poultry (except the liver), such as the tongue and the heart. When a chicken is slaughtered, its liver is preserved for pregnant woman in the home to consume (Riang'a et al., 2017). Nutrition interventions should primarily include women due to the importance of proper nutrition during pregnancy and its relationship to the growth and development of children as well as their primary responsibility for deciding who eats what meat products when.

Conclusion

This review demonstrates that women and youth play a central role in smallholder poultry value chains in Kenya. There are multiple strategies for increasing productivity of farms. One is increasing access to credit for women and youth. Also, the formation of cooperatives can aid in decreasing risks in business. Education regarding food safety knowledge and its application to poultry production in developing countries plays a critical role in improving productivity and profitability in small-scale poultry producers.

Youth also experience barriers to poultry production due to limited knowledge of business opportunities of poultry farming, which includes access to financing. Youth need training on the production and management of poultry but their attendance at existing training programs tends to be low. Utilizing technology (e.g., creation of digital trainings) can make training more accessible and appealing to youth. For example, including pictures improves accessibility for individuals with low literacy levels. Trainings also need to address social norms around which activities women and youth can engage in and ways to access financial capital and policies to support women and youth businesses.

Key activities to improve the success of women and youth in raising poultry and reducing FBD include delivering trainings on hygienic practices that target women and youth, considering their available times and locations; providing financial support for women and youth poultry production; empowering women in decision making around poultry sales; and addressing inequitable distribution of poultry meat and eggs in household consumption.

Policy implementation to support gender and youth equality and empowerment is needed at ministry, local, and national levels. Additionally, there is an increased need for sex-disaggregated data collection in order to design and implement interventions effectively. Due to their central role in poultry production, gender and youth perspectives need to be included when developing future interventions aimed at reducing foodborne pathogens in poultry production.

Author contributions

AG, EK, and KC were responsible for analysis of resources and writing. SI, CK, AB, and BK were responsible for manuscript review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Quantitative risk assessment of salmonellosis in Cambodian consumers through chicken and pork salad consumption

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Salmonella is a globally important foodborne bacterial pathogen that poses a high risk to human health. This study aimed to estimate the risk to Cambodian consumers from acquiring salmonellosis after consuming chicken and pork salad, using a quantitative microbial risk assessment (QMRA). Chicken and pork salads are typical Cambodian dishes containing raw vegetables and boiled chicken meat or pork. As previously described, chicken meat and pork samples ($n = 204$ of each) were collected from traditional markets in 25 Cambodian provinces to generate data on *Salmonella* contamination. Salad preparation and consumption practices were surveyed in 93 Cambodian households and this information was used to design an experiment to assess *Salmonella* cross-contamination from raw meat to ready-to-eat salad. In the part of the study reported here, data on consumption, *Salmonella* in salad, dose-response, and predicted salmonellosis were modeled using Monte Carlo simulations at 10,000 iterations. The prevalence of *Salmonella* in chicken meat and pork were set to 42.6 and 45.1%, respectively, with average most probable number (MPN) per gram of *Salmonella* in chicken meat was 10.6 and in pork 11.1 MPN/g, based on an earlier study. Half of the interviewed households cooked meat for the salad directly after purchase. The QMRA model showed that the modeled annual risk of salmonellosis from consuming chicken salad, pork salad and both chicken and pork salad were 11.1% probability of illness per person per year (90% CI 0.0–35.1), 4.0% (90% CI 0.0–21.3), and 14.5% (90% CI 0.0–33.5), respectively. The factors most influencing the estimate were cross-contamination while preparing the salad, followed by the prevalence of *Salmonella* in chicken meat and pork at the market. The wide confidence interval for the incidence was mainly due to the variability in reducing bacteria concentration by cooking and salad consumption. The predicted risk of

salmonellosis due to chicken and pork salad consumption is high, and the study provides evidence supporting control measures of improving the safety of retailed chicken and pork obtained from markets to households and improving food preparation methods in the household.

KEYWORDS

ASF consumption, Cambodia, QMRA, cross-contamination, Cambodian chicken and pork salad, traditional market

1. Introduction

The World Health Organization (WHO) has estimated that foodborne diseases (FBD) cause 33 million disability adjusted life years (DALYs) globally, and a loss of more than US$110 billion in productivity and medical expenses each year in low- and middle-income countries (LMIC) (Havelaar et al., 2015; Devleesschauwer et al., 2018). Annually, around 200 different types of foodborne pathogens cause disease in 600 million people, and FBD has been reported to result in around 420,000 deaths every year (World Health Organization Regional Office for South-East Asia., 2016). People living in LMIC are at particular risk for contracting FBD due to challenges related to insufficient hygiene practices, poor knowledge and reduced access to safe food (Grace, 2015; Varijakshapanicker et al., 2019). FBD thus constitute a significant health challenge globally, and non-typhoid *Salmonella* serovars have been reported as the most common foodborne bacteria causing FBD (Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Boqvist et al., 2018).

Most *Salmonella* serovars are human pathogens and may cause a wide range of symptoms, of which diarrhea is the most common (Oscar, 2004; Majowicz et al., 2010; Crump and Wain, 2017). Animal-source food (ASF) is often implicated in human salmonellosis. It is estimated that globally *Salmonella* causes approximately 230,000 deaths annually, mainly in elderly and children under 5 years (Majowicz et al., 2010; Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Devleesschauwer et al., 2018). *Salmonella* is carried by many animal species and can be transmitted by ASF, contributing to food safety concerns in LMIC (Unger et al., 2020).

In Cambodia, ASF (especially chicken meat and pork) are essential parts of the diet eaten by all age groups (General Directorate of Animal Health Production of Cambodia, 2021) and contribute important micronutrients (Tum, 2008; Sary et al., 2019). Chicken and pork are commonly sold at traditional markets where most people buy fresh food. Meat is commonly stored without chilling at the markets and in most households (People in Need, 2015; Rortana et al., 2022). Several popular dishes are prepared from boiled chicken meat or pork mixed with raw vegetables (Baker, 2009; Saorath, 2019; Cambodia Recipe, 2020).

In LMIC, chicken and pork are easily contaminated with *Salmonella*, which can occur at slaughterhouse facilities, markets, and storage facilities with insufficient cooling (Cliver, 2006; Carrasco et al., 2012; Aizaabi and Khan, 2017; Possas et al., 2017). Storing of meat in warm temperatures provides good conditions for the growth of *Salmonella* (Possas et al., 2017; Dang-Xuan et al., 2019). Improper handling and poor practices also contribute to the transmission of bacteria along the food chain, especially from markets to ready-to-eat (RTE) foods (Kristina and Sophal, 2018)). In addition, recent studies have found that poor handling of meat before and during cooking causes bacterial cross-contamination to RTE food, including chicken salad in Cambodia (Rortana et al., 2022) and boiled pork in Vietnam (Dang-Xuan et al., 2018). In Cambodia, a recent study detected 43% prevalence of *Salmonella* in chicken meat, 41.9% on chicken cutting board, 45% on pork, and 30% on pork cutting board; and the mean MPN of *Salmonella* per gram was 10.6 MPN/g in chicken and 11.1 MPN/g in pork samples (Rortana et al., 2021).

Quantitative microbial risk assessment (QMRA) can estimate health consequences and help in food safety management and communication. In Cambodia, QMRA has been conducted on *Salmonella* and different hazards and food type, but there are, to our knowledge, no publications on the risk of salmonellosis related to chicken meat or pork (Tum, 2008; Kristina and Sophal, 2018; Walia et al., 2018; Food and Agriculture Organization of the United Nations, 2021) although QMRA models of salmonellosis have been developed in other countries (Dang-Xuan et al., 2016; Perez-Rodriguez, 2020; Oscar, 2021a,b).

In Cambodia, there is a lack of comprehensive and solid evidence on the impact of FBD that can guide policymakers on health hazards related risks, and support meat production and donors to tackle food safety issues and public health notices (Tum, 2008; Public Health of Canada, 2017; Lam et al., 2019). Moreover, the household knowledge of FBD is low in Cambodia, and most people associate food safety challenges mainly with chemical contamination (Brown et al., 2022; Duong et al., 2022). This study aimed to estimate the risk of consumers acquiring

Salmonella infection through consuming contaminated pork and chicken salad at the household level to generated new and actionable information.

2. Materials and methods

2.1. Study location

The study was conducted in Cambodia, located in the Mekong sub-region in Southeast Asia. In 2019, the total population of Cambodia was around 15 million (National Institute of Statistics of Cambodia, 2019). The country is influenced by tropical monsoon winds and has two seasons, the dry season (November–April) and the rainy season (May–October). In the rainy season, the average temperature in 2019 was 29°C, ranging between 27 and 36°C, with a humidity between 45 and 80 % (Department of Meteorology of Cambodia, 2019). Data for *Salmonella* contamination used in this QMRA was collected from all 25 provinces in Cambodia.

2.2. Study design

The QMRA model was built according to the Codex Alimentarius Commission quantitative microbial risk assessment framework (Codex Alimentatius Commission, 1999), consisting of hazard identification, hazard characterization, exposure assessment, and risk characterization (CAC/GL-30, 1999). This QMRA model was designed using data published earlier. Firstly, a cross-sectional market survey of the *Salmonella* prevalence in chicken meat and pork had been conducted in traditional markets in all 25 provinces in Cambodia (Rortana et al., 2021). Secondly, a household survey had been carried out in four provinces and cities (Battambang, Preah Sihanouk, Phnom Penh and Siem Reap) to explore handling and consumption patterns of chicken and pork salad in Cambodian (Rortana et al., 2022). Thirdly, experiments to identify cross-contamination scenarios during the preparation of chicken and pork salad at the household level had been done at the National Animal Health and Production Research Institute (Phnom Penh, Cambodia) (Rortana et al., 2022) and in Vietnam (Dang-Xuan et al., 2018). Lastly, data for the hazard characterization, of bacteria growth and dose-response model were obtained from the literature (Teunis et al., 2010; Velugoti et al., 2011). These surveys and experiments were conducted from November 2018 to June 2020 and are described briefly below.

2.3. Salmonellosis risk assessment model

2.3.1. Hazard identification

In a publication from WHO on the global burden of FBD, salmonellosis was identified as the most important bacteria hazard in DALYs (Havelaar et al., 2015). Salmonellosis is also considered one of Cambodia's most critical FBD (Rortana et al., 2021). The hazard identification of this study was made using data from a systematic literature review (Kristina and Sophal, 2018), from key stakeholder meetings in Cambodia (including representatives from a national food safety working group, policymakers, and international partners) (Nguyen-Viet, 2018), from a multi-hazard survey (Rortana et al., 2021), and from the cost of hospitalization for FBD performed by the Ministry of Health in Cambodia (Srey, 2019).

2.3.2. Hazard characterization

Non-typhoid *Salmonella* serovars are the most common foodborne bacteria causing FBD (Havelaar et al., 2015; World Health Organization Regional Office for South-East Asia., 2016; Boqvist et al., 2018). Most *Salmonella* serovars are human pathogens and may cause a wide range of symptoms, of which diarrhea is the most common (Oscar, 2004; Majowicz et al., 2010; Crump and Wain, 2017). Moreover, invasive *Salmonella* infection has been reported in Cambodia (Emary et al., 2012; Vlieghe et al., 2012; Kimsean et al., 2017; Kristina and Sophal, 2018; Kuijpers et al., 2018). *Salmonella* contamination in ASF is often implicated in human salmonellosis infection (Botteldoorn et al., 2003; Carrasco et al., 2012; Havelaar et al., 2015). In this study, the Beta-Poisson dose-response model developed from *Salmonella* outbreak data was used (alpha = 0.00853; beta = 3.14) (Teunis et al., 2010). That dose-response model presented an infection ID50 of 7 colony forming unit (CFU) and an illness ID50 of 36 CFU.

2.3.3. *Salmonella* exposure assessment

2.3.3.1. Meat sampling at market

A previous study investigated the prevalence and concentration of *Salmonella* in chicken meat and pork sold in traditional Cambodian markets (Rortana et al., 2021). In brief, samples from chicken meat ($n = 204$) and pork ($n = 204$) from markets in 25 provinces in Cambodia were included. The prevalence of *Salmonella* from all the markets in chicken meat was 42.6% and in pork 45.1%. The mean MPN of *Salmonella* was 10.6 MPN/g in chicken meat and 11.1 MPN/g in pork samples.

2.3.3.2. Cross-contamination study

Cross-contamination of *Salmonella* has been described in two published papers on chicken salad (Rortana et al., 2022) and boiled pork (Dang-Xuan et al., 2018). According to a recent study, cross-contamination of *Salmonella* in chicken salad was common in the four scenarios or sets of household practices used for salad preparation in Cambodia (Rortana et al., 2022). Briefly, *Salmonella* occurrence on cutting boards, knives and hands under four preparation scenarios (Table 1) was assessed. Similarly, *Salmonella* cross-contamination from raw pork to boiled pork *via* a hands and kitchen utensils was examined in

Vietnam (Dang-Xuan et al., 2018). The similarity of the four scenarios is described in detail in Table 1. The proportion of households using each scenario, as well as the probability of contamination, were part of the modeling.

2.3.3.3. Chicken and pork salad consumption

Chicken and pork salad consumption were assessed using focus group discussion (FGD) among 93 households in four provinces (Siem Reap, Preah Sihanouk, Battambang, and Phnom Penh) in Cambodia (Rortana et al., 2022). Three FGDs (with participants chosen to represent rural, peri-urban, and urban areas) in each of the four provinces were conducted by randomly selecting households within one commune. A discussion outline was developed in English and translated to Khmer language for FGD and back translated into English for analysis. The FGD was led by trained researchers using flipchart and notes, and lasted about 1.5 h. The information of chicken and pork salad consumption was determined for children below 5 years, youth (6–15 years old), adults (16–60 years old), and the elderly (above 61 years old).

2.3.4. Risk characterization of *Salmonella* infection

The data presented above was integrated into a stochastic risk model, including different input parameters (Table 2). The risk of salmonellosis (health outcome) was defined as the probability of illness per year per person, simulated by combining different transmission pathways through chicken and pork salad consumption. The parameters, statistics, distribution, and data sources used in the QMRA model are presented in Table 2 and Figure 1. In step 1, the prevalence of *Salmonella* from samples collected at the markets was used as representative of bacterial contamination in fresh chicken meat and pork (Rortana et al., 2021). In step 2, the rate of *Salmonella* entering chicken and pork salad was estimated at the household level, the temperature at the study site, duration of storage until cooking, and the laboratory experiment to measure the level of *Salmonella* in RTE chicken salad (Rortana et al., 2022) and boiled pork (Dang-Xuan et al., 2018). In step 3, the consumption rate was assessed including how often people consume chicken/pork salad and age groups (result from this study).

2.4. Data management and analysis

Data were managed and processed using MS Excel (Office 365). Descriptive statistical analysis was used to describe *Salmonella* prevalence using RStudio version 3.2.2 (R Core Team). The risk model was developed, and Monte Carlo simulation was performed using @Risk (Version 8.1, Palisade, Corporation, USA) for 10,000 iterations. The sensitivity analysis was conducted by selecting all the uncertainty parameters and running 1000 iterations at seven quantile values. Consumption data, prevalence and concentration of pathogen were described as mean and median. Final risk estimates were presented as mean and median with 90% confidence interval (CI).

2.5. Ethical considerations

Ethical approval of this study was done under the Safe Food, Fair Food Cambodia project and granted by the National Ethical Committee of Cambodia, coded 300NECHR, dated 26th December 2017. The participating researchers were informed and instructed on the safety procedures and provided their signed informed consent prior to starting the experiment. For the FGDs, participants invited to the discussion were asked for their written consent agreement prior to starting.

3. Results

3.1. Exposure assessment

The consumer survey on consumption of chicken and pork salad was conducted among 93 households in 12 FGDs. Detailed salad eating frequency (times/month) and amount of salad consumed (gram/meal) by age groups are presented in Table 3. In brief, the median frequency of consuming either chicken or pork salad was 1.6 times per month, ranging from 0–24, and the average amount consumed per meal was 130 grams per person (Table 3).

3.2. Risk characterization

The modeled annual incidence rate of salmonellosis was higher for chicken salad (11.1% probability of illness per person per year; 90% CI: 0–35.1) than for pork salad (4.0%, 90% CI: 0–21.3); considering consumption of both chicken and pork salad the annual incidence rate was 14.5% (90% CI: 0–33.5, Table 4; Figure 2). Adults had the highest modeled annual incidence rate (19.1%; 90% CI: 0–48.3): incidence by age categories and types of salad are shown in Table 4.

3.3. Sensitivity analysis

The sensitivity analysis found the most important influencer of the annual incidence rate of salmonellosis was the probability of cross-contamination in preparing salad in scenario 3 (wash chicken and pork first, use same utensils). This was followed by the prevalence of *Salmonella* on chicken at the market; probability of cross-contamination in scenario 1 (wash vegetables first, use same utensils for cutting salad and raw chicken and pork); prevalence *Salmonella* in chicken and pork

TABLE 1 Description of the four scenarios where cross-contamination of *Salmonella* may occur when preparing chicken and pork salad.

Description of scenarios	Procedure when preparing chicken and pork salad	Probability of cross contamination to RTE salad (%)		Concentration of *Salmonella* on RTE salad (CFU/g)	
		Chicken [a]	Pork [b]	Chicken [a]	Pork [b]
Scenario 1	- Wash and chop vegetables; then wash and cut the raw chicken or pork; and wash the cutting board, knife and hands (once with detergent). - Use the same washed cutting board, knife and hands to debone and slice cooked chicken and pork and mix the salad.	77.8	77.8	37.3	0.71
Scenario 2	- Wash and chop vegetables; then wash and cut the raw chicken or pork; and wash the cutting board, knife, and hands (once with detergent). - Use separate cutting board and knife to debone and slide cooked chicken and pork, and mix the salad.	11.1	0.0	0.36	0.0
Scenario 3	- Wash and cut raw chicken or pork; then wash and chop vegetables; wash the cutting board, knife and hands (once with detergent). - Use the same washed cutting board, knife to debone and slide cooked chicken and pork, and mix the salad.	22.2	22.2	0.36	0.12
Scenario 4	- Wash and cut raw chicken or pork; then wash and chop vegetables; wash the cutting board, knife and hands (once with detergent). - Use separate cutting board, knife to debone and slide cooked chicken and pork, and mix the salad.	0.0	66.7	0.0	2.49

This table is adapted from authors (Dang-Xuan et al., 2018[b] and Rortana et al., 2022[a]) which aimed to model the cross-contamination rate of bacteria entering ready-to-eat (RTE) food from raw animal-source food. There were only slight differences between the experiments for chicken and pork and therefore they are described as similar.

from the market; and probability of cross-contamination in scenario 4 (wash chicken and pork first, use different utensils, Figure 3; Table 5). The scenarios are described in detail in Table 1.

4. Discussion

This study developed a QMRA model from retail-to-table pathways predicting the likelihood of salmonellosis owing to consumption of chicken and pork salad in the Cambodian setting. The two most crucial factors for bacterial contamination of consumed food were the probability of cross-contamination during preparation in scenario 3 and the *Salmonella* prevalence in meat from markets. According to Rortana et al. (2022), most (86–96%) households practice preparing salad according to scenario 3, which had less contamination than scenario 1, but the fact that this practice is so common, gave it a larger influence in the model. The high influence of this common practice in the model also shows that there is a great scope of improvements. If the risk of cross-contamination at household level could be reduced, or people could change their habits completely to scenario 2 or 4, the risks could be reduced. All scenarios included rinsing the chicken, since it was the common practice, even if this step should be completely discouraged, as it increases the risks for salmonella contamination.

Most of the meat in Cambodia is sold in traditional markets where temperatures are suitable for bacterial growth (Sary et al., 2019; General Directorate of Animal Health Production of Cambodia, 2021; Rortana et al., 2022). Earlier studies found that meat and vegetables were frequently contaminated with *Salmonella* at this level (Rortana et al., 2021; Schwan et al., 2021). People in urban and peri-urban areas commonly purchase meat in the morning and cook it the same day (Brown et al., 2022; Rortana et al., 2022), while people in rural areas often keep meat longer before cooking (Duong et al., 2022). The focus of this QMRA was on meat purchased in the traditional value chain, as this is still the most common source of food in Cambodia, and where the prevalence was found the highest (Rortana et al., 2021). The model was built according to how people handle meat in their daily life. Another study in Cambodia used QMRA of *Salmonella* for risk assessment, specifically on the consumption of cricket powder to treat undernutrition in infants and children (Walia et al., 2018), while our study is the first to build

TABLE 2 Summary of the parameters, statistics, distribution, and data sources used in the QMRA model to estimate the risk of salmonellosis through chicken and pork salad consumption in Cambodia.

Parameters (symbol)	Distribution	Unit	Source
Traditional market			
Salmonella prevalence in chicken meat (P_{ck}) and pork (P_{po}) at market	P_{ck}: 87/204 (42.6%); described as Beta (87+1, 204-87+1) P_{po}: 92/204 (45.1%); described as Beta (92+1; 204-92+1)	-	Dang-Xuan et al., 2018; Rortana et al., 2021
Salmonella concentration in chicken meat and pork at the market (Cck and Cpo)	LogNormal (mean, 0.32) Chicken meat 16.7 CFU/g (0.36-120) Pork 17.3 CFU/g (0.36-120)	Log CFU/g	Dang-Xuan et al., 2018; Rortana et al., 2021
Status of *Salmonella* contamination in chicken meat (Sck) and cut pork (Spo)	Chicken meat: Binomial (1, P_{ck}) Pork: Binomial (1, P_{po})	-	Dang-Xuan et al., 2018; Rortana et al., 2021
Growth model at household			
The temperature when storing raw pork in the refrigerator at household (Tre)	Fixed at °C	°C	Present survey
The temperature when storing raw pork at ambience condition at household (Tro)	Normal (28, 4)	°C	Department of Meteorology of Cambodia, 2019
Duration of storage of raw pork at household before cooking (Hst)	Actual data (mean, min, max)	hour	Present survey
Salmonella grow rate in food matrices (h0)	Normal (2.14, 0.71)	Log CFU/g	Baranyi and Roberts, 1994
Cooking and consumption at household			
Probability of *Salmonella* cross-contamination after preparing chicken or pork salad in each cooking scenario (P_{sc})	Scenario 1: P_{sc1} =Beta(7+1, 9-7+1) Scenario 2: P_{sc2} =Beta(1+1, 9-1+1) Scenario 3: P_{sc3} =Beta(2+1, 9-2+1) Scenario 4: P_{sc4} =Beta(0+1, 9-0+1)	-	Dang-Xuan et al., 2018; Rortana et al., 2022
Status of *Salmonella* cross-contamination after boiling chicken/pork in cooking scenarios (C_{sc})	Scenario 1: C_{sc1} =Binomial(1, P_{sc1}) Scenario 2: C_{sc2} =Binomial(1, P_{sc2}) Scenario 3: C_{sc3} =Binomial(1, P_{sc3}) Scenario 4: C_{sc4} =Binomial(1, P_{sc4})	-	Dang-Xuan et al., 2018; Rortana et al., 2022
Probability of Cambodian consumer eating chicken/pork salad every meal (P_{eat}, $0< P_{eat} \leq 1$)	Non-parametric bootstrapping from household data (using DUniform)	-	Present survey and Dang-Xuan et al., 2018
Status of eating chicken/pork salad in the meal by Cambodian consumer (S_{eat})	Binomial (1, P_{eat})	-	Present survey and Dang-Xuan et al., 2018
Quantity of chicken/pork salad consumed per meal by Cambodian consumer (Qty)	Non-parametric bootstrapping from household data (using DUniform)	gram/meal	Present survey and Dang-Xuan et al., 2018
Illness (salmonellosis) probability from dose response model (I_{pro})	Beta Poisson (α,β) equation, α =0.00853 and β = 3.14	-	Teunis et al., 2010

CFU, Colony Forming Unit.

a QMRA on commonly consumed meat. In the future, as supermarkets get more common, it would be interesting to include the origin of the meat in the model, but this was not done here.

This study found that prevalence of *Salmonella* in the market was the major driver of risk of salmonellosis to salad consumers. As already described, salmonellosis is one of the leading foodborne diseases globally, as well as in Cambodia (Shiowshuh and Cheng-An, 2011; Yates, 2011; Nair and Johny, 2019). This study also found that the prevalence of *Salmonella* in food sold in markets was an important determinant of the incidence of *Salmonella* infection, adding insight to discussions on which points in the value chain food safety interventions should target. Recent studies have detected high prevalence of *Salmonella* in chicken meat and pork in markets in Cambodian (Rortana et al., 2021) and Vietnam (Dang-Xuan et al., 2019; Ngo et al., 2021). Moreover, other studies in Cambodia have found *Salmonella* in chicken meat (Nadimpalli et al., 2019) and vegetables that are in contact with meat during the selling period at the market (Schwan et al., 2021). In the current study, the prevalence of *Salmonella* in meat was relatively high compared to studies in nearby countries, including studies from Vietnam and Thailand (Dang-Xuan et al., 2019; Poomchuchit et al., 2021). Pathogenic *Salmonella enterica* have also been isolated

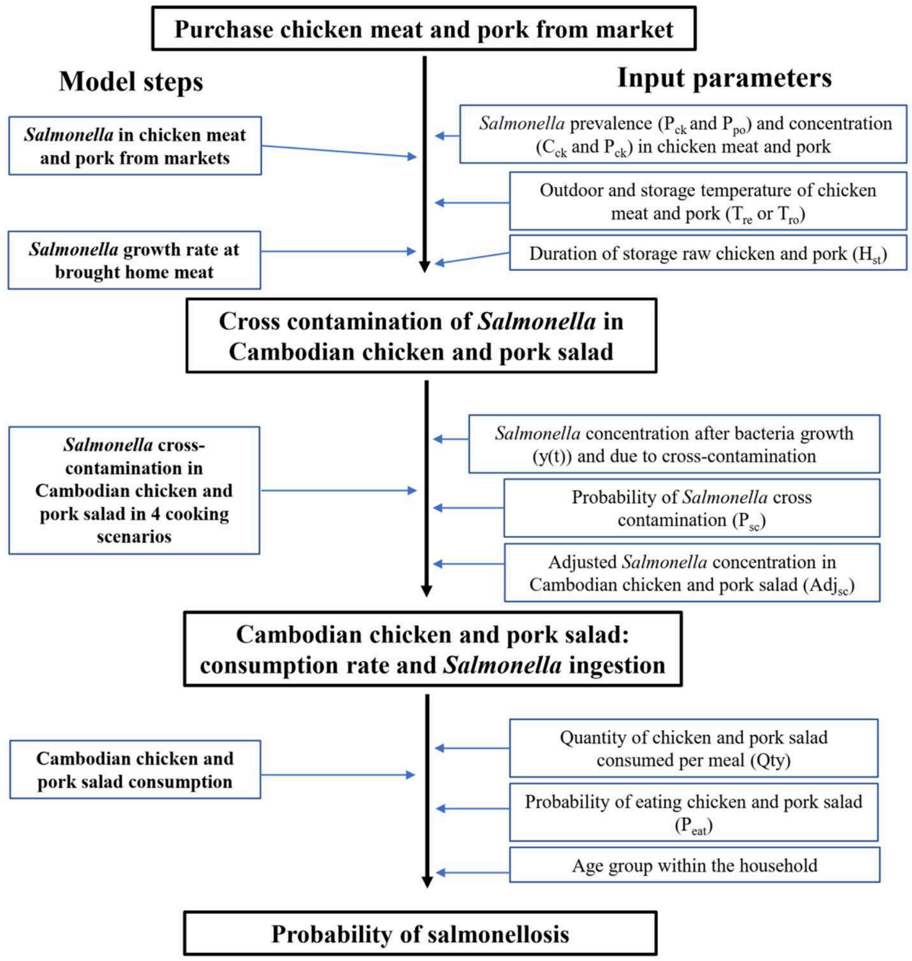

FIGURE 1
Model steps and input parameters in *Salmonella* QMRA from market to household related to chicken and pork salad preparation and consumption.

from multiple sources, including humans, animals, and food in Cambodia (van Cuyck et al., 2011; Schwan et al., 2021). Even *S. enterica* serovar Paratyphi infections have been found earlier in Phnom Penh, Cambodia (Vlieghe et al., 2013). The study found that the average incidence of salmonellosis in adults was higher than in children, youth, and the elderly, which is probably because adults more commonly consume chicken salad. Chicken salad and other similar salads are common foods in Asian countries, including Cambodia (Rortana et al., 2022) and Vietnam (Dang-Xuan et al., 2016). Even though most *Salmonella* does not cause severe disease in humans, regular exposure to these bacteria could be harmful over long time periods (Bollaerts et al., 2008; Perez-Rodriguez, 2020). Earlier studies also support that the cooking conditions and procedures such as moisture, contact time and pressure could result in higher transfer between the surface of contaminated objects (Cliver, 2006; Pérez-Rodríguez et al., 2008; Van Asselt et al., 2008). Chicken salads and similar salads (e.g. with beef, fish, shrimp, octopus) are very common at ceremonies such as traditional weddings in Cambodia. There is also an earlier report that a group of people got sick from a foodborne pathogen after eating salad in a wedding reception in Kampong Speu province (Vandy et al., 2012).

Two previous studies found that Cambodian people worry more about chemical food safety than microbial contamination (Duong et al., 2021; Brown et al., 2022). Most households also believe that chemicals (additives substances) used to make ASF products look good is the only cause of foodborne illness, leading them to care less of microbially contaminated in ASF (Brown et al., 2022). They tend to pay more attention to purchasing chemical free food than to proper storage, cooking, and good practice to reduce

TABLE 3 Frequency and the average amount of chicken and pork salad consumption by age groups in Cambodian households.

Information	Eating chicken salad	Eating pork salad	Eating either pork or chicken salad
Frequency of consumption by age group [times/month, *median (min-max)*]			
Children (under 5 years old)	0 (0–1.6)	0 (0–3.3)	0 (0–3.3)
Youth (6-15 years old)	0.3 (0–3.3)	0.8 (0–3.3)	1 (0–6.6)
Adult (16-60 years old)	0.3 (0–12)	0.8 (0–12)	1 (0–24)
Elder (over 61 years old)	0.5 (0–2.5)	0.6 (0.1–2.5)	1.5 (0.2–5)
Overall	0.8 (0–12)	0.9 (0–12)	1.6 (0–24)
Average consumption amount [g/person/meal, *(mean ± standard deviation)*]			
Children (under 5 years old)	46 ± 22	46 ± 20	46 ± 20
Youth (6–15 years old)	93 ± 62	93 ± 65	92 ± 59
Adult (16–60 years old)	124 ± 71	141 ± 79	134 ± 70
Elder (over 61 years old)	85 ± 62	81 ± 51	83 ± 54
Overall	141 ± 79	124 ± 71	130 ± 75

TABLE 4 The annual incidence rate of human salmonellosis due to chicken and pork salad consumption by age groups in Cambodia.

Age groups	Estimated annual salmonellosis incidence rate (%, Mean, 90% Confidence interval)		
	Consume chicken salad only	Consume pork salad only	Consume chicken and pork salac
Children (under 5 years old)	5.3 (0–31.8)	1.7 (0–12.3)	6.6 (0–25.3)
Youth (6–15 years old)	6.5 (0–35.7)	2.6 (0–19.0)	8.2 (0–29.4)
Adult (16–60 years old)	14.6 (0–51.9)	5.3 (0–32.9)	19.1 (0–48.3)
Elder (over 61 years old)	5.9 (0–35.9)	1.8 (0–12.9)	7.9 (0–28.8)
Overall	**11.1 (0–35.1)**	**4.0 (0–21.3)**	**14.5 (0–33.5)**

microbial contamination (Kristina and Sophal, 2018; Brown et al., 2022). Yet this study shows a high risk of FBD from a bacterial hazard. Changing perceived risks requires awareness raising data. This in turn, demands a food surveillance system, which is not yet in place in Cambodia (Thompson et al., 2021). Slaughterhouse hygiene improvements are still under development in the country, and the government has only started to monitor microbial contamination in slaughterhouses (Tum, 2008; Thompson et al., 2021) and markets (Rortana et al., 2021) to identify critical control points and prevent cross-contamination.

As consumption varies with age, different age categories were used in this study. However, the dose-response model used did not take differences in susceptibility between age groups into account (Teunis et al., 2010). Therefore, the separate dose-response model of *Salmonella* according to the categories of age and health condition was uncertain and could not be used as a formal analysis (Bollaerts et al., 2008; Marks and Coleman, 2017; Sanaa, 2021).

Food safety regulations vary between countries, but the usual goal is to combat foodborne diseases (Kunthear, 2022). In Cambodia the government recently adopted the National Plan on Food Safety. Six ministries are currently involved in governing national food safety, and coordinated by a multi-ministries team, the Technical Working Group for Food Security and Nutrition, which included representatives from each ministry. In June 2022, the law on food safety which addresses the entire food chain was adopted and brings Cambodia in line with international food safety standards (Food and Agriculture Organization of the United Nations, 2022; Kunthear, 2022). The law authorizes food inspection and provides a legal basis for action where food safety hazards are identified. This study provides scientific evidence that cross-contamination of *Salmonella* in the food chain (in this case from market to preparation of RTE salads) is a significant factor for human salmonellosis. This data is of relevance for local and national authorities and could be used to guide future policies, surveillance, and intervention to improve food safety along the food chain.

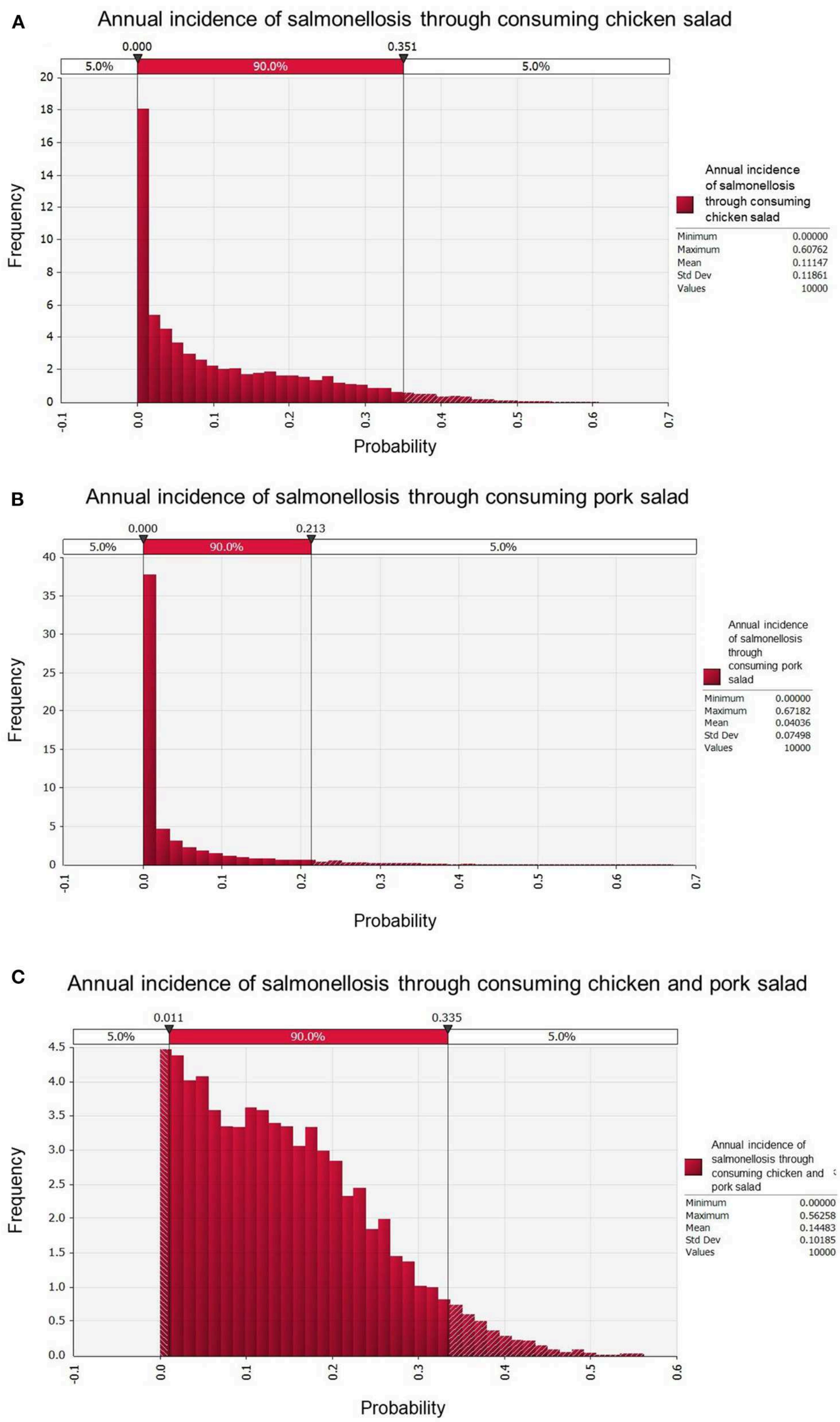

FIGURE 2
Annual incidence rates of salmonellosis in Cambodian households eating chicken salad **(A)**, pork salad **(B)**, and both chicken and pork salad **(C)**.

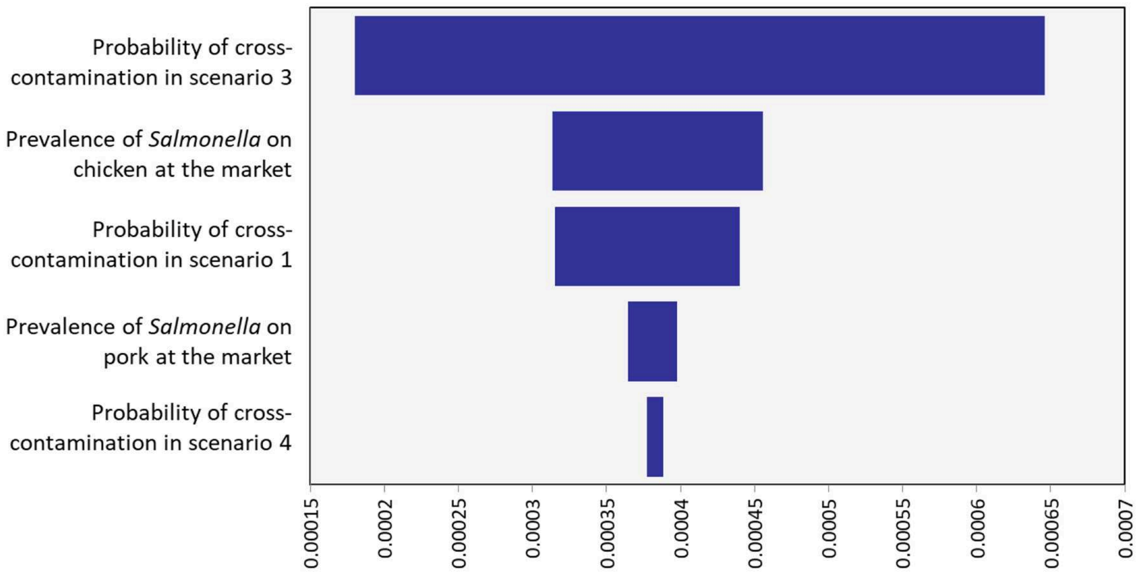

FIGURE 3
Sensitivity analysis of influence factors on annual salmonellosis incidence due to consuming both pork and chicken salad.

TABLE 5 Sensitivity analysis of influence factors on daily salmonellosis incidence due to consuming pork and chicken salad.

Rank	Influence factors	Values at 50th (1st–99th) percentiles	Mean (90% CI) daily incidence of salmonellosis per 10,000 people
1	Probability of cross-contamination in scenario 3	0.24 (0.07–0.5)	4.70 (0.35–12.57)
2	Prevalence of *Salmonella* on chicken at the market	0.43 (0.35–0.51)	4.76 (0.32 –12.3)
3	Probability of cross-contamination in scenario 1	0.76 (0.5–0.93)	4.77 (0.3–12.21)
4	Prevalence of *Salmonella* on pork at the market	0.45 (0.37–0.53)	4.75 (0.31–12.39)
5	Probability of cross-contamination in scenario 4	0.04 (0.00–0.22)	4.73 (0.32–12.45)

CI, Confidence interval.

5. Conclusions

The study presents new results on the risks of contracting salmonellosis after eating chicken and pork salad in Cambodia. It describes household practices that may facilitate *Salmonella* contamination of RTE food and estimates the probability of salmonellosis caused by consumption of this food. The QMRA suggests that changing meat storage and handling practices from market to household can reduce the likelihood of foodborne disease. The results are evidence for use as a basis for adapting policies in Cambodia. The new knowledge can guide implementation of appropriate and effective intervention strategies to prevent and control the undesirable consequences associated with microbial contamination in animal source food until RTE. Through enhancing food safety practices and responsibility among actors across the value chain, targeting markets, households and RTE food providers and restaurants, the findings can contribute to reduce the burden of FBDs in ASF in Cambodia and elsewhere.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved under the Safe Food, Fair Food Cambodia project and granted by the National Ethical Committee of Cambodia, coded 300NECHR, dated 26th December 2017. The participants provided their written informed consent to participate in this study.

Author contributions

Conception: DG, HN-V, ST, FU, JL, SD-X, SB, and CR. Data curation and writing—original draft: CR and SD-X. Formal analysis: CR, SD-X, and JL. Investigation: CR, SD-X, CT, and ST. Methodology: CR, CT, and SD-X. Supervision: SB, HN-V, ST, SD-X, DG, and JL. Writing—review and editing: SD-X, SB, HN-V, FU, ST, DG, KO, and JL. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Quantitative analysis of knowledge, attitude and practice of workers in chicken slaughter slabs toward food safety and hygiene in Ouagadougou, Burkina Faso

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Introduction: In low- and middle-income countries, chicken serves as a cheap source of protein and an income source for many households. It is particularly important in the capital, Ouagadougou, Burkina Faso, where chicken is regularly consumed. However, hygiene standards are very low, posing a significant public health risk.

Methods: To better understand the food safety situation, we conducted a cross-sectional survey of the hygienic practices of 155 randomly selected chicken slaughter outlets and carcass shops with a semi-structured questionnaire.

Results and discussion: Of the outlets visited, 59% are not licensed, and 63% are not regularly inspected, operating in the dominant, largely unregulated informal sector. More than 80% of the chickens are sourced from village production systems, but around 6% of the birds die during transport. The monetary loss due to chicken death during transportation is around four million USD annually. Market hygiene is poor; 86% of the holding pens have no hard floor and are not washed regularly. Almost all (92%) chickens are slaughtered on bare earth floors; bleeding, plucking, and evisceration are done on a wooden table that is rarely washed. On average, the same scalding water is used for 33 birds, seven scalded at a time. Most respondents (49%) thought that plucking and evisceration were the major cause of contamination of carcasses with foodborne pathogens. Most operators only washed their hands and knives with tap water at the beginning of the slaughtering process. Some shops use refrigerators and freezers to store carcasses before selling. However, they store carcasses with other foods like fish, beef and vegetables, facilitating further cross-contamination. There were rats (26%), cats (39%) and dogs (30%) present at outlets, roaming for food, especially roadside outlets. Training schemes on hygienic food handling practices were favoured by outlets as an approach to improve matters; however, improvements in food safety will be limited without significant upgrades in infrastructure and facilities. Slaughter slabs need a standard house with stable energy, adequate airflow, clean water, toilets, detergents and freezers. Also, they need equipment like knives, tables and dishes made of high-quality, easy-to-clean materials.

KEYWORDS
chickens, food safety, KAP, Ouagadougou (Burkina Faso), slaughter outlets

1. Introduction

Foodborne diseases cause a huge global socioeconomic and health burden. Each year one in 10 people get ill from contaminated food, resulting in 600 million illnesses, 420,000 deaths and the loss of 33 million healthy years of life globally, a burden comparable to tuberculosis or malaria (WHO, 2015). In Africa, with the highest foodborne disease (FBD) burden, the 31 foodborne hazards caused 1,200–1,300 DALYs per 100,000 individuals in which nearly 70% of the burden is estimated to be due to non-typhoidal Salmonella and Enteropathogenic and Enterotoxigenic *Escherichia coli* (Mensah et al., 2012; Makinde et al., 2020).

The burden of Foodborne diseases in low and middle-income countries is increasing. Besides health impact, food safety has become a precondition for access to global food markets and increasingly for high-value domestic markets in lower-income countries (Hoffmann et al., 2019). In Sub-Saharan Africa, the dominant informal food sector standards are absent or poor, with little regulatory oversight. Although animal products are at higher risk in terms of foodborne diseases, they are vital components of the diets and livelihoods of the undernourished majority across Sub-Saharan Africa. An improved supply of safe foods is needed to improve food security, with processing and slaughter particularly important with a high risk of microbial contamination of meat with feces and dirt or microbes from hands, equipment and surfaces (Roesel and Grace, 2014).

In urban settings, food selection is determined by availability, affordability, nutrition, and convenience (Wong et al., 2017). Chicken meat is widely consumed in Burkina Faso, especially in Ouagadougou, where it is regularly eaten in chicken restaurants ("maquis"), or cooked chicken is taken home, ready-to-eat, at the end of the working day and during celebrations and festivals (Dione et al., 2021). As well as being sold in restaurants, consumers, restaurants and hotels often buy raw chicken carcasses slaughtered and prepared at the market and roadside outlets. However, studies report high levels of bacterial contamination in these settings, with 90% of carcasses contaminated with *Campylobacter* species and 100% of carcass washing solutions being contaminated with *Salmonella* species (Kagambèga et al., 2018).

Previous studies from the same ILRI "Pull-Push project"[1] found consumer concerns about chicken safety (Dione et al., 2021). Disease-causing microorganisms can jump at any stage in the food chain of chicken processing. Among these stages, slaughtering is a key where microbes can be introduced from slaughterers, the environment, working equipment or washing water. Keeping the process hygiene clean at this stage is critical in ensuring chicken safety for consumers. These include clean hands, equipment like knives and containers, clean water, appropriate sanitiser and a clean working environment like a clean table and surfaces can play a paramount role in chicken safety. However, existing standards and practices are poorly described. This information is needed to understand what is required to improve the situation. In light of this, we conducted a quantitative knowledge, attitude and practices (KAP) survey amongst chicken slaughter points and retailers in Ouagadougou.

1 Urban food markets in Africa: Incentivising food safety using a Pull-Push approach www.ilri.org/research/projects/urban-food-markets-africa-incentivizing-food-safety-using-pull-push-approach.

TABLE 1 Sample size distribution across outlet types.

Market type	Outlet type in the market	Sample size
Outlets with live birds, including those conducting slaughter ($N = 1,331$, 107 randomly selected)	Slaughter place in a market	66
	Slaughter place adjacent to the market (but not in the market)	20
	Roadside slaughter place not in or by a market	5
	Others	16
Carcass outlets ($N = 273$, 48 randomly selected)	Formal shop/supermarket	48
Total		155

2. Methods

2.1. Study design and sampling approach

A cross-sectional survey with stratified sampling was carried out in Ouagadougou, the capital of Burkina Faso. First, chicken slaughter points in Ouagadougou were mapped to create a sampling frame in which the city's main roads were georeferenced, and a route was defined for trained enumerators. The enumerator then surveyed the route and recorded the location of each eligible outlet. At the end of the mapping, 81 live chicken markets and 273 chicken carcass outlets were recorded. The official list of markets in Ouagadougou was obtained from the municipal authorities to capture more outlets providing twenty-five (25) additional live chicken markets, each with many outlets, giving 106 live chicken markets. All markets with less than two sellers and without slaughtering sites were excluded, leaving 50 markets with 1,331 outlets (including those inside and outside markets). In a separate sampling stratum, there were 273 chicken carcass outlets (shops) with no chicken slaughtering. The inclusion criteria for the sampling frame were that an outlet has to slaughter chickens or at least have a slaughtering place or have to trade chicken carcasses. Based on that, a total of 155 outlets were included (Table 1). Of the 155 outlets included, 65 slaughter chickens themselves, 46 purchase carcasses from other outlets and re-sell them, while the rest 44 were involved in live poultry trading. However, these markets have slaughtering points so that chicken buyers can hire slaughterers and get their chickens slaughtered since there are many slaughter teams in a market. Some outlets have a slaughter team (young men) in which the outlet owner is often involved. Sampling points in the city are depicted in Figure 1.

2.2. Survey instrument

A semi-structured questionnaire was delivered using tablets with Open Data Kit by trained enumerators. The survey consisted

of three sections; (1) background information, including demographics, regulatory information and live bird source and trading, (2) process hygiene, including slaughtering, personal and processing practices; and (3) personal knowledge and perception of food safety.

2.3. Questionnaire administration

A list of outlets was developed for each enumerator to visit over 21 days (conducted in June 2021). A list of extra outlets was selected and used to replace those who did not consent to the survey. Slaughter outlets were located using GPS information from the mapping exercise and visited. The questionnaire was administered to individuals working at the outlet knowledgeable about chicken slaughter and carcass handling practices.

2.4. Data management and analysis

The collected data with ODK-installed tablets were stored in a centralized hub and exported to MS Excel (Microsoft, 2021), cleaned and checked before analysis started. Then it was summarized with descriptive statistics using STATA 14 (STATA, 2016) and the summary of results was presented in tables and graphs.

3. Results

3.1. Demographics of participants and regulatory information

Of the 155 participants, 128 (82.6%) were males, and most (104/155, 67%) were owners of the outlets. Only six (3.2%) participants had attended food safety related training (Table 2).

TABLE 2 Demographics of participants and outlet/market characteristics.

Background		*N*	%
Age category	<18	7	4.5%
	19–40	73	47.1%
	41–54	52	33.6%
	>55	23	14.8%
Interviewee role	Owner	104	67.1%
	Employee	32	20.6%
	Owners relative working at the outlet	19	12.2%
Main income source	Livestock keeping	114	73.6%
	Sale of chicken	30	19.4%
	Other	11	7.1%

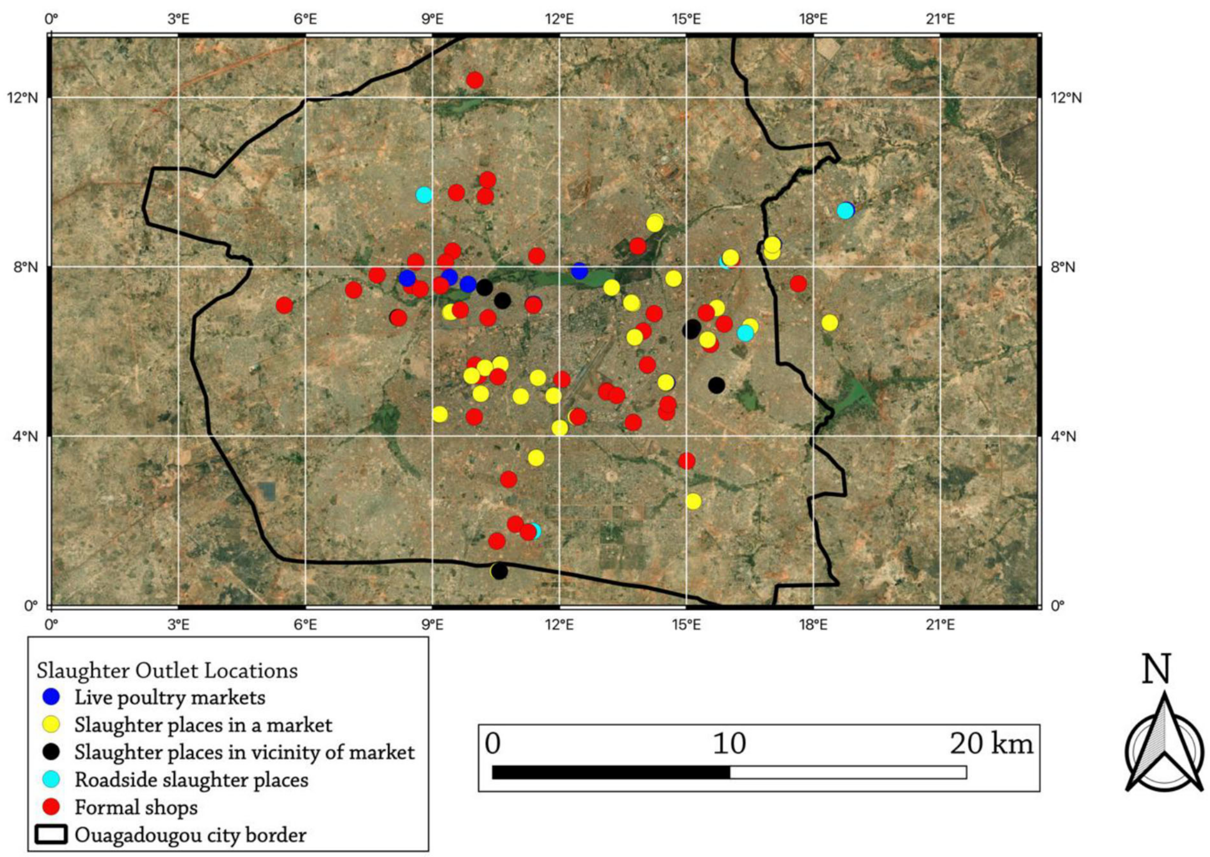

FIGURE 1
Map with an expanded view of Ouagadougou showing locations of sampled chicken slaughter establishments.

TABLE 3 Regulatory information by type of outlet (N = 155).

Variable	Variable category	Outlet type *n* (%)					
		Slaughter place in a market	Slaughter place adjacent to market (but not in the market)	Roadside slaughter place not in or by market	Formal shop/supermarket	Others	Total
Gender	Male	62 (40)	18 (11.6)	4 (2.6)	29 (18.7)	15 (9.7)	128 (82.6)
	Female	4 (2.6)	2 (1.3)	1 (0.6)	19 (12.3)	1 (0.6)	27 (17.4)
Licensed to work	Yes	19 (12.3)	2 (1.3)	3 (1.9)	31 (20)	2 (1.3)	57 (3,648, 31.8)
	No	47 (30.3)	18 (11.6)	2 (1.3)	17 (11)	14 (9)	98 (63.2)
Trained in hygiene and food safety	Yes	3 (1.9)	–	–	1 (0.6)	1 (0.6)	5 (3.2)
	No	63 (40.6)	20 (12.9)	5 (3.2)	47 (30.3)	15 (9.7)	150 (96.8)
Regularly inspected	Yes	12 (7.7)	1 (0.6)	1 (0.6)	27 (17.4)	–	41 (26.5)
	No	54 (34.8)	19 (12.3)	4 (2.6)	21 (13.5)	16 (10.3)	114 (73.5)
Regularly sell cooked chicken	Yes	10 (6.5)		2 (1.3)	1 (0.6)		13 (8.4)
	No	56 (36.1)	20 (12.9)	3 (1.9)	47 (30.3)	16 (10.3)	142 (91.6)
Keep other live animals	Yes	55 (35.5)	9 (5.8)	3 (1.9)	3 (1.9)	10 (6.5)	80 (51.6)
	No	11 (7.1)	11 (7.1)	2 (1.3)	45 (29)	6 (3.9)	75 (48.4)
Keep live chickens for their business?	Yes	61 (39.4)	16 (10.3)	4 (2.6)	3 (1.9)	16 (10.3)	100 (64.5)
	No	5 (3.2)	4 (2.6)	1 (0.6)	45 (29)	–	55 (35.5)

Others are primarily involved in live poultry trading, but they have a slaughtering place near their site so that when a live chicken buyer wants it slaughtered, there are young guys to process the carcass.

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TABLE 4 Slaughter outlet information, keeping live birds and other species.

Variable	Variable category	Outlet type					
		Slaughter place in a market (*n*, %)	Slaughter place adjacent to the market (*n*, %)	Roadside slaughter place not in or by market (*n*, %)	Formal shop/supermarket (*n*, %)	Others (*n*, %)	Total (*n*, %)
Slaughter own chickens	Yes	46, 29.7	12, 7.7	5, 3.2	2, 1.3		65, 41.9
	No	20, 12.9	8, 5.2		46, 29.7	16, 10.3	90, 58.1
Sell fruit or vegetables	Yes	1, 0.6			2, 1.3		3, 1.9
	No	65, 41.9	20, 12.9	5, 3.2	46, 29.7	16, 10.3	152, 98.1
Purchase chicken carcasses for retail	Yes	3, 1.9	1, 0.6		42, 27.1		46, 29.7
	No	63, 40.6	19, 12.3	5, 3.2	6, 3.9	16, 10.3	109, 70.3
Slaughter sick chicken and sell the meat	Yes	34, 21.9	9, 5.8	3, 1.9	2, 1.3	–	48, 73.8
	No	12, 7.7	3, 1.9	2, 1.3	–	–	17, 26.2
Holding pen where birds stay before slaughter has a hard floor	Yes	8, 5.2		1, 0.6	–	–	9, 13.8
	No	38, 24.5	12, 7.7	4, 2.6	2, 1.3	–	56, 86.2
Holding pen ever been washed	Yes	2, 1.3				–	2, 3.1
	No	44, 28.4	12, 7.7	5, 3.2	2, 1.3	–	63, 96.9
Source of carcass purchase	Direct from the farm to you	3, 1.9	–	–	4, 2.6	–	7, 12.7
	From another market	6, 3.9	2, 1.3	–	4, 2.6	1, 0.6	13, 23.6
	From a middle man who has kept them for up to a few days	16, 10.3	2, 1.3	1, 0.6	10, 6.5	1, 0.6	30, 54.5
	From a middle man who brings them directly from farm to you	2, 1.3	1, 0.6	–	1, 0.6		4, 7.3
	Other	–	–	–	1, 0.6	–	1, 1.8

TABLE 5 Information on live bird price, health and transport.

Variables	Minimum	Maximum	Most likely (mean)
Sale price of improved live chicken (XOF)	1,982	3,410	2,328
Journey times for live chickens to reach slaughter outlet in hours	7	22	12.5
Percentage of birds that die during transport or within 24 h of arrival	3	12	6
Percentage of birds that die of disease that you own	2	5	2
Percentage of birds that you own become sick or weak but not die	3	9	5
Number of chickens slaughtered per day	31	106	54
Days chickens stay at outlet before slaughter	1	4.5	2

Only 57 (36.8%) participants held a license to trade chicken or an appropriate health certificate, while the rest were not authorized or licensed to work; also, only 41 (26.4%) reported that the authorities regularly inspected them. Outlets that slaughtered chickens kept live chickens for some time [1–5 days, average (mean) of 2 days] before slaughter. Besides, 80 (36.8%) kept other live animals at their slaughter slab or shop (Table 3), most commonly guinea fowl, turkey and ducks (Supplementary Table 1).

Outlet types were stratified by their involvement in chicken slaughter and trade. Based on this, most participants were from slaughter places in the market or adjacent to the market. Of the total outlets visited, 65 slaughtered chicken themselves, 46 purchase carcasses for retail, while the rest, 44, were involved only in live chicken marketing and hence not included in the slaughtering section (Table 4).

Sixty percent of the outlets transported chickens to the place of slaughter by motorbike, with tricycles and wooden cages used by 32 and 30% of outlets, respectively. The mean price of a live bird sold by an outlet is 2,328 XOF, equivalent to 3.5 USD (1 USD = 664.56 XOF as of September 2022 exchange rate); chicken sale prices varied between 1982 XOF to 3410 XOF (Table 5). The journey time from the bird's place of purchase to the outlet where it is slaughtered takes more than 12 h on average (mean) (7 h on the minimum and 22 h on the maximum) with a mean travel distance of 100 km. On average, 6% of the birds die during transport or within 12 h of arrival at their market and more than 3% of the birds become weak or sick after arrival (Table 5). Chickens typically stay at the outlet for 2 days before slaughter. From the 155 outlets, 65 (42%) slaughter chickens themselves, while 46 (30%) obtain carcasses from other slaughter slabs. The rest, 44 (28%), were involved only in live chicken trading but not slaughtering and carcass trading. On average, they slaughter 54 chickens per day (31–106). The majority (73.8%) (48/65) of outlets slaughter birds even if they are sick. They use various medicines to treat sick birds. Among the common medicines, antibiotics 30% and vitamin 10% are their favorites to treat sick birds (Table 6).

3.2. Process hygiene (hygienic practices from slaughter to sale of chickens)

3.2.1. Chicken slaughter

Sixty/sixty-five (92%) of the participants slaughter chickens on a bare earth floor (Table 6). Most slaughter slabs bled birds on the floor or in barrel containers (Figure 2A). The bleeding surfaces were mainly made of metal, plastic, or bare earth (Figure 2B). Most respondents said they dispose of blood and blood washings on-site or throw them away on the floor without proper disposal.

3.2.2. Scalding, plucking and evisceration of chickens

On average, 33 birds use the same scalding water, while seven birds are scalded at once in the scalding tank. The scalding water is changed once a day at most outlets. Outlets estimated that 25 L of water is used in the scalding tank; on average, nearly 20 outlets share the slaughtering site. Most of the respondents use a table as a plucking surface 48 (73.8%), which is made of wood 53 (70.7%) (Table 6). After removing the feathers (plucking), most slaughters (23%) dispose of them in a bin or dump them, e.g., on the street or in the market (20%) or a combination of the two (17%) (Supplementary Table 3).

3.2.3. Cross-contamination sites

After plucking, the next stage in carcass processing is evisceration. Most respondents (49%) do pluck and eviscerate in the same place; scalding and plucking were done at the same location in 12.3% of outlets. Among the concerning practices reported in this study was that 50% said that they just drop solid slaughter and processing waste on the ground, followed by outside the market and down the drain (Table 7).

3.2.4. Equipment, hand washing, and waste disposal

Most 22/65 (33.8%) of the respondents wash knives at the beginning of slaughter and the end of the day, while some 6/65 (9%) wash after each batch of chickens. A quarter of slaughterers wash their hands at the end of slaughtering, with fewer washing at the start (16%) (Supplementary Table 3). Most respondents (31%) use tap water to wash knives, followed by carcass rinse water (15.4%) and then store wash water (17%). Most respondents (52%) said they throw solid slaughter waste outside the market (not in a specified waste disposal site). Only seven (11%) prepare the waste material to be taken by waste collection services (Table 7).

3.2.5. Carcass purchase and sale

3.2.5.1. Carcass purchase

Sixteen percent of outlets sell frozen chicken. For shops that purchase, freeze and resell carcasses, the mean transport time from

TABLE 6 Slaughtering, plucking and surface type and materials used to construct the surface ($N = 65$).

Surface type per slaughter stage	Materials to make the surface	N (%)
Slaughter surface type	Floor	60 (92)
	Table and other	2 (3)
	Table	1 (2)
	Other	2 (3)
Materials used to make the slaughter surface	Bare earth	50 (77)
	Others	8 (12)
	Wood	4 (6)
	Non-corrosive metal (stainless steel or aluminum)	1 (2)
	Bare earth and Wood	1 (2)
	Non-corrosive metal	1 (2)
Chicken plucking place type	Floor	48 (73.8)
	Wood	4 (6)
	In a basin	4 (6)
	Table and other	3 (4.6)
	Table, in basin and floor	3 (4.6)
	Other	3 (4.6)
Material types used to make plucking surface	Wood	52 (80)
	Metal	7 (11)
	Plastic	4 (6)
	Bare earth	2 (3)
Evisceration place type	Table	54 (83)
	Table and a basin	4 (6)
	Basin	3 (4.5)
	Other	3 (4.5)
	Floor and table	1 (1.5)
Material types used to make evisceration surface	Wood	54 (83)
	Others	4 (6)
	Metal and plastic	3 (5)
	Plastic and wood	2 (3)
	Plastic	2 (3)
Medicines use to treat sick birds ($N = 100$)	Antibiotics only	22 (22)
	Vitamin	4 (4)
	Antibiotic and antiparasitic	4 (4)
	Antibiotics and vitamin	4 (4)
	Do not use medicine	66 (66)

place of purchase to their shop was 30 min. On average, they sell 77 chicken carcasses per week. They keep frozen chicken carcasses for about 35 days before selling them (Table 8).

Only 22% said carcasses are individually packed when receiving them (Table 9). They use plastic bags followed by sacks and cardboard boxes to transport carcasses to households and street restaurants ("maquis") (Figure 3A).

Only 40% of the participants said they washed carcasses before selling. Most respondents put the carcass on a wooden table before and after washing. They use the main water supply to wash the carcass. Carcasses are typically stored in refrigerators or freezers if kept for a prolonged period (37 and 35%, respectively). However, they keep fish and other meat together with the chicken carcass in the same refrigerator/freezer, and power cuts are frequent (Table 9).

3.2.5.2. Carcass sale

Households are the leading customers of fresh chicken carcasses. Local breeds are the most commonly sold frozen chicken type, followed by imported broilers and locally reared improved breeds. Furthermore, households are the leading buyers of frozen chicken, followed by restaurants. Most (70%) of outlets said there is no refrigerated transportation mechanism for carcasses; 54% of the respondents said that carcasses come in contact with other carcasses during transportation (Table 9).

3.2.5.3. Hygiene at retail shops

Out of the 46 carcass retailers, 17 (37%) wash carcasses immediately after receiving them, washing on-average 20 carcasses at a time. The majority (61/155, 39.5%) of the respondents frequently wash their hands with soap. However, 3% of participants admitted they never or rarely wash their hands (Figure 3B).

3.2.6. Contamination

There were rats, cats and dogs roaming around certain outlets searching for food; from roadside outlets, 40/155 (26%) reported rats, 61/155 (39%) reported cats, and 47/155 (30%) reported that most of the time dogs will come close to food preparation surfaces. However, only 17, 17, and 10% have control measures for these pests, respectively. Poisons are the most common control method, followed by traps and other approaches like covering the carcasses tightly to prevent access. Also, they use fly sprays and other insect killers (Table 10). However, slaughter places in the market were free from rats, cats and dog visits, with zero reports of these animals from the informants (Supplementary Table 4).

Interviewers observed several animals during their visits. Among the animal species observed, sheep, cats, dogs, and goats were most often seen wandering around the outlets. Most informants had access to toilets and water, often in the market or a public toilet; 63% reported the toilet was adequate and 85% reported they had access to water at the toilet (Table 10).

3.3. Food safety perception and knowledge

Ninety percent of interviewees stated that they had not heard of food poisoning incidents from chicken consumption and knew that temperature is important for the safety of carcass storage. Furthermore, 98% know water hygiene is important for food safety (Figure 4).

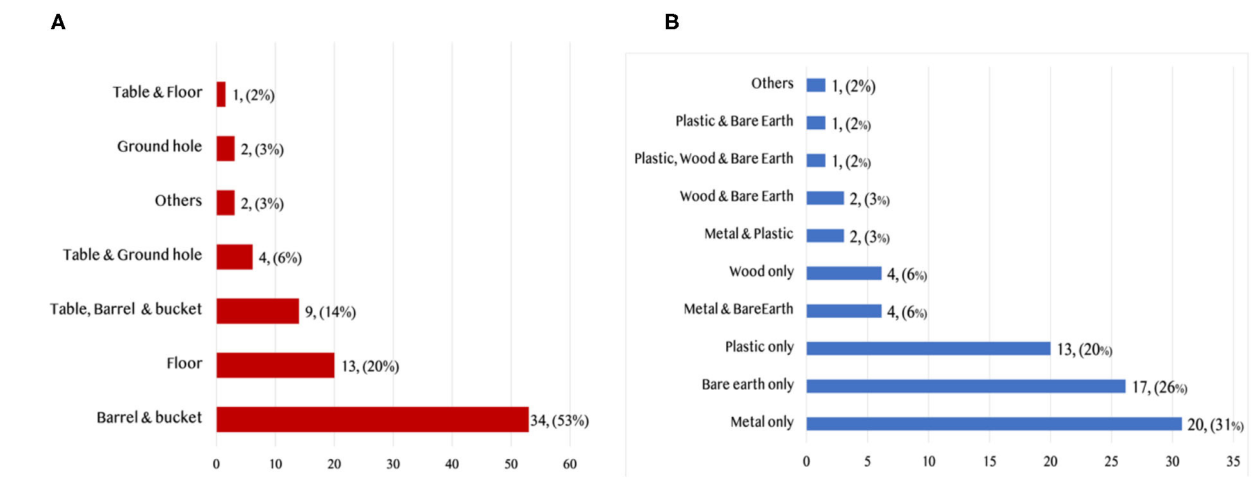

FIGURE 2
(A) Chicken bleeding surface types (N = 65), **(B)** materials used to make the bleeding surface (N = 65).

TABLE 7 Cross-contamination possibilities through multiple steps performed at the same point, equipment washes and waste disposal methods (N = 65).

Questions	Response categories	*N* (%)
Cross-contamination (activities done at the same point)	Plucking and evisceration	32 (49)
	Scalding and plucking	8 (12)
	Scalding and evisceration	6 (9.2)
	Scalding, plucking and evisceration	19 (30)
Solid slaughter waste disposal places	Outside market	34 (52)
	Taken by waste collection service	7 (11)
	Throw outside market or taken by waste collection service	5 (7.7)
	Throw in the market	3 (5)
	Throw in pond or lake	2 (3)
	Combination of all the above	14 (21)

TABLE 8 Information on frozen carcass transport and selling.

Variables	Most likely	Minimum	Maximum
Carcass transport time in minutes	32	26	64
Number of carcass purchases per week	77	50	122
Carcass storage days before selling	36	11	82

4. Discussion

Chicken is a highly consumed animal source of food in Ouagadougou, Burkina Faso. However, the hygienic practice in slaughtering outlets and carcass shops is very poor (Figure 5). Among the risky sanitary practices and perceptions observed in this study, most participants were not licensed to work and were not regularly inspected by authorities; almost all workers have no training on food safety and related topics. Most outlets keep live chickens and other birds like guinea fowl, turkey, and ducks in and around outlets. Also, rodents and other mammals, such as cats, dogs and goats, many stressed and in poor health, are near food preparation areas creating a favorable environment for disease transmission. The house where birds stay before slaughtering is rarely washed or sanitized, exacerbating risks of transmission of zoonotic disease pathogens like avian influenza that could affect people and birds. The unhygienic handling practices in live markets before slaughtering can be a hotspot for the possible emergence of new pathogens that can cause local or global pandemics unless there is improvement in housing, marketing and transportation of birds in the city.

Various studies in chicken slaughtering, processing, and consumption in sub-Saharan African countries indicated below-standard chicken handling practices, in line with this study. Ovai et al. (2022) reported in Gahanna that consumers transport chicken meat with other items, store it in the same refrigerator, and have low hand and equipment washing habits. Birgen et al. (2020) have also discussed that chicken consumers in Kenya had a medium hygienic practice level. Also, many similar unhygienic practices were reported in street-vended chicken, and loads of bacterial contamination of chicken carcasses were found to be high due to poor handling practices. Also, the presence of pests and flies, contaminated vending places, lack of appropriate clothing and the use of unclean water to wash carcasses are the main malpractices that are causing cross-contamination in chicken slaughtering, vending and consumption chains (Hessel et al., 2019; Mpundu et al., 2019; Musawa et al., 2020).

Most slaughter outlets get chickens from local markets. On average, chickens travel more than 12 h to reach the city's market. During transportation, a large number of birds die due to stress-associated diseases. The mode of transport of the chickens to the slaughter point with birds hung together from motorbikes or other vehicles not only stresses the birds but also contributes to increased pathogen shedding and animal suffering; the close contact between the birds increases cross-contamination with fecal microbes such as *Campylobacter* species (Neri et al., 2019). Also, long-distance

TABLE 9 Carcass storage, packaging, transport means and storage surfaces before and after washing and storage facilities.

Items/questions	Response category	*N*	%
During transport, carcasses are packed in	Plastic bag and Sac	13	28.3
	Plastic bag	8	17.4
	Cardboard box	6	13.0
	Others	5	10.9
	Sac and card box	4	8.7
	Sack	3	6.5
	Plastic bag and card box	3	6.5
	Sack and plastic box	1	2.2
	Sac plastic box and card box	1	2.2
	Sac and other	1	2.2
	Plastic box and other	1	2.2
Carcasses are individually packaged when you receive them	Yes	10	22
	No	36	78
Outlet sells frozen chicken	Yes	25	16
	No	40	84
Wash chicken carcasses after purchasing them	Yes	18	39
	No	28	61
Carcasses placed temporarily before washing	Table	45	54.2
	Container	26	31.3
	Table and container	9	10.8
	Other	2	2.4
	Floor	1	1.2
Carcasses placed temporarily after washing	Container	35	42.17
	Table	28	33.73
	Floor and table	13	15.64
	Floor	2	2.41
	Floor and other	2	2.41
	Table and others	2	2.41
	Other	1	1.2
Carcass washing water sources	Main water supply	79	89.8
	Borehole	4	4.5
	Rainwater collection	3	3.4
	Spring/well	2	2.3
Chicken carcass storing facilities	Refrigerator	17	37
	Freezer	16	35
	Refrigerator and freezer	12	26
	Refrigerator, freezer and open-air at ambient temperature	1	2
Other foodstuffs stored with chicken	Fish	20	76.9
	Other meat	4	15.4
	Other	2	7.7

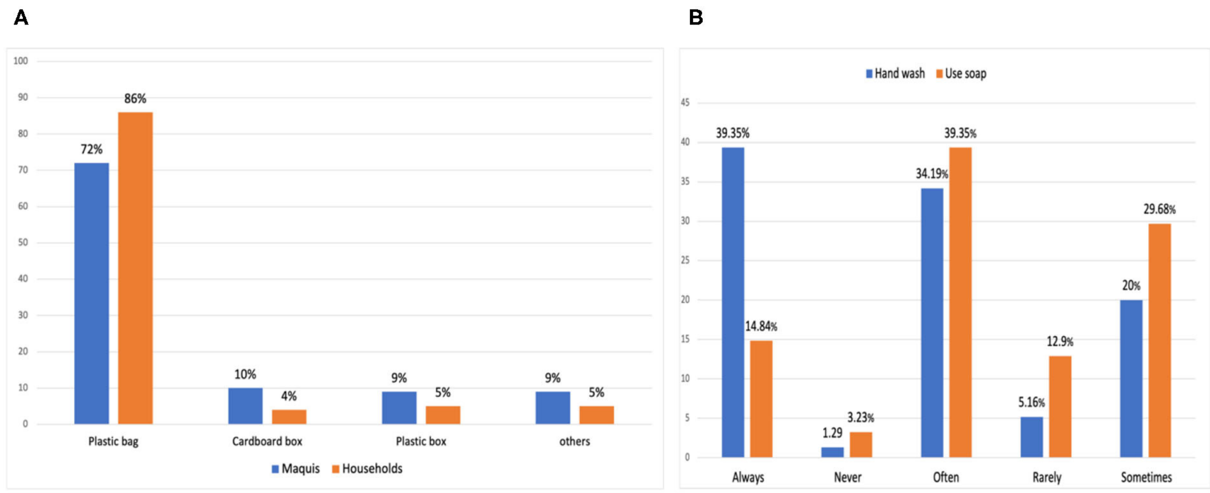

FIGURE 3
(A) Percentage of carcass carrying materials during transport to deliver to households and restaurants/maquis (n = 65). **(B)** How often do respondents wash their hands (blue) and use soap (orange) during the working day (N = 155).

transportation is associated with an increased mortality rate of birds (Caffrey et al., 2017; Dos Santos et al., 2017, 2020). Around 6% of the birds die during transport or within 24 h of arrival. To have an intuitive of the loss due to death, we used a simple calculation to estimate the amount of the monetary loss in the city alone. According to Somda et al. (2018), 50,000 chickens are consumed daily in Ouagadougou. Eight per cent loss of this amount is around 3,000 chickens with an average estimate of chicken price of 2,328 XOF per bird; the daily loss in the city is XOF 7 million per day, equivalent to more than four million USD per year that instead have to contribute to the national economy. Not all of this loss is preventable—but it can easily be reduced with better transport, which will also reduce cross-contamination of birds with FBD pathogens like non-typhoidal *Salmonella* and *Campylobacter*, reduce stress which precipitates pathogen shedding and improve animal welfare.

If a bird is diseased during transport, they administer medications like antimicrobials, vitamins and antiparasitic drugs at the site. Inappropriate antimicrobial use without considering the withdrawal period poses a significant public health risk in terms of drug residue. Studies indicated that tetracyclines, aminoglycosides and fluoroquinolones are commonly used antimicrobial classes to treat various diseases in poultry in sub-Sahara Africa (Azabo et al., 2022). Various organisms that have veterinary and public health importance have been reported to develop antimicrobial resistance to these drugs mainly due to irresponsible use (Economou and Gousia, 2015). There were numerous diabolical practices with little to no concept of hygiene. Many outlets slaughter and sell sick chickens, a practice that poses significant risks to consumers. Food preparation surfaces were not food-grade; if they were cleaned, mostly they were not, they would still harbor high microbial loads. When there is cleaning, they rarely use detergents. Tap water alone will not eliminate microbes at an acceptable level. Strong detergents that have low residual risk to public health, like Lactic Acid, Acetic Acid, and Trisodium Phosphate sprays, must be used to effectively kill most pathogens from the surface, utensils and meat (Sallam et al., 2020; Nkosi et al., 2021).

Almost all of the tables used to slaughter, bleed, scald, and eviscerate chickens were made of either bare earth or wood. Many stages of slaughter and dressing, such as plucking and evisceration, were conducted in the same place or by the same person, with no cleaning of surfaces, equipment or hands. Slaughtering and dressing are the main sources of contamination (Althaus et al., 2017; Emanowicz et al., 2021). Plucking, which is removing the feather, can contaminate the worker's hand and, when done together with evisceration, can contaminate the carcass. Scalding and other activities should be segregated from evisceration to reduce cross-contamination because most contamination with pathogens like *Salmonella* spp., *E. coli* and *Campylobacter* species are introduced at the evisceration stage (Mpundu et al., 2019; Zeng et al., 2021). On average, twenty outlets share the same scalding water. Mixing different chickens will promote cross-contamination between birds, and the non-standardized approach to heating the scalding water would not ensure a consistently high temperature that would inactivate microbes. Certain disease causing agents are known to spread in scalding tanks unless additives are used to sterilize the water (Projahn et al., 2018; Mpundu et al., 2019). Scald additives like sodium hydroxide significantly reduce the bacterial load by creating an unsuitable alkaline environment in the tank, which must be practiced in slaughtering outlets visited and elsewhere (McKee et al., 2008). Most workers wash their hands and knives at the beginning of the day with only tap water, contributing to massive cross-contamination. Given the tenacity of some microbes, tap water alone cannot eliminate all pathogens unless standardized detergents are used. There was also an issue of environmental contamination with blood and feathers dumped near the site or in the market, exposing the public and animals to diseases and attracting vermin. Humans and free-rooming urban livestock can get infected from the inappropriately disposed waste and keep the disease transmission cycle alive (Cook et al., 2017).

TABLE 10 Carcass contact, frequency of using refrigerator, animal control methods, and animals observed during the interview.

Questions	Response categories	*N*	%
Carcasses' physical contact with other carcasses	Never	3	12.5
	Rarely	2	8.3
	Sometimes	2	8.3
	Often	4	16.7
	Always	13	54.2
Carcasses refrigerated during transport	Never	17	70.8
	Rarely	2	8.3
	Often	3	12.5
	Always	2	8.3
Animal control method			
Rat ($n = 27$)	Rat poison	14	51.9
	Trap	10	37
	Other	3	11
Cat ($n = 27$)	Rat poison	13	48
	Trap	9	33
	Other	5	18.5
Dog ($n = 17$)	Rat poison	3	23.
	Trap	3	23
	Other	7	53.85
Fly ($n = 36$)	Fly spray	15	41.7
	Other insect killers	19	52.8
	Fly tape	2	5.6
Animals observed during the interview wandering around the outlet	No animals observed	69	44.5
	Sheep	21	13.6
	Cat and dog	18	11.6
	Dog	8	5.2
	Goat	8	5.2
	Cat	7	4.5
	Sheep and goat	8	5.2
	Cat, dog and pig	3	2.6
	Wandering hens	3	1.9
	Combinations of the above animals	9	5.8
Toilet place used by the outlet workers	Market or public toilet	63	41
	Others private toilet	33	21
	Private toilet at stall/shop	29	19
	Outside (open air)	14	9
	Another private toilet	14	9
	Private or public	2	1
Action when a worker has stomach upset (e.g., diarrhea)	Do not go to work	52	34
	Wash hands more thoroughly	45	29
	Wash hands more thoroughly and minimize handling of produce	28	18

(Continued)

TABLE 10 (Continued)

Questions	Response categories	N	%
	Do not go to work, wash hands more thoroughly and minimize handling of produce	22	14
	Minimize handling of produce	6	4
	Do not go to work and other response	2	1
Toilet reported as adequate	Yes (%)	98	63
	No (%)	57	37
Water is available	Yes (%)	132	85
	No (%)	23	15
Have to pay for toilet	Yes (%)	78	50.3
	No (%)	77	49.7

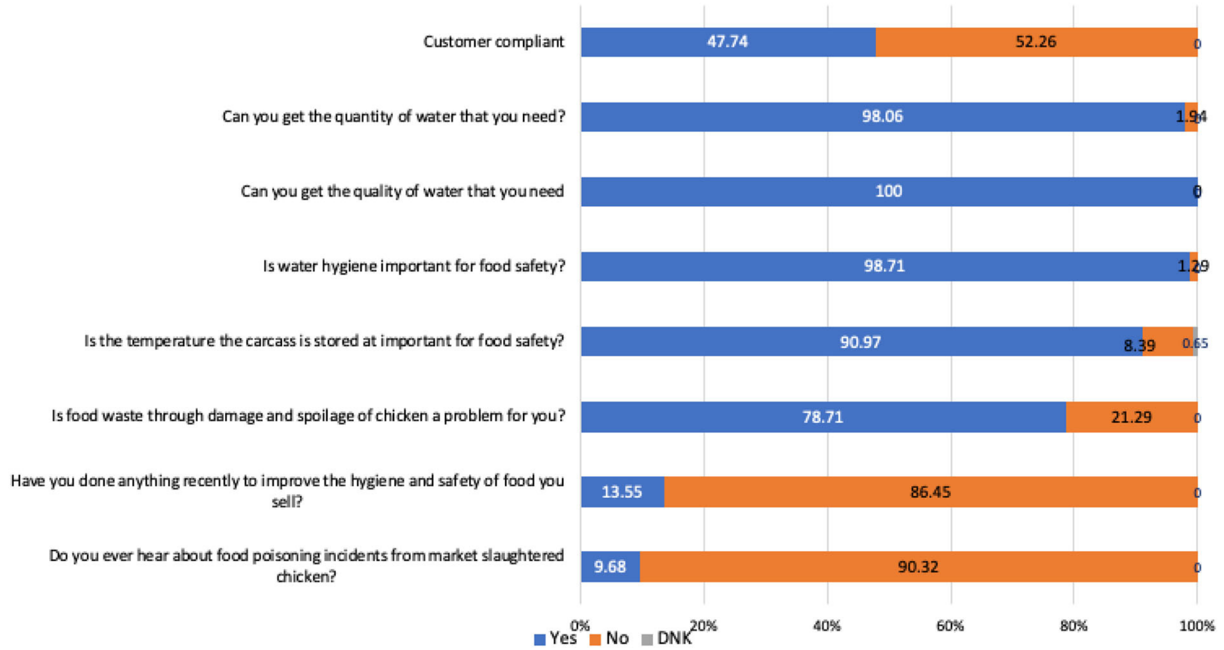

FIGURE 4
Knowledge and perception of participants on food safety.

From chicken carcass shops visited, a limited number of the outlets receive individual-packed carcasses, while most receive carcasses mingled. After receiving, less than half of the outlets wash before selling, contributing to cross-contamination between carcasses from single or multiple sources. They keep the cold chain by storing the carcass in refrigerators and freezers but with other foodstuffs, which can cause cross-contamination among foods. Even though cooking may kill most organisms, cross-contamination will happen without proper storage protocol. Rodents and pests could access food storage and preparation areas. Unhygienic and unmanaged places are likely to be visited by animals and insects carrying nasty zoonotic pathogens. The first line measure must manage the outlet by making it neat and hygienic, which is unfavoured by rodents and flies. However, they prefer to use unsafe approaches like poisons and insecticides, increasing the public health risk with active poisoning and long-term residual effects.

Although many described toilets as adequate, they are, in fact, little more than holes in the ground that are not cleaned, and given that most have to pay to use these toilets, open excretion is likely. Clean water with detergents is definitely among the easiest yet most effective measures to reduce foodborne diseases, but water is not available in most toilets, contributing to the poor hygiene of the working environment of the outlets. 34% do not go to work when workers feel sick, which is good practice. However, a significant number (29%) continue to work, posing risks to consumers through contamination with pathogens the sick worker may be shedding, which is exacerbated by the shortage of clean water in most outlets.

Even though most food safety practices were exceptionally poor, participants had some knowledge of basic food safety and hygiene requirements. They understood the importance of the cold chain, although carcasses were often kept at ambient temperatures. Chicken eaten in restaurants is typically thoroughly cooked, killing foodborne

FIGURE 5
Typical carcass processing work environment. (As it can be observed from the picture, the working area has substandard hygiene levels; worker is not properly dressed (at least no gloves, shoes and mask), and the table is unclean and messed with different equipment and garbage. The washing water is not changed with an imminent probability of contaminating the carcasses. The ground is polluted and contaminated with visible dirt).

microbes. Given the poor upstream hygiene, this microbial kill-step before consumption is critical. However, massive microbial loads will be introduced into the food preparation area on the carcass, contaminating other foods stored or prepared with the chicken, such as salads commonly eaten with chicken. Cooked carcasses may also become re-contaminated through unwashed hands, utensils and surfaces. This is a problem in homes, where preparation practices will be highly variable, and children, the most vulnerable to foodborne disease, routinely eat at restaurants, where food poisoning can impact many customers.

Low income and lack of resources could account for the poor standards. However, some improvements could be made with little to no investment, such as processing in sequential, linear steps with separation of clean and dirty areas and wiping clean surfaces. However, the impact of such measures on food safety may be limited, given the lack of food-safety prerequisites, such as clean water, pest control, and clean and cleanable environments. Given that preventing cross-contamination of cooked and ready-to-eat food prepared at home and in restaurants and adequate cooking of meat are the main control points that will limit the impact of poor hygiene upstream, it is advisable to promote awareness of measures to ensure appropriate practices at these control points. The focus on reducing this risk is not on what the average person does but on reducing the percentage that does not apply appropriate measures or utilize particularly hazardous approaches.

5. Conclusions

Massive and preventable loss of birds was observed, through poor handling and transport, resulting in undue economic loss for the outlet and supply chain, and appalling animal welfare, in turn exacerbating zoonotic disease risks, including foodborne. Concerning food safety, standards are so poor and infrastructure so limited that major improvements would require massive investment to improve facilities and train slaughterers and other value chain actors, combined with an appropriate level of regulation. There are, in fact, many stages that intervention can be done to safeguard consumers from foodborne illnesses. The easiest and possibly cheapest measure can be awareness creation for workers because most faulty practices can be reverted with simple educational campaigns. Importantly there needs to be much greater consumer awareness of the dangers of eating unhygienically prepared food. This may create consumer demand for safer food, with market forces beginning to drive improvements and sustaining investments in improved food safety and infrastructure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

AA: cleaned the data, performed analysis, and wrote the manuscript's draft. MD: developed survey tools and supervised the surveys. GI: trained enumerators and supervised and conducted the surveys. VL: trained enumerators and conducted the surveys. BG: participated in data analysis and commented on the first draft of the manuscript. DG: conceptualized the project. TK-J: conceptualized and supervised the overall project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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State of the art of breeding, milking, and milk processing for the production of curdled milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products

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Introduction: This study aimed to identify the factors favoring the contamination of raw cow's milk, curdled milk, and *Wagashi Gassirè* cheese during their production and preservation in order to develop strategies to improve their quality.

Methods: A cross-sectional survey of 401 randomly selected stakeholders encompassing all levels of the dairy production chain in the Nikki and Dassa-Zoumé communes of Benin was conducted. The data obtained were analyzed using the SAS software for the calculation of frequencies and the R software for classifying the stakeholders based on the hygiene practices they adopted during the production and conservation of raw cow's milk, curdled milk, and *Wagashi Gassirè*.

Results and discussion: The study identified three types of dairy farmers based on how they medically treated their cattle and implemented hygiene practices, including farmers who (1) relied on themselves or received help from veterinarians trained in animal husbandry and milking to monitor the animals on their farms; (2) relied only on veterinarians; and (3) relied only on themselves. The majority of these dairy farmers felt that hygienic milking practices were very restrictive and difficult to implement. In addition, three groups of *Wagashi Gassirè* producers were identified: (1) producers trained in good hygiene practices who did not boil or sundry the cheese; (2) producers lacking the infrastructure to protect from weather exposure who used all parts of Calotropis procera for colored *Wagashi Gassirè* production; and (3) producers who did not often filter the milk and boiled the *Wagashi Gassirè* in bags before immersion in simple water or whey. The sanitary quality of milk and milk products is influenced by the diverse handling practices employed by producers. These practices must be considered according to the types of farmers and processors when suggesting improved intervention policies.

KEYWORDS

milk, cheese, hygiene, quality, Benin (West Africa)

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1. Introduction

Like most West African countries, Benin's economy is based on agricultural production (Okry et al., 2017). The agricultural sector employs about 70 percent of the labor force, contributes nearly 23.3 percent to GDP, and provides about 75 percent of export earnings and 15 percent of government revenue (MPD, 2022). This sector is considered to have numerous potentialities that must be judiciously exploited to support national economic growth and thus contribute to effectively fight poverty. Most importantly, agriculture ensures Benin's food security (MAEP, 2017). In the agricultural production sector, livestock occupies a predominant place and contributes to the livelihood and food security of the population (Sounon et al., 2019). In 2012, the live-stock subsector, which plays an important role in the incomes of northern Beninese people, generated a revenue of USD 180.6 million (2.4% of GDP). In 2013, the national livestock population was estimated to be 4.6 million head, including 2.1 million cattle and 2.5 million small ruminants (MPD, 2022). However, the output of this subsector is plagued by the strong influences of various constraints, such as climatic, nutritional, sanitary, and genetic factors, that are at the root of major problems, including the low productivity of livestock (Soule et al., 2017). To overcome these obstacles and increase production, the government has implemented a series of projects including the Milk and Meat Sector Support Project (PAFILAV), which is part of the Growth Strategy for Poverty Reduction and the implementation of which has helped to increase production in the milk industry [Gouvernement du Bénin, 2022a; Groupes de la Banque Africaine de Développement (GBAD), 2022]. Indeed, the average number of lactating cows in Benin increased from about 495,000 in 2011 to more than 580,000 in 2015, and milk production increased from ∼102,000 tons in 2011 to 113,000 tons in 2015 (FAO, 2020). To boost milk production, the Government of Benin through its actions program for the period from 2021 to 2026, under pillar 2 relating to the pursuit of the structural transformation of the economy has planned to increase milk production through the development of the milk sector and the promotion of livestock farming enterprises (Gouvernement du Bénin, 2022b). With this program, the livestock sector is flourishing and interest in milk production has increased [Groupes de la Banque Africaine de Développement (GBAD), 2022]. Although milk production is increasing and makes a significant contribution to economic growth and to the food and nutritional security of the population, the marketed and sanitary quality of the milk is not always satisfactory and milk spoils rapidly under the influence of high environmental temperature in Benin (Sessou et al., 2013). To circumvent its quick and high adulteration, milk produced in Benin is often transformed into curdled milk and *Wagashi Gassirè* cheese using traditional processes. These products are important sources of protein and other essential nutrients and could help to eliminate protein deficiency in low-income populations (Dossou et al., 2016). They are part of the dairy products recognized worldwide as excellent sources of nutritional components (proteins, minerals, vitamins, lipids, and carbohydrates) and important part of the human diet (Verruck et al., 2019). Raw cow milk as well as curdled milk obtained *via* the spontaneous fermentation of raw cow milk are often consumed without any treatment by people in Benin in order to benefit from their nutritional elements (Farougou et al., 2012; Boko et al., 2016; Sessou et al., 2019). The traditional cheese *Wagashi Gassirè* is one of the most widespread dairy products in both rural regions and big cities and is widely appreciated by consumers in the Republic of Benin. It is also produced and consumed in many other West African countries, including Burkina Faso, Ghana, Nigeria, and Togo (Dossou et al., 2016; Zannou et al., 2018; Sessou et al., 2019). While these milk and traditional dairy products are enjoyed by consumers, they are also important in contributing to the nutritional health status of the population. In contrast to these potential benefits, it should also be recognized that these dairy products are perceived to be a major public health risk. Indeed, the practices generally used for the production and preservation of milk and milk derivatives do not always ensure their sanitary and nutritional quality. Contamination, particularly microbiological contamination, which leads to poor sanitary, nutritional, and marketable qualities, is commonly observed (Sessou et al., 2013). Since the demand for these pastoral dairy products, including *Wagashi Gassirè* and curdled milk, in local markets is increasing (Chabi Toko et al., 2015), it is essential to develop strategies to provide consumers with products of high sanitary and nutritional quality. Designing efficient strategies to improve the quality of these products requires knowledge of the different production and preservation practices adopted by all stakeholders in the milk production chain. Here, with this perspective, we analyzed the production and preservation practices that can cause the contamination of milk and milk products in two Beninese communes, Nikki and Dassa-Zoumé.

2. Materials and methods

2.1. Survey of dairy farmers, dairy producers, and consumers in the Beninese milk sector

A cross-sectional survey of dairy farmers, dairy producers, and consumers of the milk sector in the Beninese communes of Dassa-Zoumé and Nikki was conducted between May and July 2020 (Figure 1). The choice of these two areas was done since they are among the main cattle-breeding areas located in the center and north of Benin, respectively. A total of 401 participants, including cattle dairy farmers, dairy producers, traders, and consumers of curdled milk and *Wagashi Gassirè*, were surveyed (Table 1). The producers of fermented milk and cheese were predominantly women. Data were collected through guided interviews with each randomly selected participant using a structured questionnaire. This random approach was used as described in our previous paper (Dossou et al., 2022). The structured questionnaire was implemented using the Epicollect5 software. Field information concerning the hygienic practices implemented at all steps, i.e., milking, the production of cheese and curdled milk, preservation, and marketing, was obtained. Different questions specific to the production step were also asked. For instance, dairy farmers were asked questions regarding the hygiene measures implemented during milking and the therapeutic interventions used to treat sick cattle. The questionnaires included a list of various diseases in the local Fulani language; the questions and the answers were translated to French by trained veterinarians. The questions asked to dairy producers, traders, and consumers identified the existing production conditions and the product preservation methods being employed. During the study, the participation of respondents was voluntary, and participants provided informed consent before attempting the questionnaire (Dossou et al., 2022).

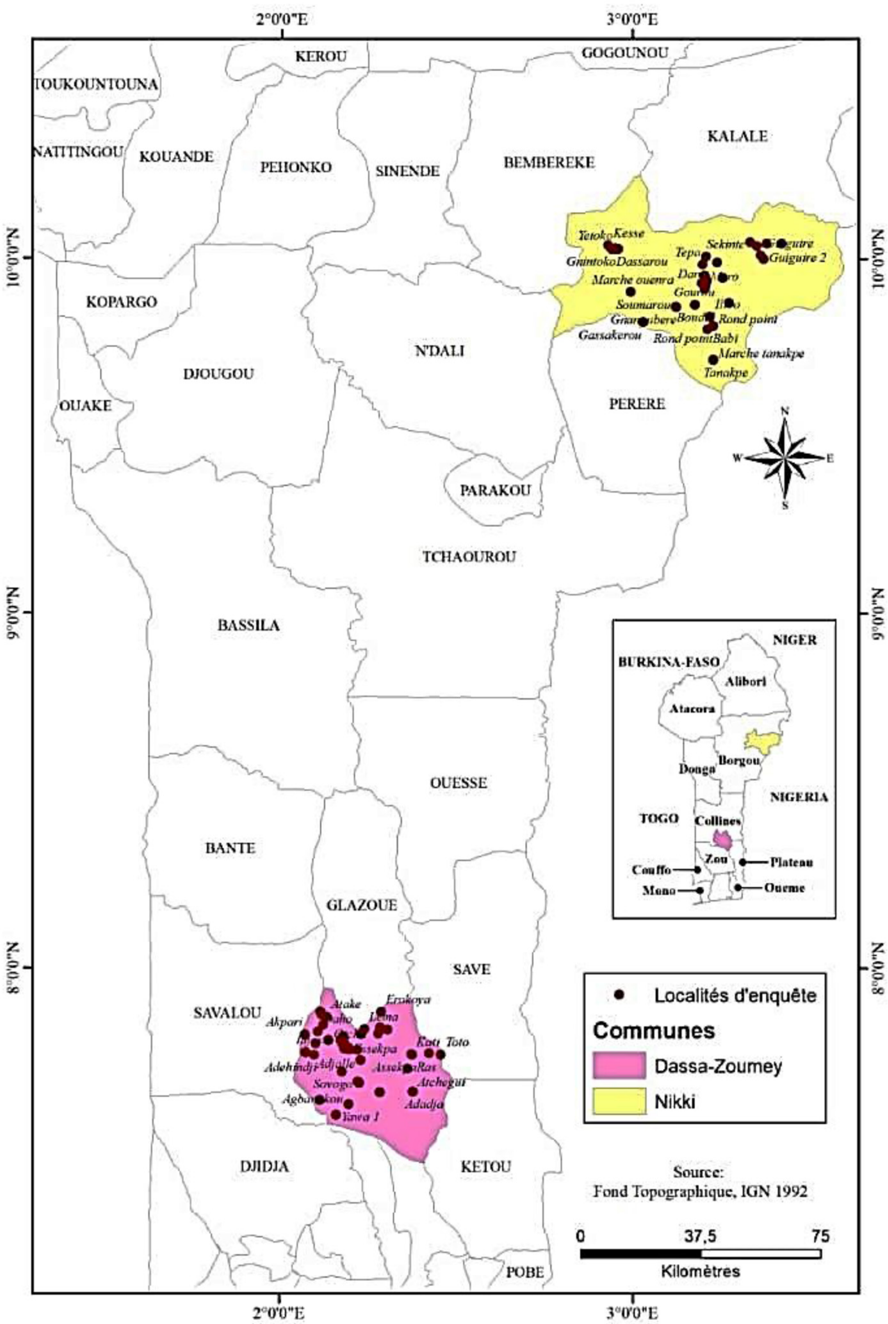

FIGURE 1
Map of the two study areas: Nikki in northern Benin and Dassa-Zoumé in central Benin.

2.2. Statistical analysis

The collected data were analyzed using the SAS (SAS, 2013) and R4.0.2 software. The PROC FREQ procedure of SAS was used to calculate frequencies, and the Chi² test to specify the significance of the common factor for the variables considered. For each relative frequency, a 95% confidence interval (CI) was calculated using the following formula:

$CI=1.96\sqrt{([P(1-P)]/N)}$, where P is the relative frequency and N is the sample size.

The multiple correspondence analysis (MCA) function of R's FactoMineR library was used for multiple correspondence analysis (Husson et al., 2017; Cornillon et al., 2018) on a contingency table crossing the individuals in row and the variables in column. The variables considered are:

- For farmers: application of sanitary measures, implementation of hygienic measures during milking, hygienic measures observed during milking, hygiene training, availability of processing materials.

TABLE 1 Distribution of respondents according to stakeholder category in each commune.

Stakeholder category	Commune		Total
	Dassa-Zoumé	Nikki	
Dairy farmers	54	30	84
Wagashi Gassirè producers	90	75	165
Curd producers	02	09	11
Traders of *Wagashi Gassirè* and curd	35	18	53
Consumers	56	32	88
Total	237	164	401

- For *Wagashi* producers: ethnicity, marital status, hygiene training, boiling and drying practices, immersion in whey, coloring of cheese, dye used, preservation method, filtering of milk, coagulant used.
- For traders: ethnicity, equipment, preservation methods and situation of sale point.
- For consumers: level of education, level of satisfaction and preservation methods. A hierarchical ascending classification (HAC) of the MCA components was then performed using the hierarchical clustering on principal components (HCPC) function. Stakeholder categories were then identified and characterized. The correspondence analysis (CA) function in R's FactoMineR library was used for factorial correspondence analysis (FCA) (Husson et al., 2017; Cornillon et al., 2018) on a contingency table crossing individuals in a row with variables in the column. The variables considered for the FCA are the level of education, ethnicity, and level of training on hygiene of the actors. The individuals are represented by the breeders, traders, *Wagashi* producers and consumers.

3. Results

3.1. Sociodemographic characteristics of the surveyed actors in the milk sector

The responses showed that milk farming is practiced exclusively by men (100%), the majority of whom are married and belong to the Fulani (87%) and Gando (13%) sociocultural groups in the two communes. Cattle rearing is therefore primarily carried out by the Fulani in the two communes surveyed. Overall, 93% of farmers were uneducated (91% in Dassa-Zoumé and 97% in Nikki). Women were the exclusive *Wagashi Gassirè* producers in both communes. In the Dassa-Zoumé commune, these women belonged to the Peulh (95%), Idaatcha (3%), and Fon (2%) sociocultural groups. In the Nikki commune, they be-longed to the Gando (77%) and Peulh (23%) sociocultural groups. Curd production was carried out mostly by women of the Peulh sociocultural group, with 100 and 89% in Dassa-Zoumé and Nikki, respectively. The survey revealed that the female producers were all uneducated, which could negatively influence the sanitary quality of their milk products due to a lack of knowledge on the safe handling of milk and dairy products. The analysis of the data showed that the *Wagashi Gassirè* traders belonged to the Baatonou, Dendi, Peulh, Fon, and Idaatcha tribes. In Nikki, 83% of traders were exclusively uneducated, whereas 43 and 31% of the traders in Dassa-Zoumé were uneducated and had a primary level education, respectively.

3.2. Dominant diseases in cattle identified by dairy farmers and their in-farm treatment strategies

As shown in Table 2, several diseases were found to be prevalent in the cattle farms surveyed. Arranged in descending order of importance, the dominant diseases were foot and mouth disease (FMD; 87%), pasteurellosis (62%), dermatophilosis (27%), trypanosomiasis (27%), lumpy skin disease (21%), and contagious bovine pleuropneumonia (18%). A comparison of the results obtained in the two communes (Table 2) revealed that pasteurellosis was significantly more prevalent ($p < 0.05$) in the commune of Nikki than in Dassa-Zoumé. These diseases were identified during the survey when a question was asked about the diseases often encountered on the participants' farms. The majority (64%) of dairy farmers reported that they diagnosed diseases themselves before administering treatment to the animals. When faced with these different diseases, the farmers adopted different treatment methods (Table 3). The majority (64%) of cattle dairy farmers treated their sick animals themselves, compared to 36% who relied solely on veterinarians. Some of the cattle dairy farmers who diagnosed and treated their animals themselves claimed to be self-taught, having learned either from their peers or from veterinarians, and 36% of cattle dairy farmers said that they had received training in treating animals. Two treatment types were identified in the survey: modern treatments and traditional treatments using bark and plant leaves. All the dairy farmers surveyed used modern treatments and obtained drugs from veterinary clinics by describing the symptoms of their animals to the veterinarians or pharmacists. Of these, 68% combined modern and traditional treatments. An inventory of the antibiotics used to treat animals based on the medicine labels found in the dairy farmers' homes showed that the most commonly used antibiotics were penicillin, streptomycin, oxytetracycline, tylosin, and sulphonamides. These antibiotics were also purchased from veterinary pharmacies, as the quality of products on the black market is doubtful. A total of 59% of the respondents estimated the weight of the animal and the dosage without referring to the manufacturer's instructions, and the rest (41%) determined the dosage according to the manufacturer's instructions by estimating the weight of the animal. In addition, all respondents did not respect the post-treatment waiting period for milking. The majority (98%) of the surveyed dairy farmers said that milk from cows treated with antibiotics was used either for direct human consumption or for processing into *Wagashi Gassirè* or curdled milk, in contrast to the remaining 2% who rejected this type of milk (Table 4). Compared to Nikki, the Dassa-Zoumé commune had a significantly higher proportion of dairy farmers who treated animals without veterinary help and used milk collected during the treatment period. Consequently, the probability of having dairy products contaminated with drug residues is higher in Dassa-Zoumé than in Nikki.

TABLE 2 Dominant diseases identified in farm animals and disease diagnosis in the communes of Dassa-Zoumé and Nikki.

Variable	Both communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Dairy cattle diseases (local dialect)										
Foot and mouth disease (*Tchabou*)	73	86a	8	46	91a	8	27	78	16	NS
Pasteurellosis (*Hinrin*)	73	62b	11	46	52	14	27	78	16	*
Nodular dermatosis (*Borla*)	73	21c	9	46	26	13	27	11	12	NS
Dermatophilosis (*Goungnan*)	73	27c	10	46	22	12	27	37	18	NS
Brucellosis (*Konedjé*)	73	7d	6	46	9	8	27	4	7	NS
PPCB (*Otel*)	73	18c	9	46	24	12	27	7	10	NS
Trypanosomiasis (*Samorin*)	73	27c	10	46	30	13	27	22	16	NS
Calf plague (*Tchibel*)	73	1d	3	46	2	4	27	0	0	NS
Other	73	7d	6	46	7	7	27	7	10	NS
Diagnosed and treated by who										
Dairy farmer	84	64b	10	54	72	12	30	50	18	*
Veterinarian	84	36a	8	54	28	10	30	50	15	NS

*$p < 0.01$; *N*, number; NS, not significant; %, percent of respondents; CI, confidence interval; a, b, c, d—values from the same column followed by the same letter were not significantly different at the 5% level. Chi^2- tests were performed for variables from both communes in the same line.

TABLE 3 Mode and type of treatment of listed livestock diseases and sources of drugs.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Basis for treatment choice by dairy farmers who diagnose and treat diseases themselves										
Self-taught	54	19b	10	39	17	12	15	7	13	NS
Professionally trained	54	36ab	13	39	28	14	15	43	25	NS
Mixed (self-taught and professionally trained)	54	45a	13	39	55	15	15	50	25	NS
Type of treatment used										
Modern only	54	32b	12	39	31	15	15	33	24	NS
Modern and traditional	54	68a	12	39	69	15	15	67	24	NS
Supply sources of drugs										
Veterinary office	54	87a	10	39	85	11	15	93	13	NS
Informal source	54	13b	9	39	15	11	15	7	13	NS
Non-compliance with waiting period	54	100	0	39	100	0	15	100	0	NS
Choice of timeframe										
Self-taught	54	74a	12	39	72	14	15	80	20	NS
Veterinarian's instructions	54	17b	10	39	15	11	15	20	20	NS
Mixed (self-taught and veterinarian's instructions)	54	9b	8	39	13	11	15	0	0	NS

N, number; NS, not significant; %, percent of respondents; CI, confidence interval; a, b—values followed by the same letter were not significantly different at the 5% level. Chi^2 tests were performed for variables from both communes in the same line.

3.3. Hygiene measures implemented by dairy farmers during milking

In Benin, all farmers use a hand milking procedure. The hygiene measures implemented by dairy farmers during milking are shown in Table 5. The analysis of the data on milking equipment revealed that most of the respondents (96%) used milking equipment only for milking. Milking equipment was either stored in rooms, hung on trees, or placed on open racks, exposing it to environmental microorganisms. Plastic equipment was used more frequently ($p < 0.001$) in Dassa-Zoumé than in Nikki. In contrast, calabashes were used more ($p < 0.001$) frequently in Nikki than in Dassa-Zoumé.

TABLE 4 Use of milk from cows treated with antibiotics without respecting the withdrawal period.

Variable	Both communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Consumption/transformation	42	98a	4	31	97	6	11	100	0	NS
Rejection	42	2b	4	31	3	6	11	0	0	NS

N, number; NS, not significant; %, percent of respondents; CI, confidence interval; a, b—values followed by the same letter were not significantly different at the 5% level, while means followed by different letters were significantly different at the 5% level. Chi^2 tests were performed for variables from both communes in the same line.

TABLE 5 Types of equipment used for milking and curd production.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Milking material										
Plastic	73	67a	11	46	96	6	27	19	15	***
Calabash	73	29b	10	46	2	4	27	74	17	***
Plastic and calabash	73	3c	4	46	0	0	27	7	10	NS
Stainless steel	73	1c	2	46	2	4	27	0	0	NS

***$p < 0.001$; *N*, number; NS, not significant; %, percent of respondents; CI, confidence interval; a, b, c—values followed by the same letter were not significantly different at the 5% level. Chi^2 tests were performed for variables from both communes in the same line.

The majority (98–100%) of the dairy farmers washed the milking utensils after milking, whereas only a minority (2–5%) washed them just before milking. Only 38% of the dairy farmers surveyed claimed to wash their hands before milking. Approximately 11% of the dairy farmers claimed to be trained in good milking hygiene practices, but only 5% implemented these practices. The majority who did not implement them felt that these practices were very restrictive and difficult to implement.

3.4. *Wagashi Gassirè* production practices

The unit operations of the *Wagashi Gassirè* production process that could influence its sanitary quality were investigated. Only 28 and 8% of the cheese producers in Dassa-Zoumé and Nikki, respectively, had cheese-making rooms that were laid out for improved protection from weather (Table 4). Consequently, although the *Wagashi Gassirè* cheese produced in both communes was exposed to external pollutants, including microbiological and physical hazards, it was exposed to a significantly greater extent in the Nikki commune ($p < 0.01$). While 72% of the cheese producers in Dassa-Zoumé filtered the milk used for cheese production, only 59% implemented this step in the Nikki commune. *Calotropis procera*, which is used for milk coagulation, was procured from markets (or known clients) in 72% of cases in Dassa-Zoumé. The majority of Nikki cheese producers obtained *Calotropis procera* directly from the fields (99%). After molding, *Wagashi Gassirè* is stored immersed in either water or whey, and 92 and 50% of the producers in the Dassa-Zoumé and Nikki communes, respectively, followed the immersion in whey method of storing. In addition, 50% of the female producers immersed the cheese in plain water after molding. Generally, the cheese is colored using different techniques and raw materials. In Dassa-Zoumé, the coloring of *Wagashi Gassirè* cheese was mostly performed (69.33%) using sorghum panicles procured from markets, while in Nikki, sorghum ears were used for coloring. Touhi bark or teak leaves (*Tectona grandis*) were also used for coloring by many female producers. For coloring, sorghum panicles added to potash (followed by 67% of producers in Dassa-Zoumé) or to salt/bicarbonate (followed by a smaller proportion of producers) were used in either hot (cooking) or cold (trituration of the dye in water) methods. Importantly, very few cheese producers (9% in Dassa-Zoumé and 9% in Nikki) were trained in hygienic *Wagashi Gassirè* production practices.

3.5. Conditions of curd production

The milk used for curd production was mostly filtered in both communes. Curd producers in Dassa-Zoumé exclusively used plastic equipment for fermentation (Table 5). In contrast, in the Nikki commune, stainless steel or enamel equipment was also used in addition to plastic. Moreover, the fermentation equipment was not solely reserved for curd making; this multipurposing could lead to the cross-contamination of the dairy products.

3.6. Market conditions for selling *Wagashi Gassirè*

The results showed that *Wagashi Gassirè* was sold by traders untrained in good hygiene practices. They also lacked the infrastructure to protect the cheese from environmental contamination. As a result, it was exposed to microbiological, physical, and chemical hazards. This study considered consumers with varying levels of education and of both sexes. In both Dassa-Zoumé and Nikki, most respondents were educated. According to the educated respondents, the hygiene conditions at the time of sale of *Wagashi Gassirè* were less than satisfactory (Table 6). In contrast, the uneducated consumers responded that hygiene conditions at the time of sale of *Wagashi Gassirè* were satisfactory. After buying, the consumers in the commune of Nikki made significant use ($p <$

TABLE 6 Milking hygiene measures implemented on farms.

Variable	Both communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Training in milking hygiene	84	11	7	54	13	9	30	7	9	NS
Implementation of hygiene practices	84	5	5	54	29	10	30	100	0	*
Hand washing before milking	73	38	11	46	37	14	27	41	19	NS
Other use of milking materials	84	4	4	54	4	5	30	3	6	NS
Washing of utensils after use	73	100	0	46	100	0	27	100	0	NS
Washing of utensils just before use	73	7	6	46	2	4	27	15	13	*

*$p < 0.05$; *N*, number; NS, not significant; %, percent of respondents; CI, confidence interval. Chi^2 tests were performed for variables from both communes in the same line.

TABLE 7 Consumer assessment of hygiene conditions at the time of sale and preservation methods.

Variable	Both communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	*N*	%	CI	*N*	%	CI	
Hygiene conditions during the sale										
Satisfactory	88	42b	10	56	45	13	32	38	17	NS
Unsatisfactory	88	58a	10	56	55	13	32	62	17	
Preservation method										
Cooking	86	48a	11	54	59	13	32	28	16	**
Refrigeration	86	7d	5	54	11	8	32	0	0	NS
Frying	86	30bc	10	54	37	13	32	19	14	NS
Smoking	86	41ab	10	54	43	13	32	38	17	NS
Cooking in salted water	86	21c	9	54	19	10	32	25	15	NS
Sun drying	86	33bc	10	54	11	8	32	69	16	***

**$p < 0.01$; ***$p < 0.001$; *N*, number; NS, not significant; %, percent of respondents; CI, confidence interval; a, b, c, d—values followed by the same letter were not significantly different at the 5% level. Chi^2 tests were performed for variables from both communes in the same line.

0.001) of sun drying to pre-serve *Wagashi Gassirè*, compared to daily heating in water, which was utilized by consumers in Dassa-Zoumé. Other methods used by consumers to preserve cheese included refrigeration, frying, and hot smoking. The level of microbiological contamination in *Wagashi Gassirè* would therefore depend on the preservation method used, especially because sun drying exposes the cheese to more microbes (Table 7).

3.7. Classification of actors according to hygiene practices

3.7.1. Classification of dairy cattle farmers

This study characterized the dairy cattle farmers and classified them into different groups. The first three axes were used to interpret the MCA results. The total inertia of the three axes was 57.97%, with 27.24% on the first axis, 21.39% on the second axis, and 9.34% on the third axis (Figure 2). Group 1 dairy cattle farmers treated their animals themselves or with help from veterinarians. These farmers also practiced milking hygiene measures. For example, they washed and dried the collection utensils and washed their hands before milking. Group 2 consisted of dairy cattle farmers who used veterinarians to monitor their animals and who had received training in animal husbandry and milking, which they implemented on their farms. Group 3 consisted of dairy farmers who treated their animals themselves, without help from veterinarians. These individuals had not received any training in milking hygiene. Training in good hygiene practices should be implemented for all farmers, but especially for the latter group, and a rigorous follow-up of the application of these good practices should be carried out to improve the sanitary quality of the milk produced in these breeding areas.

3.7.2. Classification of female *Wagashi Gassirè* producers

This study identified three groups of female *Wagashi Gassirè* producers. The first three axes were used to interpret the MCA results. The total inertia of the three axes was 36.98% with 21.98%, 10.38%, and 4.61% on the first, second, and third axes, respectively (Figure 2). Axis 1 contrasts the female producers in Groups 1 and 3 with those in Group 2. Axis 2 compares female producers in Group 3 to those in Groups 1 and 2 (Figure 3). Group 1 consisted of women who were trained in good production hygiene practices. They did not scald or sun dry their cheese. Most also did not immerse the cheese in whey and did not color it. In addition, they put the

cheese in salted water but did not smoke it. Group 2 consisted of single female producers who did not have infrastructure, such as properly laid out cheese-making rooms, to protect the cheese from weather exposure. They used filtered milk to make *Wagashi Gassirè* by coagulating the milk with the leaves, stems, and sap of *Calotropis procera*. They mainly produced red cheese. The cheese was colored with residues of sorghum (panicles and ears) and teak (Touhi bark and leaf). These producers were of the Gando and Peuhl ethnic groups, and they also sun-dried the cheese. Group 3 consisted of female producers from the Idaatcha ethnic group. They often skipped the milk filtration step and sometimes used papaya leaves to coagulate milk. They used vegetables and plastic strainers for draining. They preserved the *Wagashi Gassirè* by boiling in bags and storing the cheeses immersed in plain water and whey. They also smoked the *Wagashi Gassirè* for better preservation. Therefore, it is important to assess the sanitary quality of products according to these groups and to initiate training on good hygiene practices for these groups according to their individual needs.

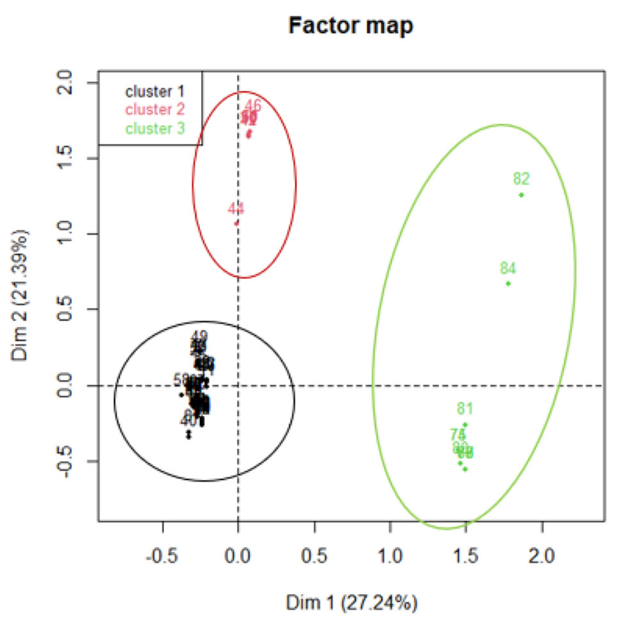

FIGURE 2
Classification of cattle dairy farmers in Dassa-Zoumé and Nikki.

3.7.3. Classification of female *Wagashi Gassirè* traders

This study identified two groups of female *Wagashi Gassirè* traders. The first two axes were used to interpret the MCA results. The total inertia of the three axes was 22.79%, with 12.72% on the first axis and 10.07% on the second axis (Figure 4). The female traders were opposed on axis 2. The first group comprised female traders from the Baatonou, Fon, Mahi, and Peuhl tribes who worked either on the edge of the streets, at crossroads, or as street vendors. They preserved cheese by drying it in the sun and cooking it daily in salted water. The products sold by these groups could therefore be contaminated by environmental microorganisms and chemical contaminants from vehicles on the streets and intersections. The second group consisted of vendors from the Idaatcha, Yoruba Dendi, and Mina tribes. They preserved *Wagashi Gassirè* by boiling it either enclosed in or without plastic bags. Contamination from the plastics used for cooking is possible under these conditions, affecting the sanitary quality of these products at the chemical level and weakly at the microbiological level.

3.7.4. Classification of consumers

The first three axes were used to interpret the MCA results. The total inertia of the three axes was 44.10 with 17.52%, 15.12, and 11.45% on the first, second, and third axes, respectively (Figure 5). Axis 2 contrasted the female consumers in Group 3 with those in Groups 1 and 2 (Figure 5). Group 1 consisted of

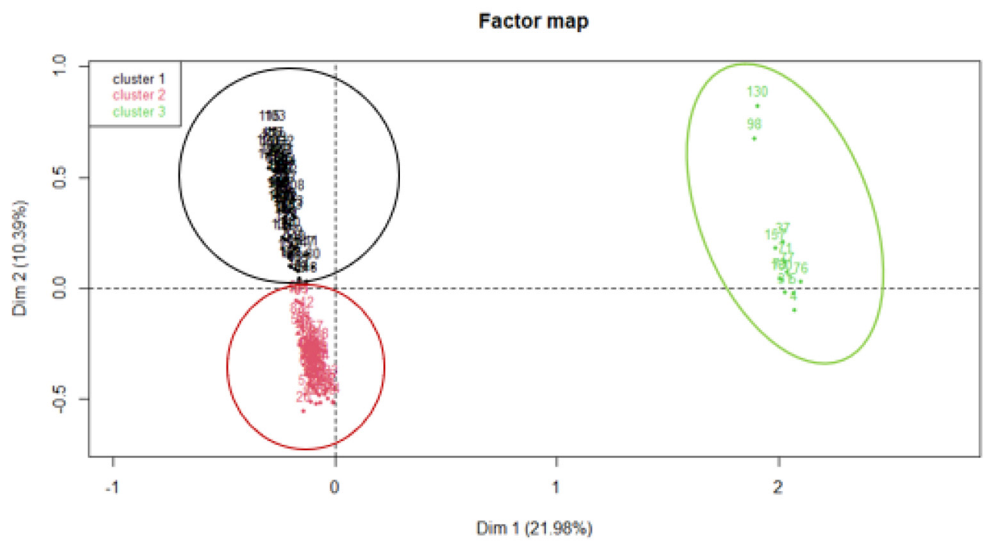

FIGURE 3
Classification of female *Wagashi Gassirè* producers in Dassa-Zoumé and Nikki.

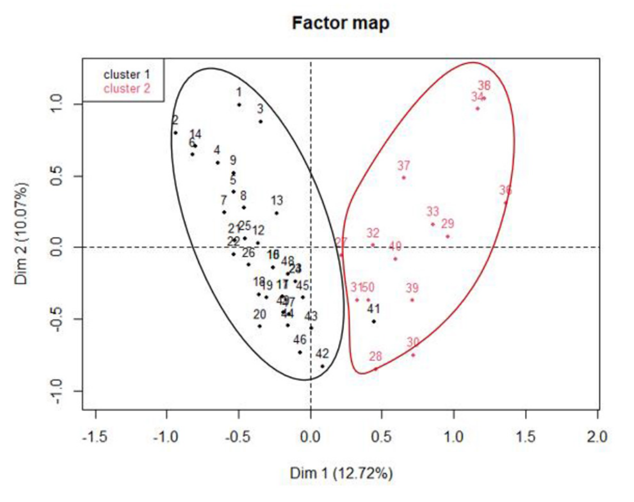

FIGURE 4
Classification of *Wagashi Gassirè* traders in Dassa-Zoumé and Nikki.

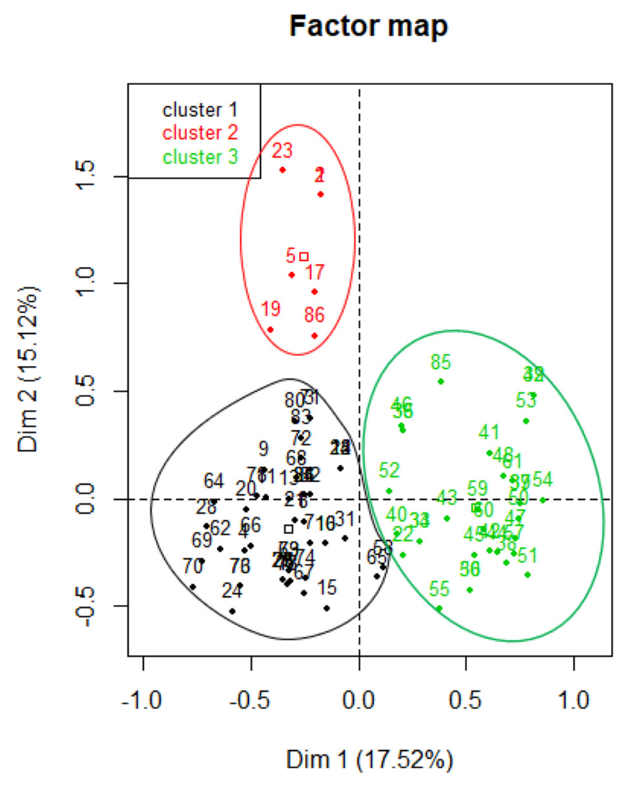

FIGURE 5
Classification of *Wagashi Gassirè* consumers from Dassa-Zoumé and Nikki.

secondary-school-level educated consumers who practiced frying and sun-drying *Wagashi Gassirè*. Group 2 consisted of university-level educated consumers who cooked and refrigerated *Wagashi Gassirè* for preservation and were not satisfied with the hygienic conditions under which the *Wagashi Gassirè* was sold. Group 3 consisted of uneducated consumers who smoked and cooked the cheese in salted water for storage; they were satisfied with the hygiene conditions of the market. Therefore, the appreciation of the level of hygiene depends on the education level of the consumers; however, abnormally, the uneducated consumers fortunately seem to adopt practices for treating the cheese that support the destruction of microbiological contaminants.

3.8. Presentation of stakeholders according to level of education, ethnicity, and hygiene training

The analysis of the dairy stakeholders according to their sociocultural group as well as their level of education and training on hygiene practices during the milking, production, and preservation of curdled milk and *Wagashi Gassirè* revealed that the *Wagashi Gassirè* trade was practiced by the Dendi and Baatonou in Nikki and the Fon and Idaatcha in Dassa-Zoumé. Milk farming was practiced by mostly uneducated Fulani farmers, some of whom had received training in hygiene, while the female producers of *Wagashi Gassirè* were mostly uneducated and from the Fulani and Gando tribes.

4. Discussion

The husbandry and hygiene practices adopted in the production, processing, and distribution of milk not only affect the quality of the raw milk and its byproducts, but also directly affect human and animal health. Moreover, they also have economic implications (Singh and Ramachandran, 2020). Here, the various stakeholders in the dairy chain were characterized and factors that may affect the sanitary quality of milk and its derivatives in Benin were identified. This study revealed that cattle rearing is practiced mostly by uneducated men of the Fulani group in the two communes considered. In agreement with these results, Dahouda et al. (2019) reported that the majority (62.73%) of cattle dairy farmers in Nikki were of the Fulani ethnic group. The lack of education among most farmers acts as a handicap in the implementation of good husbandry and hygiene in milking practices. According to Gökdai et al. (2020), the education level of stakeholders influences the observance of good hygiene practices on the farms; uneducated farmers fail to master good hygiene and husbandry practices and lack an understanding of the risks associated with bad practices. Several diseases, mainly foot and mouth disease, pasteurellosis, dermatophilosis, trypanosomiasis, lumpy skin disease, contagious bovine pleuropneumonia, brucellosis, and calf plague, were found to be prevalent in the surveyed cattle farms, corroborating the findings of Kpodékon et al. (2015), who showed that foot and mouth disease is the dominant viral disease in cattle farms in northern Benin. Sow (2014) also observed that (arranged in decreasing order of importance) foot and mouth disease, pasteurellosis, trypanosomiasis, botulism, lumpy skin disease, mastitis, and symptomatic anthrax were the dominant diseases encountered in dairy cattle farms in the regions of Thiès and Diourbel in Senegal. These diseases cause significant economic losses in terms of milk production and skin condition in the affected animals. Apart from these economic consequences, some of these diseases encountered in animals, such as brucellosis, can affect the quality of milk and meat and consequently the health of consumers. They are diagnosed and

treated by the farmers themselves, mostly with antibiotics purchased from pharmacies or veterinary clinics. Consistently, in another study, 76.7% of farmers used antibiotics without veterinary assistance for the treatment of diseases encountered on cattle farms in northern Benin (Mensah et al., 2019). Similarly, Dognon et al. (2018) observed that only 49% of farmers used the veterinary services in northern Benin for the treatment of their animals. While 59% of farmers obtained veterinary drugs from local markets, 41% procured them from veterinary pharmacies. Here, the antibiotics commonly used to treat animals were penicillin, streptomycin, oxytetracycline, tylosin, and sulphonamides. These results agree with the reports of Dognon et al. (2018) and Mensah et al. (2019), who observed that tetracyclines, penicillin, sulphonamides (47%), and macrolides (17%) were the antibiotic families most used by livestock keepers in northern Benin for treating sick cattle. Most respondents in this study did not follow the manufacturer's instructions when estimating weight and the dosage of the medicine. This proves the existence of the rampant misuse of antibiotics on cattle farms in the surveyed communes. The guidelines for the post-treatment waiting period were disregarded by all respondents, and the milk from antibiotic-treated cows was used either for direct human consumption or for processing into *Wagashi Gassirè* or curdled milk. These practices suggest an increased likelihood of the contamination of dairy products with antibiotic residues and antibiotic-resistant bacteria, which can adversely affect consumer health (Dognon, 2018). The practice of the unhindered consumption or processing of raw milk contaminated in this manner by concerned stakeholders could possibly be explained by a lack of understanding of the associated health risks by the high number of uneducated respondents in this survey. Plastics and calabashes are the most commonly used materials for milking equipment. The use of calabashes favors the formation of microbial biofilms that can participate in the rapid fermentation of raw milk. Unlike plastic, which can be properly cleaned when soap and drinking water are available, calabashes cannot be disinfected easily (Gagara et al., 2019). According to Kebede et al. (2007), the use of different types of containers for milk collection significantly influences the diversity of the flora present in curdled milk and the characteristics of the resulting milk product. Only a minority of the responders complied with many hygiene measures; in particular, hand washing procedures were not followed during the production and preservation of raw milk, curdled milk, and *Wagashi Gassirè*. Group 3, which consisted of dairy cattle farmers who treated their animals themselves without help from veterinarians and had no training in milking hygiene, was the most targeted as not following good hygiene practices. The absence of good hygiene practices reported by majority of respondents and the non-washing of hands, which are reservoirs of microorganisms including *Staphylococcus* in wound infections and fecal coliforms, highlighted that the probability of the contamination of milk and dairy products by pathogenic germs is very high; this was confirmed in the works of Farougou et al. (2012), Boko et al. (2016), Gouissi et al. (2017), Sessou et al. (2019), and Djobo et al. (2021). Indeed, the work of Djobo et al. (2021) showed that raw cow milk from Benin was of unsatisfactory quality, with 1.8×10^8 CFU/mL for total aerobic mesophilic flora (TMC), 4.0×10^7 CFU/mL for fecal coliforms (FC), 3.5×10^7 CFU/mL for *Escherichia coli*, 2.8×10^7 CFU/mL for total coliforms (TC), 2.1×10^7 CFU/mL for fecal Streptococci (FS), 1.6×10^7 CFU/mL for yeasts and molds (YM), 1.7×10^7 CFU/mL for sulfur-reducing anaerobic bacteria (SRA), and 1.2×10^7 CFU/mL for *Staphylococcus* spp. The same observations were recorded by Farougou et al. (2012), where raw cow milk samples collected from Dassa-Zoumé were determined to be unsatisfactory regarding the total mesophilic aerobic flora (1.1×10^8 CFU/ mL), fecal coliforms (9.2×10^2 CFU/mL1), *Escherichia coli* (0.4×10^1 CFU/mL), and *Staphylococcus aureus* (4.0×10^1 CFU/mL). Boko et al. (2016) revealed that the average fecal coliforms in curdled milk made from raw cow milk varied from $11.313 \pm 13 \times 10^3$ CFU/mL at 30°C to $0.983 \pm 1.228 \times 10^3$ CFU/mL, while the average *Escherichia coli* count was 0.34 ± 0.89 CFU/mL. Gouissi et al. (2017) and Sessou et al. (2019) showed that *Wagashi* cheese was of poor microbial quality when some pathogens were present. The presence of *Escherichia coli* used as reliable indicator of fecal contamination indicates possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. The low proportion of stakeholders who implement hygiene measures indicates a lack of awareness regarding the risks associated with non-compliance. Therefore, increasing stakeholders' awareness of good hygiene practices in milking and milk handling is necessary to obtain safe, high-quality dairy products. The results obtained regarding preservation methods corroborate the findings of Sessou et al. (2013), who inventoried the methods studied here and found limitations in *Wagashi Gassirè* production and preservation methods, reflected by their low sanitary quality. The existence of stakeholder subcategories indicates diversity in the employed husbandry and hygiene practices, and consequently diversity in the sanitary and nutritional quality of the target dairy products. In view of these findings, the products targeted may present potential risks for public health during their consumption, given their contamination with the above-mentioned microorganisms and findings reported in other studies (Michael and Mullan, 2019; Idland et al., 2022). Indeed, several studies have identified milkborne pathogens including Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica* in dairy products. The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk or eat these uncooked dairy products. These bacterial pathogens are a substantial source of health loss globally (Ahmadi, 2019; Idland et al., 2022). To improve the safety quality and shelf-life of dairy products, factors that contribute to microbiological contamination must be considered at the level of every stakeholder cluster in the dairy chain. The critical points found in this study will be used to improve hygiene quality of dairy products in Benin. Although this study has provided valuable information on the milk sector in Benin for the improvement of dairy products, there are some limitations to our study due to the selection approach of our participants; there was an uneven distribution of participants from different area. Future studies should use more rigorous sampling methods, for example in a nationally representative household survey, specifying balanced and adequate representation of important demographic groups. On the other hand, the reluctance of farmers to report truthful status could bias the information obtained.

5. Conclusions

This study assessed the production and storage practices of raw cow's milk, curdled milk, and *Wagashi Gassirè* in two Beninese communes. Several factors were found to affect the sanitary quality

of dairy products in these regions. These included the misuse of antibiotics on farms, the non-implementation of good hygiene practices by stakeholders, the sale of products in the open air (as a source of contamination), and the non-availability of coagulants, especially in the Dassa-Zoumé area. This study also confirmed the prevalence of several diseases on cattle farms in these regions, which can influence the quality of their dairy products. A large cluster of dairy cattle farmers treated their animals themselves, without help from veterinarians. These individuals had not received any training in milking hygiene. Training in good hygiene practices should be implemented for all farmers, and a rigorous follow-up of the application of these good practices should be carried out to improve the sanitary quality of the milk produced in these breeding areas.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GK, AD, BM-TSO, PS, SF, JM, M-LS, and AC: conceptualization. GK, AD, and BM-TSO: methodology and investigation. IY: software and formal analysis. PS, PA, JH, JM, M-LS, AC, and SF: validation, writing—review and editing, and supervision. SF and AC: resources, project administration, and funding acquisition. GK, PS, JM, and SF: data curation. GK: writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1050592/full#supplementary-material

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Foodborne disease hazards and burden in Ethiopia: A systematic literature review, 1990–2019

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Background: Foodborne disease (FBD) affects millions of people each year, posing a health burden similar to malaria, tuberculosis or HIV. A recent World Bank study estimated the productivity losses alone attributed to unsafe food within Africa at $20 billion in 2016, and the cost of treating these illnesses at an additional $3.5 billion. Ethiopia faces multiple food safety challenges due to lack of infrastructure and basic pre-requisites for food safety such as clean water and environment, washing facilities, compounded by limited implementation of food safety regulations, and a lack of incentives for producers to improve food safety. A consolidation of our understanding and evidence of the source, nature and scale of FBD in Ethiopia is needed to inform policy and future research. We performed a Systematic Literature Review (SLR) of publications on FBD occurrence in Ethiopia including hazard presence and impact.

Method: The SLR followed Cochrane and PRISMA guidelines. We searched PubMed and CAB-Direct for relevant publications between 1990 and 2019 (inclusive). Observational studies and reviews were included. Two reviewers screened titles and abstracts, and retained publications were reviewed in full for quality and data extraction.

Result: In total 128 articles met the inclusion criteria. Most articles focused on the identification of biological hazards in food. High levels of microbial contamination in different food value chains were often found in the small, *ad hoc,* observational studies that dominated the literature. Raw milk (22/128, 17.0%) and raw beef (21/128, 16.4%) were the most studied food products. Foodborne (FB) parasites were often found at higher rates in food than bacterial and viral pathogens, possibly due to differences in ease of identification. High levels of bacterial contamination on the hands of food handlers were widely reported. There were no reports on the incidence of human FBDs or resulting health and economic impacts.

Conclusion: Our findings reflect existing concerns around food safety in Ethiopia. A lack of substantial, coordinated studies with robust methodologies means fundamental gaps remain in our knowledge of FBD in Ethiopia, particularly regarding FBD burden and impact. Greater investment in food safety is needed, with enhanced and coordinated research and interventions.

KEYWORDS

food borne diseases, value chains, hazards, burden, Ethiopia

1. Introduction

Foodborne diseases (FBDs) are illnesses caused by contaminated, or naturally harmful, food. A foodborne (FB) hazard is anything present in food that can harm consumers' health. They are usually categorized as: biological hazards, which are pathogenic organisms and the toxins they produce; chemical hazards, which may be artificial or natural; and physical hazards, such as foreign objects in food (Grace et al., 2018). The most comprehensive estimates of the health burden of FBDs in African countries are those provided by the World Health Organization (WHO) Foodborne Disease Epidemiology Reference Group (FERG) (Havelaar et al., 2015), which estimated that FBD burden is comparable to that of malaria, HIV/AIDS or tuberculosis. Nearly all of this burden (98%) is borne by low-income countries and most of it (97%) is due to biological hazards (Havelaar et al., 2015), with diarrheal disease agents being the most important contributor to overall FBD burden in African region E (which includes Ethiopia), followed by helminths and invasive bacteria (Havelaar et al., 2015).

Accurate information on the health and burden associated with FBDs is critical for countries to guide food safety risk management strategies and to justify allocation of resources. The lack of reliable data from surveillance systems for FBD in low and middle income countries limits the ability to conduct risk-based food safety management. FBD burden is thought to be high in Ethiopia (Pieracci et al., 2016; Keba et al., 2020; Belina et al., 2021; Mekonnen et al., 2021). *Salmonella*, *Listeria monocytogenes*, *Escherichia coli (E. coli)*, *Campylobacter* spp. and *Shigella* are among the most common FB pathogens reported from Ethiopia and food-producing animals are the major reservoirs (Belina et al., 2021). Reports of food poisoning outbreaks in Ethiopia are often linked to consumption of raw meat and milk (Kassahun and Wongiel, 2019), However, cases of FB illnesses are underreported and are rarely investigated in detail (Ayana et al., 2015).

Given the lack of knowledge around this vital topic, this Systematic Literature Review (SLR) was conducted to investigate the existing literature and collate the evidence on FB hazards in Ethiopia. The SLR did not look at specific pre-specified hazards or specific foods, but instead explored available literature on any FB hazards and any foods. This SLR is one early output of a portfolio of research projects[1] looking to improve understanding and control of FBD in Ethiopia. This review was used to inform these projects which then went on to examine FBD using various approaches, including modeling, molecular and field-based approaches.

1

- Urban food markets in Africa: Incentivizing food safety using a pull-push approach ("pull-push" project), led by the International Livestock Research Institute.
- The assessment and management of risk from non-typhoidal Salmonella, diarrheagenic *Escherichia coli* and *Campylobacter* in raw beef and dairy in Ethiopia (TARTARE), led by Ohio State University.
- Foodborne disease epidemiology, surveillance and control in African LMIC (FOCAL), led by Technical University of Denmark.
- Ensuring the safety and quality of milk and dairy products across the dairy value chain in Ethiopia (ENSURE), led by Addis Ababa University.

2. Methods

2.1. Research question

This SLR aimed to inform the design of further studies by addressing the following research questions:

- What biological and chemical hazards have been identified in foods in Ethiopia?
- What is the prevalence (i.e., percent of contaminated products) and concentration of these hazards in foods in Ethiopia?
- What is the incidence of FBD and what is the associated health burden in Ethiopia?

2.2. Search strategy

We conducted an SLR following the methods suggested by the Cochrane Collaboration and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009). A comprehensive search on PubMed and CAB Direct was performed for articles published in English from the 1st of January of 1990 to the 30th of September 2019, inclusive. The search was done on 30th of September 2019. The search syntax with different search terms and Boolean Operators including the following search terms:

i foodborne OR "food borne" OR food-borne OR "food safety" OR "food related" OR "food associated" OR "food derived" OR "food* illness" OR "food* disease*" OR "food* intoxica*" OR "food pathogen" OR "food* poison*" OR "food* microb*" OR "food* vir*" OR "food parasit*" OR "food* toxin."

ii AND Ethiop*.

The syntax was left broad and generic to capture all the literature covering the various aspects of interest (e.g., prevalence, impact, risk factors, and control). Inclusion and exclusion criteria are shown in Table 1.

2.3. Article selection process and quality assessment

Lists with the identified titles and abstracts were downloaded to an excel file and checked for duplicates using Mendeley (https://www.mendeley.com/download-reference-manager/windows). After the removal of duplicates, titles and abstracts were reviewed independently by two reviewers against the inclusion and exclusion criteria. All titles considered relevant by both reviewers were included; articles considered relevant by just one reviewer were reviewed by a third reviewer who made a final decision on article inclusion.

Full articles for the included titles were obtained, when available. The full articles were then subjected to a two-step review process; Articles were reviewed against (1) inclusion/exclusion criteria (as

above) and (2) quality criteria. The quality criteria considered the paper's merit on five aspects: (1) selection of subjects, (2) study methods, (3) data analysis, (4) reporting of methods and results, and (5) reliability of results (Table 2). Quality of the papers was rated as "Good" (scoring positively to all five quality criteria), "Moderate" (scoring positively in three or four of the quality criteria) or "Poor" (scoring positively in two or less of the quality criteria). However, an overall reviewer's overall impression could over-ride this rating. Poor-quality articles were excluded from data synthesis.

2.4. Data extraction and analysis

An excel template was designed to allow standardized data extraction by different reviewers (Supplementary material). Ten percent ($n = 13$) of the full articles were systematically selected by dividing the total number of included articles by 13 so each 10th article in the list was selected. Then theses were reviewed in parallel by two reviewers and outputs were compared to ensure the review process was standardized and comparable across reviewers. After standardization, the remaining articles were reviewed by a single reviewer. Given the large diversity of foods, organisms, and studies, it was not possible to conduct any meaningful meta-analysis.

TABLE 1 Inclusion and exclusion criteria (inclusion also require Ethiopia focus and published on or after 1st January 1990 but not after 30th September 2019).

Criteria	Included articles	Excluded articles
Study design	Observational studies (cross-sectional, case-control or cohort studies), reviews, randomized control trials (RCT) and experimental studies	Laboratory based/experimental studies
Study focus	FBDs incidence, health burden, prevalence in food products	Non-foodborne illness/hazards, non-food samples (i.e., feces from animals, serology, or carriage in vectors), antimicrobial resistance studies; and studies on aspects of basic science (i.e., immunology/gene sequencing/chemistry)

3. Results

3.1. Literature search

The database searches returned 760 and 244 records from PubMed and Cab Direct, respectively. Out of 519 unique articles, 307 were excluded at title and abstract review, and 3 were not available as full manuscripts (Figure 1). From the remaining 209 full articles, 53 were excluded based on the inclusion/exclusion criteria and 28 based on the quality criteria. These were excluded due to poor scientific quality, mostly from biased selection of subjects, inappropriate data analysis and incomplete and/or inaccurate results.

Data was ultimately extracted from 128 selected articles.

3.2. Profile of the reviewed publications

Articles reported studies conducted in Oromia (42/128, 32.8%), Amhara (30/128, 23.4%), Addis Ababa (29/128, 22.6%), and Southern Nation Nationalities People (SNNP; 21/128, 16.4%) region. Few studies had been done in the other regions of the country. These were generally conducted in major cities and were not representative of all regional states (Figure 2).

Most articles were conducted in the capital city, Addis Ababa ($n = 29$), followed by Debre Zeit ($n = 14$), Awassa ($n = 9$), Jimma ($n = 9$), Gondar ($n = 9$), Bahir Dar ($n = 7$), Haromaya ($n = 6$), and Arba Minch ($n = 5$) the location of well-established universities.

The number of articles relevant to the topic identified in our review increased over time (Figure 3).

Majority of the articles included in the review (119/128, 93%) focused on biological hazards and six articles looked at presence of chemical hazards in food. Among the 119 articles focused on biological hazards, 82/119 (68.9%) investigated presence of pathogens in food, 48/119 (40.3%) presence in human samples (stool and hand swab samples) and 19/119 (15.9%) in food environments (i.e., beef slaughterhouses, butcheries, and milk shops). While half of the articles studied one food item and one hazard, other half covered various hazards and/or various matrices. None of the selected studies covered incidence of FBD or FBD burden.

The majority ($n = 119$, 92.9%) of the 128 papers examined the presence of bacteria ($n = 87$, 73.11%), parasites ($n = 23$, 19.33%), parasites and bacteria ($n = 7$, 5.88%), fungal toxins ($n = 3$, 2.52%), and viruses ($n = 1$, 0.84%) using a cross-sectional design. Nearly half of the articles (47.3%) incorporated risk factor analysis. The majority

TABLE 2 Quality criteria used for the quality review.

Quality measure	Good quality	Medium quality	Poor quality
Subject selection	Unbiased selection of subjects	Biased selection of subjects is acknowledged and accounted for	Not acknowledged biased selection of subjects
Data analysis	Data analysis is appropriate	Limitations in data analysis are acknowledged and accounted for	Data analysis is not appropriate
Study method	Methods used are scientifically sound	Methods used are scientifically sound, although may not be the most appropriate methods	Wrong or inappropriate methods are used
Reporting of methods and results	Accurate description of methods	Some details of methods are lacking, but methods are understandable, and results remain valid	Methods are not clear or are incomplete
Reliability of results	Reported results are complete and seem accurate		Reported results are incomplete and/or inaccurate

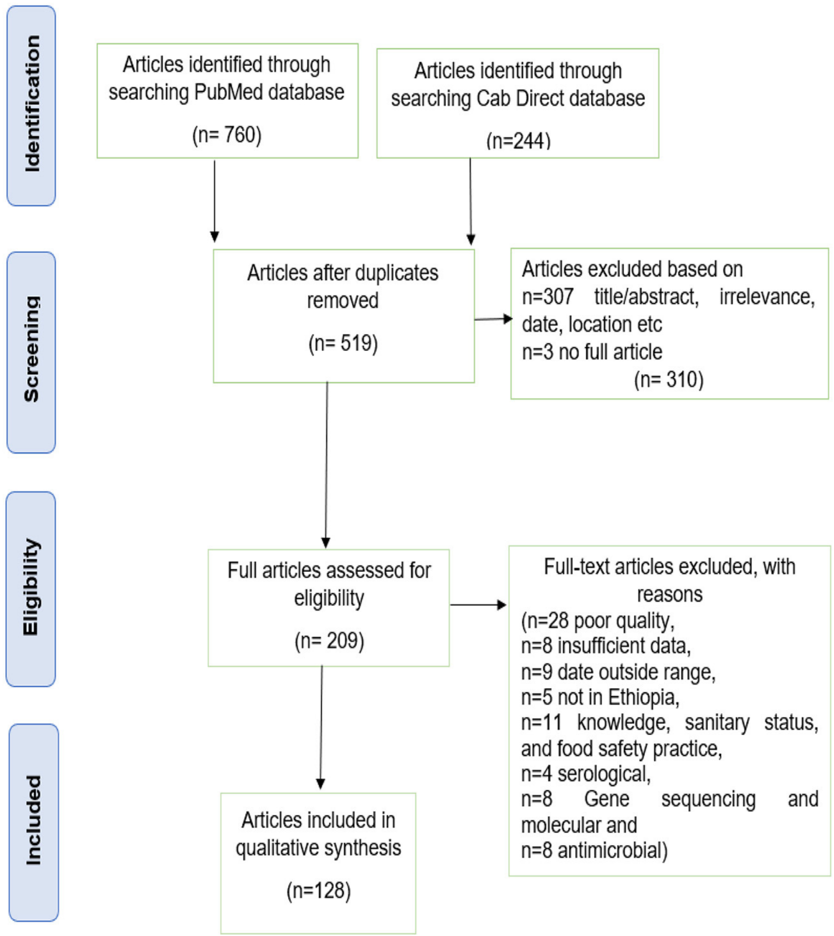

FIGURE 1
Flow chart of the process followed during the literature search for data extraction. The PRISMA flow diagram for the systematic review detailing the database searches, the number of abstracts screened, and the full texts retrieved.

of these publications (n = 32, 55.7%) concentrated on evaluating risks from food handlers, feverish patients in healthcare facilities, and randomly selected school students or community members. Of the 128 retained articles, 72 (56.25%) were mainly focused on detecting pathogens in animal source foods. Bacterial contamination was reported in food products in beef, dairy, and poultry value chains while parasites were reported from apparently healthy food handlers, from stool samples of patients of health care facilities and to some extent (4/10,40%) in vegetable value chain.

Figure 4 below, describes number of articles reporting a particular bacteria species.[2]

In terms of parasitic hazards, *Entamoeba*, *Giardia* and *Ascaris* were the most commonly studied hazards (Figure 5).

Study designs were often duplicated, to identify similar pathogens in similar populations. For example, most of the articles investigating pathogens on food handlers were conducted in student cafeterias of public universities. However, this may be more down to convenience, rather than intended inter-study comparability or the widespread use of established, optimized methods. The parasites investigated in these articles were similar, focusing on widely known FB parasites (e.g., *Ascaris lumbricoides*, *Giardia lamblia*, *Entamoeba histolytica*, *Taenia* spp. etc.).

3.3. Prevalence of foodborne hazards in the food value chains

Of the 82 articles that investigated hazards in food, the majority (64/82, 78%) focused on beef, dairy, vegetable poultry, and eggs value chains. Other value chains, such as camel, pork, fish, mutton, goat meat, fruits, crops, and street-vended locally made food (Sambusa, Bombolino, donat, and all doughnut variants) were the focus of 18 (22%) articles, the results of which are not reported on this paper (Ashenafi, 1995; Muleta and Ashenafi, 2001; Molla et al., 2005; Abdel Gadir Atif et al., 2006; Hiko et al., 2008; Kibret and Tadesse, 2013; Dulo et al., 2015; Garedew et al., 2015b; Eromo et al., 2016; Taye et al., 2016; Messele et al., 2017; Wendwesen et al., 2017; Tegegne et al., 2019) (Supplementary material). This section presents the literature findings per value chain or host, and per pathogen investigated.

2 *Salmonella* spp.—many studies did not provide the speciation of *Salmonella*, and it is possible some of these studies may or may not include Non-Typhoidal *Salmonella*., N.B. *Salmonella* spp. does not include Non-Typhoidal *Salmonella*, and *Escherichia coli* does not include *E. coli* O157:H7. N.B. articles may include more than one bacteria and parasite.

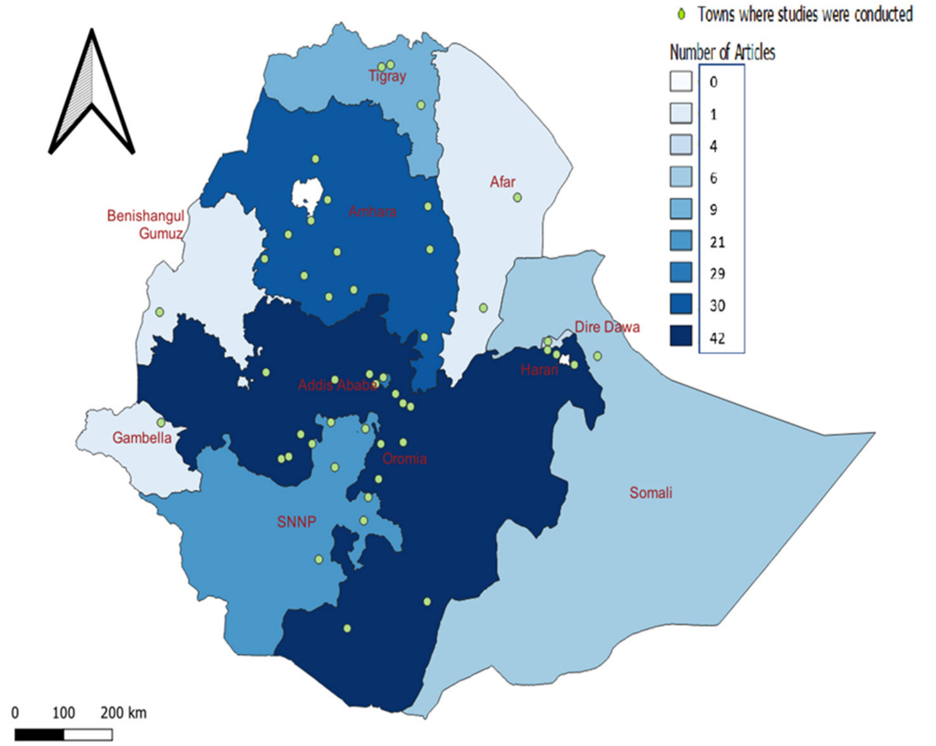

FIGURE 2
Spatial distribution of regions and towns where the studies were conducted. Concentration of included studies over regions in Ethiopia with dark blue color indicating the highest number of articles and light blue representing fewer number of articles. The green circle dots indicating the towns where the studies were conducted.

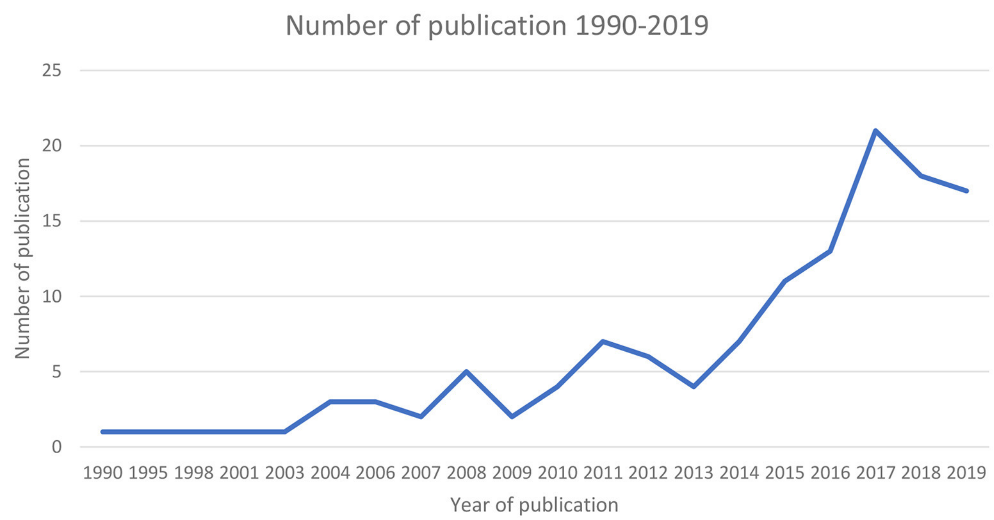

FIGURE 3
Number of retained publications on FBD in Ethiopia between 1990 and 2019. The solid line shows actual number of articles published in a particular year.

Pathogens are listed from the more frequently studied to the least, for each value chain or host.

3.3.1. Beef value chain

Close to one third of the articles investigated bacterial pathogens in the beef value chain (37/128, 28.9%).

3.3.1.1. *Salmonella* spp.

Eighteen articles (48.6%) reported information on *Salmonella* spp. *Salmonella* spp. contamination estimates ranged from 1 to 13% in raw beef samples collected from slaughterhouses in different parts of the country (Molla et al., 2003; Alemu and Zewde, 2012; Hiko et al., 2016, 2018; Edget et al., 2017; Wabeto et al., 2017; Ketema et al., 2018; Takele et al., 2018; Bersisa et al., 2019), and between

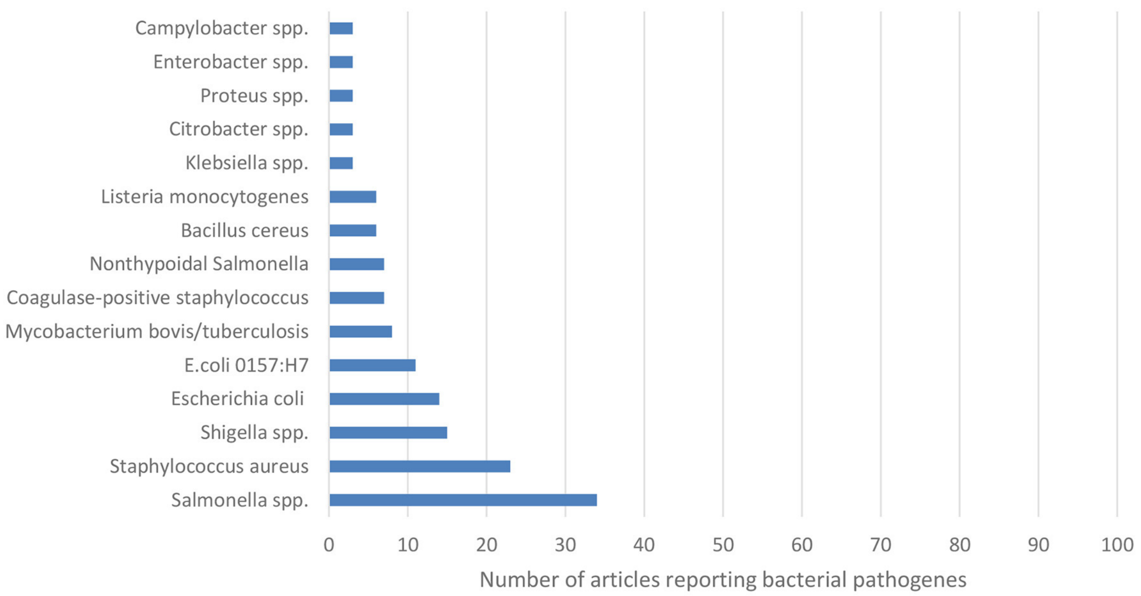

FIGURE 4
Number of articles reporting a particular bacterial pathogen. Numbers are derived from every article investigating on the pathogens. That is an article may report more than one bacteria species.

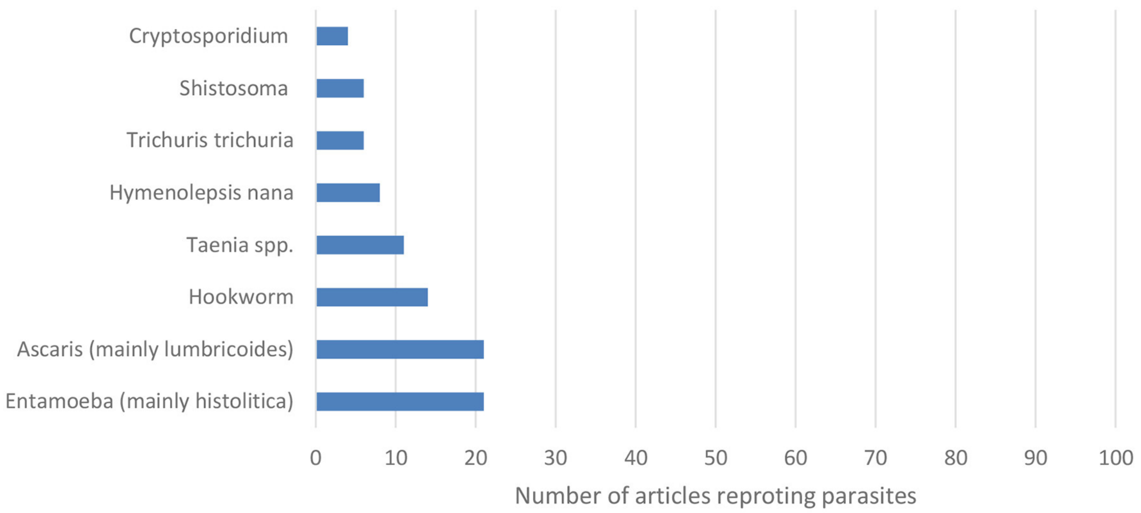

FIGURE 5
Number of articles reporting a particular parasite. Numbers are derived from every article investigating on parasites. That is an article may report more than one parasite.

1 and 35% in raw meat samples from butcheries and retail shops (Garedew et al., 2015b; Hiko et al., 2016, 2018; Edget et al., 2017). One study found 70% of fresh meat samples collected in butcher shops contaminated with *Salmonella* spp. (Azage and Kibret, 2017). Four articles investigated foodborne pathogens in "*kitfo*," a traditional Ethiopian raw (or lightly cooked) minced meat dish. About 9.8–12, 8, and 30% of "*kitfo*" samples in supermarkets, restaurants, and street vendors, respectively, were found to carry *Salmonella* spp. (Muleta and Ashenafi, 2001; Molla et al., 2003; Ejo et al., 2016; Dagnachew, 2017). One article investigated *Salmonella* spp. in processed meat (mortadella) and found 0.8% positive samples (Hiko et al., 2015).

3.3.1.2. *Escherichia coli*

Eleven articles (29.7%) reported contamination of beef with *E. coli*. About 5.5%–35.5% of raw beef samples from slaughterhouses,13.8% from restaurants, and 6%–29.4% from supermarkets were found contaminated with *E. coli* (Hiko et al., 2015; Edget et al., 2017; Messele et al., 2017; Bedasa et al., 2018; Bersisa et al., 2019). Kumar et al. (2014), reported that 62.5% of raw beef samples from butcher shops were positive for *E. coli*. Articles showed *E. coli O157:H7* detection ranged from 0.8 to 9% in raw beef samples from slaughterhouses (Hiko et al., 2016; Abdissa et al., 2017; Atnafie et al., 2017; Edget et al., 2017; Haile et al., 2017; Bedasa et al., 2018). The rate of contamination was higher in raw beef samples

from butchers (2%−18%) (Kumar et al., 2014; Hiko et al., 2016; Atnafie et al., 2017; Beyi et al., 2017). *Escherichia coli O157:H7* was detected in raw beef and minced meat samples in (0%−3.1%) from retailer shops, restaurants, and supermarkets (Beyi et al., 2017; Edget et al., 2017; Bedasa et al., 2018).

3.3.1.3. *Mycobacterium bovis*

Five articles (13.5%) looked at *Mycobacterium bovis;* postmortem inspection studies conducted in abattoirs reported 0.8%−10% of carcasses showing mycobacterial lesions. The sensitivity and specificity of the routine postmortem examination method was also reported to be lower compared to detailed inspection and culturing. Specifically, 0.7–7.5 and 2.7–19.4% of carcass negative in routine postmortem examination were found to be positive in detailed examination and by culture, respectively (Asseged et al., 2004; Teklu et al., 2004; Demelash et al., 2009; Biffa et al., 2010; Aylate et al., 2013).

3.3.1.4. *Listeria monocytogenes*

In four (10.8%) articles investigating *L. monocytogenes*, 1.6%−−2.6% of raw and minced meat samples at retailer shops in Addis Ababa were found to be positive (Molla et al., 2005; Gebretsadik et al., 2011; Derra et al., 2013; Garedew et al., 2015b). 6.7%−12% of raw meat and minced meat samples from restaurants in Gondar showed contamination with *L. monocytogenes* (Garedew et al., 2015b).

3.3.1.5. *Staphylococcus spp.*

According to findings in three (8.1%) articles, up to 11.6% of samples from slaughterhouses and 19.7% from butchers were contaminated with *Staphylococcus aureus* (Beyene et al., 2017; Adugna et al., 2018; Bersisa et al., 2019). Authors indicated continuous contamination throughout transportation from slaughterhouses to butcher shops (Tolosa et al., 2016; Beyene et al., 2017). Beyene et al. (2017) reported 10.6% carcass swabs from slaughterhouse were positive for coagulase-negative *Staphylococci* in beef.

3.3.1.6. *Shigella* spp.

Three articles (8.1%) investigated *Shigella* spp. in beef. None of the beef samples ("kitfo" and raw meat) collected from restaurants and slaughterhouses were positive for *Shigella* spp. (Muleta and Ashenafi, 2001; Bersisa et al., 2019). However, 11% of raw beef samples from butchers were found to be contaminated with this pathogen (Garedew et al., 2016).

3.3.2. Dairy value chain

Bacterial pathogens in dairy value chains were reported in 23.4% (30/128) of the articles reported.

3.3.2.1. *Staphylococcus* spp.

Different *Staphylococcus* spp. were reported in 15 articles (50%). *Staphylococcus aureus* was common in milk at the farm (14.3%−73.2%) and up to 80% of milk samples at collection centers carried this pathogen (Almaw et al., 2008; Lakew et al., 2009; Daka et al., 2012; Haftu et al., 2012; Makita et al., 2012; Tigabu et al., 2015; Abebe et al., 2016; Tolosa et al., 2016; Ayele et al., 2017; Beyene et al., 2017). Coagulase-negative *Staphylococcus* was found in 5%−15% of milk samples collected from farms (Almaw et al., 2008; Lakew et al., 2009; Beyene et al., 2017). Baby bottle (made of cow milk, powder, and cereal blend) samples collected from outpatient infants visiting public clinics in Addis Ababa were contaminated with *S. aureus* (64.2%−68.3%) (Erku and Ashenafi, 1998).

3.3.2.2. *Listeria* spp.

Six (20%) articles looked at the presence of *Listeria* spp. in the dairy value chain. *Listeria monocytogenes* was detected in 4%−13% of raw milk samples collected from retailers (Gebretsadik et al., 2011; Garedew et al., 2015b). While pasteurization should kill this bacterium, an article reported that 20% of pasteurized milk samples from retailers carried this pathogen, likely derived from cross-contamination during processing (Seyoum et al., 2015). However, another article reported that none of the pasteurized milk samples at restaurants carry *L. monocytogenes* (Garedew et al., 2015b). Contamination rate of *L. monocytogenes* in locally produced cottage cheese was low (0%−1%) (Molla et al., 2005; Gebretsadik et al., 2011; Garedew et al., 2015b). On the contrary, 27% of cheese and 5% of yogurt from supermarkets were positive for *L. monocytogenes* (Seyoum et al., 2015). Also, two articles reported that 15%−20% of ice cream samples from retailers were contaminated with *L. monocytogenes* (Molla et al., 2005; Garedew et al., 2015b).

3.3.2.3. *Bacillus cereus*

According to five (16.7%) articles, 0.6 to 0.8% of milk samples collected from producers were positive for *B. cereus* (Almaw et al., 2008; Getahun et al., 2008). Up to 63% of milk samples from markets were contaminated with *B. cereus* (Ashenafi, 1990; Abraha et al., 2017). Erku and Ashenafi (1998) also identified contamination of baby bottle contents with *B. cereus*, including 38.3% of cow's milk samples, 42.8% in cereal blend and none in powder milk.

3.3.2.4. *Salmonella* spp.

Five studies (16.7%) investigated *Salmonella* spp. in the dairy value chain. Three percent to 20% of milk samples collected at dairy farms carried *Salmonella* spp. (Addis et al., 2011; Tadesse and Dabassa, 2012). While *Salmonella* spp. was absent in dairy products (cottage cheese and cream) or pasteurized milk, 6% of raw milk samples from retailers were found to be contaminated (Ejo et al., 2016). Erku and Ashenafi (1998) also identified *Salmonella* contamination of baby bottle contents (3.3% of cow's milk and 7.1% of cereal blends), but not in powder milk.

3.3.2.5. *Mycobacterium bovis*

Mycobacterium species were the focus in four (13.3%) articles. One article reported that 13% of farms with tuberculosis reactors had milk contaminated with *M. bovis* (Fetene et al., 2011). Between 3%−14% of tuberculosis infected animals were reported to shed *M. bovis* in their milk (Ameni and Erkihun, 2007; Elias et al., 2008).

3.3.2.6. *Escherichia coli*

Articles on *E. coli* were seen in four (13.3%) recently published articles on the dairy value chain (since 2017). Articles on milk found *E. coli* contamination increasing from 7% on farm to above 60% at retailer milk shops selling raw milk. This increase was attributable to post-farm contamination and lack of cold chain (Disassa et al., 2017; Bedasa et al., 2018; Haftay et al., 2018; Messele et al., 2019). An article reported that none of the pasteurized milk samples collected at cafeterias, restaurants, and supermarkets were contaminated with *E. coli* while milk derived products, like cheese (40%) and yogurt (25.7%), contained *E. coli* (Bedasa et al., 2018). Two articles detected *E. coli O157:H7* in pasteurized milk (5.7%) sampled from cafeterias,

restaurants, open markets, and supermarket but *E. coli O157:H7* was not detected in yogurt and cheese samples (Disassa et al., 2017; Bedasa et al., 2018).

3.3.3. Poultry and egg value chains

Ten (7.81%) of the included articles reported pathogens in poultry value chain. An article on chicken meat detected *E. coli* in 37% of samples from slaughterhouses (Messele et al., 2017). Off the 452 chicken meat samples from retailer shops, 11.5% were contaminated with Non-typhoidal *Salmonella* spp. (Molla et al., 2003). *Listeria monocytonenes* was detected in 1.9% of raw chicken meat samples collected from retailers (Molla et al., 2005). *Salmonella* spp. was isolated in 2.6%–18% of egg content and egg sandwich samples collected from either retailers, producers and restaurants (Muleta and Ashenafi, 2001; Bayu et al., 2013; Ejo et al., 2016; Kemal et al., 2016; Taddese et al., 2019). *Listeria monocytogenes* was detected in 4.3% of eggs sampled at retailer (Gebretsadik et al., 2011).

3.3.4. Vegetable value chain

Ten (7.81%) of the included articles investigated hazards in vegetable value chain. High parasite contamination rates were reported for a range of raw vegetables. Presence of at least one type of parasite (*A. lumbricoides, E. histolytica/dispar, G. lamblia*) in samples of raw vegetables (including green paper, carrot, tomato, cabbage, lettuce) was reported in 49%–71% samples (Bekele et al., 2017; Alemu A. S. et al., 2019; Bekele and Shumbej, 2019). Two articles looked at bacterial hazards in vegetables and reported *Salmonella* spp. in 0 to 10% of samples and *Shigella* spp. in 10%–20% of samples (Guchi and Ashenafi, 2011; Eromo et al., 2016). Contaminated irrigation water attributed to open air defecation was stated as a possible source of vegetable contamination at the farm (Alemu G. et al., 2019).

3.3.5. Prevalence of hazards in the environment

The selected articles included studies looking at food safety hazards in the environment of beef slaughterhouses (six articles), butcheries (three articles) and milk shops (two articles). However, these studies had small samples sizes (2–30 samples per study).

In slaughterhouses, *Salmonella* spp. was detected in workers hand swab (2%–50%), surfaces (50%), environmental pooled samples (6.7%), aprons (7.1%), knives (7.7%), room floor (23.5%), refrigerator (10%), beef transport truck (36.4%) and tap water (8.3%) used for washing (Sibhat et al., 2011; Edget et al., 2017; Hiko et al., 2018). *Escherichia coli* was present on 50, 23, and 50% of samples from equipment, environment pooled samples and workers hand swab, respectively (Edget et al., 2017; Bersisa et al., 2019). *Escherichia coli O157:H7* was identified on knife swabs (13.3%) and in environmental pooled samples (6.6%) at slaughterhouses (Atnafie et al., 2017; Edget et al., 2017). *Staphylococcus* spp. (*S. aureus*, *Staphylococcus intermedius*, and *Staphylococcus hyicus*) were detected in swab samples from knives (16.7%–33.3%) and hanging materials (33.3%–50%) (Beyene et al., 2017).

About 0%–6.6% cutting board swabs samples from butchers were positive for *E. coli O157:H7* (Atnafie et al., 2017; Beyi et al., 2017). Bersisa et al. (2019) reported 11.1% cutting board swab and 5.5% of knives swab in butchery shops positive for *Salmonella* species. In One article *E. coli* was found in 25% and 19.4% of cutting board and knives swab. This study also reported presence of other bacteria (*Klebsiella, Proteus*, and *Shigella* species) in butchery shops (Bersisa et al., 2019).

Articles found *S. aureus* contamination rate ranging between 12 and 25% in samples from milk buckets. Same rate was reported in milk tank samples (Ayele et al., 2017; Beyene et al., 2017).

3.4. Prevalence in clinically healthy food operators

Articles investigated (26/128, 20%) carriage of bacteria and parasites by food handlers, including workers in universities cafeteria (9/26, 34%), workers at dairy farms, abattoirs, and butchery (9/26, 34%), and workers in other food establishments (hotels, restaurants, bars, and cafeterias; 6/26, 23%). Nineteen (73%) and 12 (46%) of the 26 articles, respectively identified bacteria (mainly *Salmonella* spp., *Shigella* spp. and *S. aureus*) and parasites from stool samples collected from apparently health food handlers. Table 3 presents the range of contamination with different foodborne pathogens reported in the selected articles.

One article reported 6% of stool samples from abattoir staff carrying non-typhoidal *Salmonella* spp. (Molla et al., 2003).

Four articles reported isolation of different bacteria species on worker's hand swab samples. Results showed these swab samples were frequently positive for *Salmonella* spp., having been found in 24% of samples from butchers' shops operators and 30%–50% of samples of slaughterhouse personnel (Garedew et al., 2015a; Edget et al., 2017; Hiko et al., 2018). *Shigella* spp. was present in 13% of hand swab samples from butcher shops. *Staphylococcus aureus* was reported in 25%–32% of dairy farm milkers, and coagulase-negative *Staphylococcus* in 12%–16% of dairy and beef farm workers' hands. *Escherichia coli* was found in 50% of hand swabs taken from slaughterhouse workers (Garedew et al., 2016; Ayele et al., 2017; Beyene et al., 2017; Edget et al., 2017).

Swabs from fingernails examined for the presence of bacteria and internal parasites were often positive for coagulase-negative *Staphylococcus* (12%) and *S. aureus* (5%) (Mengist et al., 2018a).

3.5. Prevalence in non-food operators

Parasites were the most common foodborne hazard investigated in stool samples from children at school, patients visiting health centers and household members of community, including *A. lumbricoides* (4.2%–28%), *G. lamblia* (0.8%–10%), *Entamoeba* (6.7%–7.8%), *Trichuris trichuria* (0.4%–5.6%), hookworm (0.6%–1.3%) and other parasites (Desalegn et al., 2014; Alelign et al., 2015; Jejaw et al., 2015; Gebresilasie et al., 2018; Gizaw et al., 2018; Mekonnen and Ekubagewargies, 2019).

In stool samples from adult patients (mostly with enteric signs), *Salmonella* spp. (non-typhoidal *Salmonella* 7.18%–39.7%, typhoidal *Salmonella* 0.8%–39.7%, and unspecified *Salmonella* species 10.7%), *Shigella* (1.13%–13.86%), *Campylobacter* spp. (*Campylobacter jejuni* 7.3%–11.89%, *C. coli* 0.6%–3.5%), and internal parasites (*Entamoeba*, *Giardia* and *Cryptosporidium* in 24.6%–35.6% of the patients) were identified (Kassu et al., 2007; Ewnetu and Mihret, 2010; Tafa et al., 2014; Eguale et al., 2015, 2018;

TABLE 3 Pathogens detected from stool samples of food handlers working in food establishments.

Pathogens	%Min	%Max	References
Ascaris lumbricoides	1.1	45	Gebreyesus et al., 2014; Aklilu et al., 2015; Mama and Alemu, 2016; Solomon et al., 2018; Alemu A. S. et al., 2019; Asires et al., 2019; Bafa et al., 2019; Eshetu et al., 2019; Kebede et al., 2019
Entamoeba histolytica	2.9	39.5	Gebreyesus et al., 2014; Aklilu et al., 2015; Mama and Alemu, 2016; Gezehegn et al., 2017; Solomon et al., 2018; Alemu A. S. et al., 2019; Asires et al., 2019; Bafa et al., 2019; Eshetu et al., 2019; Kebede et al., 2019; Kumma et al., 2019
Taenia spp.	0.5	14.7	Gebreyesus et al., 2014; Aklilu et al., 2015; Mama and Alemu, 2016; Solomon et al., 2018; Alemu A. S. et al., 2019; Bafa et al., 2019; Eshetu et al., 2019; Kebede et al., 2019
Giardia lamblia	0.4	10.4	Gebreyesus et al., 2014; Aklilu et al., 2015; Mama and Alemu, 2016; Gezehegn et al., 2017; Solomon et al., 2018; Alemu A. S. et al., 2019; Asires et al., 2019; Bafa et al., 2019; Eshetu et al., 2019; Kebede et al., 2019; Kumma et al., 2019
Shigella spp.	0	10	Aklilu et al., 2015; Mama and Alemu, 2016; Marami et al., 2018; Mengist et al., 2018b; Bafa et al., 2019; Getie et al., 2019
Hookworm	1	8.1	Aklilu et al., 2015; Gezehegn et al., 2017; Solomon et al., 2018; Alemu A. S. et al., 2019, Asires et al., 2019; Bafa et al., 2019; Eshetu et al., 2019; Kumma et al., 2019
Salmonella spp.	0.9	6.3	Yemane et al., 2014; Aklilu et al., 2015; Mama and Alemu, 2016; Marami et al., 2018; Mengist et al., 2018b; Bafa et al., 2019; Getie et al., 2019
Enterobius vermicularis	2.3	4.9	Asires et al., 2019; Bafa et al., 2019
Trichuris trichuria	0.5	1.7	Aklilu et al., 2015; Bafa et al., 2019

Lamboro et al., 2016; Berhe et al., 2018; Deksissa and Gebremedhin, 2019). Acute gastroenteritis patients were positive for norovirus (25.3%) and less commonly for sapovirus (4.2%) (Sisay et al., 2016).

Among prison inmates in north Ethiopia, intestinal parasites were detected in 40% of sample population and the dominant parasite was *E. histolytica/dispar* (n = 60, 22.2%) followed by *G. lamblia*, 39 (14.4%) (Mardu et al., 2019). In Gondar, 37.0% of apparently healthy street dwellers carried *A. lumbricoides* (Moges et al., 2006).

An article exploring the risk of congenital transmission of *Toxoplasma gondii* showed that 85% of pregnant women monitored in a hospital in Ethiopia had seroconverted by the third trimester of pregnancy (Gelaye et al., 2015). In another article, 31.3% of pregnant women attending antenatal care at Gondar were infected with one or more intestinal parasites. The most common single and mixed parasites observed were *E. histolytica* (38.1%) and *A. lumbricoides* (24.6%) (Kumera et al., 2018). *Entamoeba histolytica*, *G. lamblia*, *Taenia species*, *A. lumbricoides*, and *Cryptosporidium parvum* were mainly identified in one article which determine the presence of intestinal parasites and associated risk factors among HIV/AIDS patients (Gedle et al., 2017).

4. Discussion

The literature on FBDs hazards in Ethiopia is patchy, mostly consisting of small *ad hoc* local investigations, with no single comprehensive overview of the topic. This is not a unique feature of Ethiopia and has been reported across Africa (Alonso et al., 2016). The majority of the studies were performed in Oromia, Amhara, Addis Ababa and Southern Nation Nationalities People (SNNP) region which may reflect local outbreaks occurring more frequently in this area due to presence of many food establishments and consumers (Addis Ababa and surrounding Oromia region). It may also reflect a biased picture, with studies performed where relevant research groups happen to be based. The Ethiopian FBD literature has focused on measuring food contamination with key biological hazards, especially bacteria, which are suggested to account for much of the FBD burden (Havelaar et al., 2015).

The identified articles focusing on the prevalence of FB hazards in humans mostly focused on parasitic infection in children (sampled from schools, health centers and community households), adult patients with enteric signs, and susceptible populations (pregnant women and HIV/AIDS patients). A few studies looked at, and reported findings from, presence of *Salmonella* spp. (typhoidal and non-typhoidal), *Campylobacter* spp. and *Shigella* spp. in blood and stool samples.

Some of the most important bacteria in terms of public health (e.g., non-typhoidal *Salmonella*, *L. monocytogenes*, and *Campylobacter* spp.), compared to *Salmonella*, *Staphylococcus* and *Shigella*, have received, to date, little attention in the country. Assessments of the amount of these bacterial pathogens present in food and food environments are very rare. Few of the included articles which reported pathogens in environment do not take representative samples which compromises the quality of work. However, such quantitative assessments are needed to estimate consumer-pathogen exposure and health and economic risk from FBD (Zaneti et al., 2021). The outputs of such assessments are a critical part of food safety management systems and are important for countries to prioritize food safety areas of interventions.

Hazards that are harder to study such as chemicals, viruses, and certain bacteria like *Campylobacter* spp., were investigated less frequently. This is a well-known challenge in low- and middle-income countries, where resources and facilities for diagnostics are often limited. In our review, we observed an increase overtime in the number of articles on FBD. This could be suggestive of an increased interest in the topic over the past few years, which could have been matched with increased funding for research in this area. It could also be a result of the quality of articles having improved overtime,

meaning that an increasing proportion of identified papers would have passed the review's quality assessment which is evidenced by increased number of included (five-fold) than excluded articles from 2015 to 2019. Both options are encouraging and suggest that more attention is being given to food safety and FBD among the national scientific and the international donor communities.

Foodborne pathogens, such as intestinal parasites, *E. coli* including O157:H7, *Salmonella* species and *S. aureus,* were commonly isolated on different foods and at different levels in various value chains. This is not surprising as the level of hygiene and the application of good practices of food quality management are highly variable across the country, but in general are limited, especially in rural areas. Even simple equipment, refrigeration, and key infrastructure, such as a reliable power and clean water supply are not available in informal food supply chains, where most people get their food (FAO, 2007; Glatzel, 2017) Even in the more up-market outlets, including supermarkets and restaurants, food safety is a challenge, given both the limited infrastructure and the relative lack of quality suppliers and quality management.

Beef and milk are widely consumed in Ethiopia and were often the target of the included articles. Although consumption of raw beef is a common practice in Ethiopia, hygiene standards in abattoirs are poor, with high levels of *E. coli* and *Salmonella* spp. For most pathogens, contamination rates are lower for samples of product collected in slaughterhouses compared to subsequent steps in the supply chain. In the case of meat, the butcher appears to be a node in the chain where levels of contamination tend to increase. Unhygienic practices, both at the slaughterhouses and retail shops, which underpin the public health risk associated with meat-borne pathogens, have been reported in Ethiopia (Gutema et al., 2021).

A variety of articles assessed hygiene and bacterial contamination of milk, typically finding high microbial contamination. Lack of cold chain and the presence of technical limitations by dairy operators were frequently reported as reasons for poor microbial quality of milk (Disassa et al., 2017; Bedasa et al., 2018; Haftay et al., 2018; Messele et al., 2019). *Escherichia coli*, *S. aureus*, and *Bacillus* spp. were the most prevalent bacteria identified in the milk value chain. A recent article showed that the proportion of contamination was significantly lower in milk collected from dairy farms when compared to milk from vendors (Berhe et al., 2020). Generally, presence of *E. coli*, *E. coli 0157:H7*, *Bacillus* spp, and *Listeria* spp was more likely in raw milk samples collected from retailers than those from producers, indicating that milk microbial quality may derived from contamination at various points of the value chain post-harvest, and that storage conditions are facilitating bacterial growth. The literature also showed contamination by foodborne pathogens of various milk derived products; it is worth noting that, in Ethiopia, these products are typically consumed without any further processing at home, meaning that no steps that could reduce the pathogen load are taken before consumption (Beyene et al., 2017; Amenu et al., 2019; Mebrate et al., 2020; Deneke et al., 2022). The presence of hazards in pasteurized milk reported in some of the studies is concerning. Pasteurization is a heat-treatment process used to decrease the bacterial load of milk. Presence of bacteria in pasteurized milk is indicative of failures in the pasteurization process, cross- or re-contamination post-pasteurization or inadequate storage after pasteurization (Garedew et al., 2012; Tekilegiorgis, 2018).

The importance of beef and milk processing points and practices to food safety, are highlighted in articles from beef and dairy value chains. Foodborne pathogens originating from fecal contamination during slaughter, such as *Salmonella* spp. and *E. coli,* can potentially contaminate the carcass and spread to the cut or raw meat intended for further processing, causing a major public health threat (Soepranianondo et al., 2019). This is supported by several of the included articles which show presence of different bacterial species in samples collected from different environmental surfaces at beef slaughterhouses (Hiko et al., 2015; Edget et al., 2017; Messele et al., 2017; Bedasa et al., 2018). However, extrapolation of these findings to the entire country could not be reliable due to the small sample sizes and geographical coverage in the majority of the articles reviewed.

Further evidence of sources of contamination along the food value chains is presented by the microbial investigations of apparently healthy food handlers. These records confirm the potential role of food handlers in the spread of FBD (Dagnew et al., 2012). Food handlers with poor health and hygiene may be infected with a wide range of foodborne pathogens and have already been demonstrated to play a role in transmitting disease to the public (Khurana et al., 2007). This is an important area in food safety research, and our results show that it deserves greater attention in the country.

There were no published assessments of FBD burden and incidence in humans. The SLR only included published literature but did not consider hospital records that are unpublished (gray literature), therefore, the study cannot assess the true burden of FBD. However, it is true that in Ethiopia, many foods are consumed raw (beef, milk) (Dagne et al., 2022; Deneke et al., 2022), therefore the risk of FBD is higher if the prevalence of pathogens in the product is high. Disease burden and cost estimates are critical for risk-based decision-making. Estimating the incidence of illness caused by FBD is a gap to be addressed in the future.

It is important for policy makers to know the burden of a disease in order to allocate appropriate resources for its control. However, FBD burden is harder to measure than food contamination, either requiring an effective FBDs surveillance system, which does not exist in many low- and middle-income countries, or well-designed, large epidemiological studies. These studies require complex analysis to overcome issues of under-reporting and imperfect diagnosis.

We acknowledge that the search being done only in two databases and required articles to be available electronically some articles may not have been detected. Therefore, information from gray literatures is not included. However, all quality research articles are expected to have been captured and inclusion of electronically available articles as a limitation to this review. Only 10% ($n = 13$) of the 128 articles were reviewed in pairs. This may also be one limitation of this review. In addition, publications since 2019 recent years are not considered due to time constraints and because the review was performed at the time to inform overarching research projects that started around 2019. Lastly, we cannot exclude the possibility that the results from our review are affected by publication bias, but we have no ability to estimate the magnitude of that potential bias.

5. Conclusion

In conclusion, little has been done to assess FBD burden in Ethiopia. The scientific literature reveals high levels of contamination, with both bacterial and parasitic pathogens,

and shows fundamental gaps in food safety for many food value chains. Pathogens that are hard to assess are largely over-looked. In both beef and dairy value chains bacterial contamination was observed with increasing prevalence from farm/slaughterhouse to point of sale. Given the findings, the following recommendations are made to improve food safety in Ethiopia:

1. More systematic and ongoing evaluation of contamination should be implemented to provide a comprehensive overview of the topic including in Benishangul-gumuz, Afar, and Somali regions.
2. Chemicals, viruses and some of the most important bacteria which are of public health concern should be investigated more.
3. Future research on FBD should thoroughly investigate risk factors.
4. The potential role of food handlers and food environment should be investigated in detail by considering representative samples.
5. In addition to assessing presence or absence of hazards, quantitative assessments of the amount of hazards present in food and food environments is required.
6. Due attention should be given to vegetables, fruits, crops, fish, sheep, goats, and camel value chains.
7. Incidence of human FBDs or resulting health and economic impacts should also be center of attention.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Conceived and designed the study protocol: DG, SA, FMu, KR, JL, KA, and MD. Carried out the screening and data extraction from records: DG, SA, FMu, KR, JL, LG, KA, FMa, PU, TG, and GI. Drafted the manuscript: LG, SA, and TK-J. All authors read, reviewed, and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1058977/full#supplementary-material

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Milk purchase and consumption patterns in peri-urban low-income households in Kenya

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Milk plays an important role in the growth and development of children. In Kenya, it is one of the most produced and consumed animal-sourced foods, but often consumed in small amounts among children of low-income families, especially in urban settings. The aim of the study was to identify household milk purchase and consumption patterns of milk, with emphasis on young children, as well as estimate key determinants of such patterns to identify areas of leverage to increase milk consumption. Results showed that 98% of households purchased unprocessed fresh milk at least once during the 7 days prior to the survey, while only 17% purchased packed pasteurized milk. Findings from the Ordinary Least Squares (OLS) model of purchase behavior suggest that the amount of unpacked milk purchased by households is positively and significantly related to household income, the number of children below the age of 4, and the budget of animal-sourced food. The price and quantities of pasteurized milk purchased were negatively related to the amount of unpacked milk purchased. Consumption patterns for children below the age of 4 showed that milk and dairy products are most commonly consumed as part of dishes than as individual products. Informal markets played a key role in meeting the milk needs of children, but consumption was below recommended amounts. The clear association of income and milk intake calls for efforts from the government to support the dairy sector with policies that promote the availability and affordability of milk, especially for a sector that feeds low-income families, as it is the case with the informal dairy markets.

KEYWORDS

informal markets, Kenya dairy, milk, purchase, consumption, OLS

1. Introduction

Milk plays a key role in early childhood development and contributes to food and nutrition security. In Kenya, it is a vital component of people's diets and a major source of animal protein (Dror and Allen, 2011). Milk consumption contributes to cognitive development, and it has been associated with reduction of dental caries, risk of high blood pressure, stroke, and heart disease (Bus and Worsley, 2003; Lönnerdal, 2003). Milk and dairy products contain various vital nutrients such as calcium, vitamins, potassium and sodium. Calcium is essential for healthy bone density and therefore a basic nutrient for preventing osteoporosis (Wham and Worsley, 2003). Vitamin D helps absorption of calcium, and vitamin A is essential for maintaining healthy vision (Black et al., 2002). Milk is therefore one of the most nutrient dense foods. Additionally, relative to other animal sourced foods, it is one of the most affordable and accessible for children in Africa (Grace et al., 2018).

Kenya is ranked as one of the biggest producers of milk in Africa. Kenya is also one of the countries with highest annual per capita consumption of milk in sub-Saharan Africa, estimated to be 110 liters (Rademaker et al., 2016), representing approximately an

average of 300 ml per capita per day. However, this falls short of the widely recommended annual per capita consumption of 220 liters (FAO, 2011). It is also below the recommended quantity of about 500 ml (2 cups) per child per day for children above age of 1 year (MoH, 2020). Based on existing data, the Kenya Dairy Board (KDB), the government agency that regulates and promotes the dairy sector in the country, anticipates a rate of growth of milk consumption of 5.8% due to increasing urbanization, rising middle class and regional milk exportation opportunities (MoALF, 2013; Gichohi, 2014; KDB Milk Intake, 2022).

Despite the significant GDP contribution by the Kenyan dairy sector, and the estimated growth patterns indicating great potential for increasing production and consumption, there are various challenges from the upstream (production) to the downstream (consumption) end of the Kenyan dairy value chain. For instance, according to KDB (2014), only 55% of total production is sold at farm gate or beyond, with the rest consumed at the farm or given to calves (KDB, 2014; Rademaker et al., 2016). Of the amount marketed, the bulk of it (over 85%) is sold unprocessed (i.e., unpacked[1] and mostly unpasteurized) in informal dairy markets (FAO, 2011; Gichohi, 2014; KDB, 2014).

Whereas the informal dairy markets offer affordable and accessible milk to middle and low-income households – they market unprocessed milk, cheaper than the processed milk, and sold in different quantities depending on consumer's affordability – such marketing channels are often operating at the margins of regulations (Nyokabi et al., 2021). This informal sector is deemed by the regulatory authorities to pose food safety and quality concerns, as it largely operates unregulated (Alonso et al., 2018; Kangethe et al., 2020; Muunda et al., 2021; Nyokabi et al., 2021). Such concerns are valid as they influence the global disease burden (Grwambi, 2021). However, the role of informal markets in food supply and nutritional security for the urban economically underprivileged and as a source of livelihood for smallholder producers and retailers cannot be ignored while addressing such concerns (Roesel and Grace, 2014; Apaassongo et al., 2016).

Due to the above challenges, the Kenyan government, dairy development, and regulatory agencies have over the recent years designed policies and development interventions aimed at streamlining the sector, in terms of safety and quality regulations, and promoting milk consumption among Kenyans. Often, such interventions advocate for the elimination of the informal dairy marketing channel, which has the largest market share, in favor of the formal dairy markets, encouraging consumers to buy processed milk or adopting punitive approaches on the informal sector operators. This step has been taken by other developing countries like South Africa (Agenbag et al., 2012), Tanzania (URT, 2007), Rwanda and Uganda (EPRC, 2012).

As well intentioned as such policies may be, previous studies looking at their impact have hardly shown improvements on food safety (Leksmono et al., 2006; Kaitibie et al., 2010). A recent study also showed that such policy intervention affects the amount of milk low-income households allocate to children and reduced general intake in the household (Muunda et al., 2021). In order to develop policies that improve food safety and maximize public health without compromising nutritional outcomes, policy makers in low- and middle-income countries need evidence on the contribution of the informal dairy markets to the milk needs of households and what factors influence dairy consumption in those households (Cornelsen et al., 2016; Dominguez-Salas et al., 2016).

Given the nutritional value of milk, and the role that informal markets play in meeting the nutritional demands of low-income households, this information would help development of more responsive regulatory policies that are meant to address food safety and quality concerns, while preserving the role of the dairy sector to support nutrition security in low- and middle-income countries. An in-depth understanding of the milk purchase and consumption patterns is important not only for food policy formulation but also in the design of successful dairy sector development interventions. Also, given Kenya's relatively low per capita milk consumption, characterizing factors associated with milk purchasing and consumption patterns can help in identifying market expansion opportunities. This study therefore aims at revealing insights into household milk purchase and consumption patterns of milk, with emphasis on young children, as well as estimate the key determinants of such purchase and consumption patterns.

2. Materials and methods

2.1. Data collection

The study uses cross-sectional data collected in Dagoretti region (including Dagoretti North and South sub-counties) between April and June 2017. The study area is a peri-urban setting characterized by low-income households and informal settlements with limited agricultural production. The study included 200 households identified through randomly selected geospatial random points generated by ArcGIS 10.4.1©. Inclusion criteria entailed households that purchased milk from informal milk markets and had at least one child between the age of 6–48 months. The targeted sample included an age group for which feeding, and nutrient intake, is critical for growth and development, while offering a window to capture different milk feeding practices in children of various ages among peri-urban dwellers. For the purposes of this study, a household was defined as a group of people that take food from the "same pot" (economic basket). A household member was someone who had eaten in the household for at least 6 months preceding the survey date, and at least half of the week in each week in those months, and at least one of the two main daily meals. Additional details are also available in Muunda et al. (2021) and as Supplementary material.

A structured questionnaire was developed on open data kit (ODK) system, pretested, and revised to collect data on 7-day household's purchase of milk and other dairy products, milk consumption by each household member, and perceptions on milk quality and safety. The survey also included questions on household income and expenditure, and demographic characteristics like household size, composition, household members' age, occupation, and educational level.

1 Unpacked milk in this paper is used to refer to unprocessed milk at the time of selling. It does not mean that children consume the milk in raw form. Research shows that over 98% of households boil milk before consumption (Walke et al., 2014) or use as part of cooked dishes like tea and porridge (Grace and McDermott, 2015).

2.2. Data analysis

Data was extracted from ODK aggregate server, processed, and analyzed using STATA version 15.1. Descriptive statistics were generated for variables of interest such as household purchases and individual consumptions of milk and dairy products. Pairwise statistics and test of independence were used to investigate the effects of household demographic and socioeconomic characteristics on consumers' milk purchase and consumption patterns. The results are presented using descriptive statistics, tables, and graph illustrations.

2.3. Model estimations

Ordinary Least Squares (OLS) model was used to estimate the extent to which the household characteristics influenced the quantity of milk household *i* purchased from informal markets. We regressed the quantity of milk purchased from the informal market (unpacked milk) as a function of socioeconomic and demographic characteristics of the households. The rationale behind focusing on the informal market is the overall bigger share that it commands in the dairy sector, and any intervention in it would affect not only the market performance of the sector but also the nutrition needs of households significantly. These models are appropriate in describing the purchase and consumption behavior when households are faced with a variety of goods with a common consumption objective and under constraint budgets. Therefore, the average amount of milk purchased by a household, and amount allocated to a child below 4 years in the i^{th} household will be determined as (Sichilima et al., 2015):

$$Q_i = f\left(Y_i, HH_i, G_vP_i, Educ_i, Z_i\right)$$

Where:
Q_i is the average amount of unpackaged milk purchased
Y_i is the household income level
HH_i is the size of the household
G_i is the gender of the household head
P_i is the price of milk purchased
$Educ_i$ is the education level of the household head
Z_i a set of the other socioeconomic and demographic factors for the ith household like, age of the household head, number of children below 4 years, education level of spouse etc.

3. Results and discussion

In this study, <1 in five of the households interviewed were female headed, and most of these were single parent households. Average household size was four members. Two thirds of household heads were over 30 years old, and half had attained at least secondary education. Employment rate was high, with most household heads having a job. Moreover, in 40% of the households, the spouse was also engaged in a remunerated activity. Table 1 shows a detailed summary of the socioeconomic and demographic characteristics of the study sample.

TABLE 1 Descriptive statistics of the sample (*n* = 200).

Variable	Categories	% or mean ± SD
Sex of respondent	Female	98.0
Sex of the household head	Male	83.0
Age of the household head	18–29 years	36.0
	30–39 years	44.5
	40–49 years	13.0
	50years old and above	6.5
Highest education level of the household head	Primary school (class 1–8)	30.0
	Vocational school	3.0
	Secondary school (form 1 to 4)	47.0
	Technical college/Diploma	18.0
	University/Degree	2.0
Marital status of the household head	Married living with spouse	84.0
	Married living separately	2.5
	Single/divorced	11.5
	Widow/widower	2.0
Primary activity of the household head	Unemployed/Retired	3.5
	Employed/laborer	69.0
	Self-employed	27.5
Primary activity of the spouse to household head	Unemployed/Retired	58.29
	Employed/laborer	21.11
	Self-employed	20.60
Number of household Members	Two	4.0
	Three	30.0
	Four	31.0
	Five	18.0
	More than five	17.0
Number of children 6 – 48 months living in the household	One	85.5
	Two	12.5
	Three	2.0
Household monthly income (USD)[a]	<101	19.10
	Between 101 and 200	36.68
	Between 201 and 300	44.22
Average household size	Number	4.3 ± 1.5
Average food expenditure in USD for the last month (per income category)	<101	53.22 ± 4.11
	Between 101 and 200	78.641 ± 3.87

(Continued)

TABLE 1 (Continued)

Variable	Categories	% or mean ± SD
	Between 201 and 300	104.66 ± 4.23
Average non-food expenditure in USD for the last month (per income category)	Less than 101	49.21 ± 6.53
	Between 101 and 200	65.54 ± 3.53
	Between 201 and 300	106.00 ± 5.46
Primary market for purchasing milk	Producer gate	12.08
	Home delivery (door vendor)	2.42
	Street vendor	5.79
	Corner shop/Kiosk	40.1
	Milk bar (Dairy shop)	11.11
	Milk dispenser	22.71
	Supermarket	4.83
	Other	0.97

[a]We use a conversion rate of 1USD = KES100, the average market exchange rate at the time of the study.

3.1. Low-income households milk purchase patterns

All the households purchased milk from informal markets on regular basis, as per the selection inclusion criteria. Almost all surveyed households had purchased milk from these markets at least once in the 7 days prior to the visit and, those few who had not purchase milk in the previous 7 days, declared consuming milk from their own dairy animals. Only 17% had purchased packed pasteurized milk. Table 2 shows a summary of the average amounts of milk and dairy products purchased in the 7 days prior to the visit, mean weekly expenditure and frequency of purchase. Average price per liter increased with increased processing of dairy products, with raw unprocessed milk costing the cheapest and powdered milk, followed by packed fermented milk, being the most expensive. Unsurprisingly, the average amount of dairy products purchased by families decreased as the level of processing and industrialization of the products increased, and hence its price, increased.

Figure 1 shows the household dairy weekly purchase patterns by income category. For most products, the weekly amounts purchased differed across income groups, with purchase being lower in lower income households (USD $\leq$ 100/month). Generally, 98.5% of the households purchased unpacked raw milk at least once in a week compared to other dairy products. This could possibly be due to our selection criteria that required households to be primarily purchasing milk from informal markets, and also based on the fact that raw milk is cheaper compared to the rest of dairy products.

An in-depth analysis of the amount of milk purchased from informal markets indicated that there were statistically significant variations across various socioeconomic and demographic factors of the households. Table 3 shows statistical associations between the quantity of milk purchased from informal markets and household characteristics. Pairwise comparisons of average milk purchased from the informal markets revealed that there was a statistically significant difference between the mean weekly purchase of the lower income tier (below 100 USD) vs. the higher income tier (201–300 USD) ($p < 0.001$) and a marginal statistically significant difference across gender of the household head ($p < 0.07$). There were no statistically significant differences from the pairwise comparison of education level of the household, primary activity and education level of the spouse and marital status of the household head (data omitted and not included in Table 3).

3.2. Factors that influence households' milk purchase

Table 4 reports that milk price, number of children in the household below 4 years, budget of animal source foods and household income were found to be key determinants of the quantity of milk purchased per week from the informal markets. The difference in the quantities purchased by households with head aged between 40 and 49 years old from the reference age (18–29) was found to be statistically significant (P-value = 0.059). There were no differences between the base level and the other age categories. Previous research shows that life cycles of consumers influence preferences and tastes and hence affect consumption patterns (Yayar, 2012). The results from the OLS model indicated that households with younger household and the oldest heads purchased more quantities compared to their middle-aged counterparts. This is likely the case because younger households tended to have more children under the age of 4 years. High and positive coefficient of spouses that have attained tertiary education was found to be statistically different from those that had attained primary education only.

The results in Table 4 indicate that the number of children under the age of 4 years in the household significantly and positively affects (coefficient: 0.096; p-value: 0.063) the amount of milk purchased from the informal markets. In other words, the higher the number of children in the household, the more unpacked milk is purchased by the household, while the amount of processed milk (pasteurized or UHT) does not change according to household size. This agrees with the findings of two studies in Turkey that characterized the determinants of packed and unpacked milk purchase decision (Akbay and Tiryaki, 2007; Yayar, 2012). Both studies also found that as the household size increased, chances of purchasing milk from formal markets (packed milk) decreased. This reveals once more the strong household dependence on the informal markets to meet their nutritional needs, and how much strong that link is as the number of household members increases and hence the food needs of the family.

The price of unprocessed milk was found to be one of the main factors influencing the quantities of milk that households purchase (Coefficient: −0.002, p-value = 0.000). Generally, low-income households are sensitive to prices of animal sourced foods (Schneider, 2018). This implies that the households with lower incomes are likely to be sensitive to prices and would likely purchase less amounts of milk should prices increase by a unit. This agrees with (Cornelsen et al., 2015) that increasing the price of milk results into reduction of the amount consumed by households.

TABLE 2 Households purchasing patterns for different dairy products in the past 7 days ($n = 200$).

Dairy product	Households purchasing the product	Frequency (days/week) purchased	Amount (liters)	Price/liter (KES)	Weekly expenditure (KES)
	% (n)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Unpacked* raw milk	98.5 (197)	5.6 (2.07)	3.8 (2.77)	76.0 (15.09)	287.5 (207.3)
Unpacked fermented milk (mala)	2.0 (4)	1.0 (0.00)	0.65 (0.23)	100.8 (27.54)	1.3 (9.68)
Unpacked yogurt	1.0 (2)	1.0 (0.00)	0.38 (0.04)	170.0 (42.43)	0.6 (6.07)
Packed pasteurized whole fresh milk	17.0 (34)	3.4 (2.10)	0.3 (0.86)	117.7 (16.70)	35.1 (103.44)
Packed fermented milk (mala)	2.0 (4)	1.5 (1.00)	1.06 (0.22)	210.0 (77.46)	3.5 (31.58)
Packed yogurt	39.0 (78)	2.2 (1.84)	0.3 (0.68)	188.9 (70.08)	61.4 (127.84)
UHT milk	7.0 (14)	3.6 (2.53)	0.1 (0.50)	135.4 (15.75)	15.0 (69.52)
Powdered milk (kg)	1.0 (2)	1.0 (0.00)	0.0 (0.01)	1000 (0.00)	0.4 (5.35)

*Unpacked milk refers to dairy products that do not undergo industrialized processes (e.g. unpasteurized milk, traditionally made value added products).

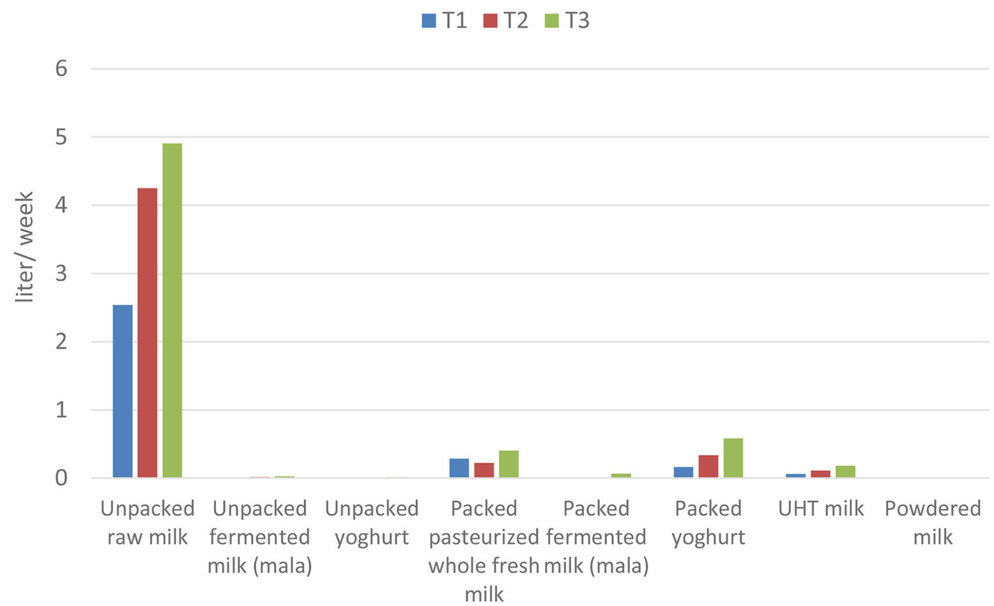

FIGURE 1
Household mean weekly purchase of dairy products, by income group. T1 = Lowest income group (<100 USD/month), T2 = Middle income group (101–200 USD/month), T3 = Highest income group (201–300 USD/month).

TABLE 3 Comparison of average amount of milk purchased from informal markets over 7 days across socioeconomic and demographic groups.

Household characteristic	Item / Comparison	Overall test statistic: Mean Difference	*P*-value	Bonferroni 95% CI	
Income	Between 101 and 200 USD vs Below 100 USD	0.423	1.000	−0.86682	1.71218
	Between 201 and 300 USD vs Below 100 USD	2.163	0.000	0.912152	3.414774
	Between 201 and 300 USD vs 101 USD and 200 USD	1.741	0.000	0.720256	2.761305
Household head gender	Male vs Female	0.965	0.069	−0.07626	2.007127
Milk market choice	Also Purchasing from formal market vs Not Purchasing from formal market	0.718	0.076	−0.07508	1.510744
Primary activity of household head	Unemployed vs Employed	−0.904	1.000	−3.49114	1.683813
	Unemployed vs Self employed	0.482	1.000	−2.19791	3.161289
	Self-employed vs Employed	1.385	0.006	0.319459	2.451245

TABLE 4 OLS estimations of determinants of amount of milk purchased form the informal market.

Socioeconomic and demographic factors		Coefficients	Std. Err.	*P*-value
Constant/Intercept		0.914***	0.169	0.000
Price of unpacked milk		−0.002***	0.001	0.000
Age of Household head	18-29years	(Omitted)		
	30-39 years	−0.022	0.056	0.702
	40-49 years	−0.166**	0.088	0.059
	50 years and above	0.038	0.106	0.722
Household head primary activity	Unemployed	(Omitted)		
	Employed	0.235*	0.139	0.091
	Self-employed	0.183	0.146	0.210
Spouse Education	Primary education	(Omitted)		
	Secondary education	−0.001	0.053	0.999
	Tertiary education	0.150*	0.081	0.067
Spouse primary activity	Unemployed	(Omitted)		
	Employed	0.004	0.072	0.960
	Self-employed	0.0374227	0.073	0.608
Household Income	Sh10,000 and below	(Omitted)		
	Sh10,001 and 20,000	0.097	0.070	0.172
	Sh20,001 and 30,000	0.150**	0.074	0.046
Animal sourced food expenditure		0.122×10^{-3}**	0.001	0.048
Quantity of milk purchased from formal sector		−0.036	0.025	0.155
Number of children below 4 years old		0.096*	0.051	0.063
Gender of household head		0.058*	0.078	0.457
		No. of observations		197
		F (16, 180)		25.17
		Prob > F		0.000
		R-squared		0.6911
		Adj R-squared		0.6637

*, **, *** Statistically significant at the 10%, 5%, and 1% levels, respectively.

Gender of household head emerged as a key factor in characterizing the purchase and children's consumption of milk from the informal sector. This is clearly shown by the differences in the quantities purchased and consumed in households led by either gender. This agrees with findings of Grace and Roesel (2015) and Flax et al. (2021) that gender roles vary in sale, purchase and consumption of food from informal markets. They concluded that gendered investigations into knowledge and preferences associated with market and food choices would be vital in addressing food safety and consumption issues at the household level.

It has been hypothesized that education level is a key determinant of knowledge, attitudes and practices and possibly an influencer of purchasing decisions. Our results showed that households with better educated spouses – of which majority are women – purchased higher quantities of milk (P-value = 0.067) compared to those with lower education level. Considering the strong association between level of education and employment status of spouses (P-value = 0.039) in our sample, better educated households are likely to be the ones with higher incomes. Hence, it is not possible to tell whether the increased milk purchase in better educated households is driven by education itself, or rather by their higher incomes. This also agrees with a study conducted to analyze determinants of purchasing decisions of mothers with young children (Flax et al., 2021).

3.3. Dairy consumption patterns of children (6–48 months)

As per the research findings reported in Table 5, only 36.8% of the children consumed dairy as is. Majority of those that consumed milk as part of other dishes took it in porridge, tea, mixed in scrambled cake or in cereals. On average, a child consumed slightly above half a liter of unpacked milk in the previous 7 days as reported in Table 6. As demonstrated in Figure 2, there was tendency of children to consume higher quantities of various dairy products as household income increased. However, this consumption is considerably below the recommended amounts of two cups (500–600ml) of milk per child

TABLE 5 Proportion of children between 6 and 48months consuming dairy products.

Dairy product	% of children consuming dairy products*	% children consuming dairy as it is*	% of children consuming dairy as part of a dish*
Unpacked raw milk	98.69	36.80	77.50
	(228)	(85)	(179)
Unpacked fermented milk (mala)	1.72	0.91	0.00
	(4)	(2)	(0)
Unpacked yogurt	0.93	0.41	0
	(2)	(1)	(0)
Packed pasteurized fresh milk	16.51	6.12	10.0
	(38)	(14)	(23)
Packed fermented milk (mala)	2.62	2.23	01
	(6)	(5)	(0)
Packed yogurt	39.42	35.51	0.00
	(91)	(82)	(0)
UHT milk	6.51	3.01	4.80
	(15)	(7)	(11)

*Count in the brackets.

TABLE 6 Mean consumption of dairy products by children between 6 and 48 months.

Dairy product	Child Mean consumption in ml/week**	Child mean consumption by percentiles (ml/week)		
		P25	P50	P75
Unpacked raw milk	693.01	288.50	576.99	961.66
	(585.34)			
Unpacked fermented milk (mala)	0.61	0.00	0.33	1.11
	(0.74)			
Unpacked yogurt	0.22	0.00	0.23	0.45
	(0.3)			
Packed pasteurized fresh milk	29.23	5.46	17.73	38.19
	(31.80)			
Packed fermented milk (mala)	0.81	0.38	0.38	0.63
	(1.03)			
Packed yogurt	58.22	14.43	28.87	49.49
	(147.27)			
UHT milk	12.90	3.77	8.62	21.12
	(11.59)			

**Standard deviation in the bracket.

per day (MoH, 2010) and below the national average (Rademaker et al., 2016).

In this study, as presented in Table 6, unprocessed products were the ones consumed in higher amounts by children. The amounts consumed declined as the level of processing of the product increased. It also decreased with declining household monthly income as shown in Figure 2. Unpacked milk was the most consumed type of dairy, and it was drunk by 98.7% of children during the 7 days previous to our visit. This is in line with the reported purchase pattern of 98.5% of the households interviewed.

Finally, from the study results demonstrated in the household's purchase and children's consumption patterns, it is evident that informal markets play a key role in meeting low-income household milk and dairy product demand. They offer affordability and easy access to an important food item that would otherwise be inaccessible given the effect of prices and household budgetary constraints. Additionally, informal markets offer a source of livelihood to smallholder actors from the rural areas (production end) to the urban markets (vendors) (Apaassongo et al., 2016). Previous studies have, however, assessed risk and reported various food safety concerns in

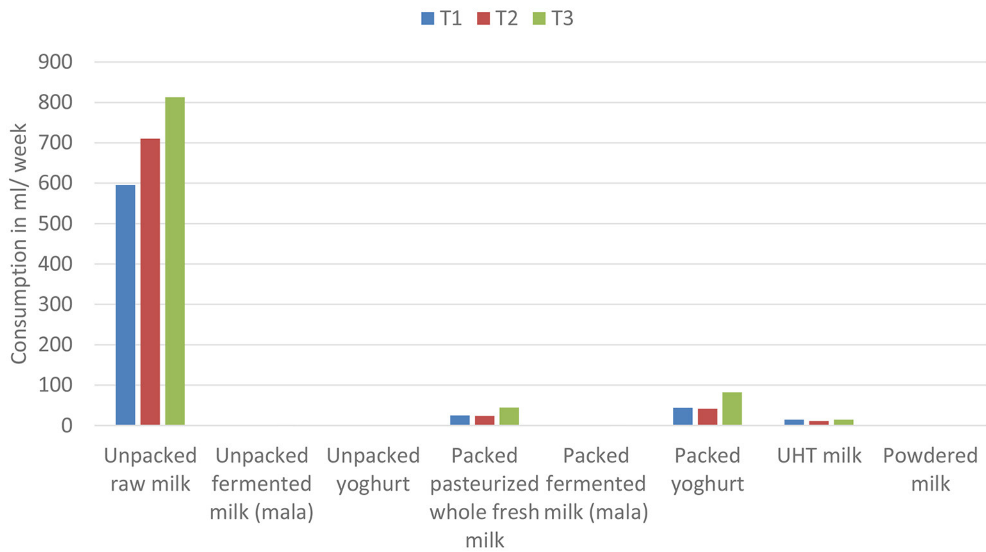

FIGURE 2
Average consumption of dairy products of children 6–48 months per week, by income groups. T1 = Group with lowest income of <100 USD/ month, T2 = Middle group with an income between 101 and 200 USD per month, T3 = Wealthiest group with an income between 201 and 300 USD/month.

these informal dairy markets in developing economies, such as Kenya (Zyl et al., 2006; Grace et al., 2010; ILRI, 2012; Grace, 2013; Lapar et al., 2014). They proposed various approaches that can address such concerns (Grace et al., 2014; Lapar et al., 2014; Johnson et al., 2015). Formulating effective dairy policies, therefore, require a broader understanding of the interlinkages within these parameters and identifying how to strengthen food markets without triggering unintended negative effects in other aspects of food security.

4. Conclusions and policy implications

This study sought to reveal insights into households' dairy purchase and consumption patterns, focusing on milk from informal markets. It also aimed at characterizing determinants of those purchase patterns, and how such determinants influence quantities of milk available to be feed to children below the age of 4 years. Data on consumption patterns of the various types of milk and milk products by children can inform interventions that target the health and nutrition status of children. It can also be used to estimate market needs for the different milk products, information relevant to governments, producers, processors and retailers.

We hypothesized that the choice and quantities of dairy purchased by the households is influenced by socioeconomic and demographic characteristics of the households. In line with other studies conducted in similar contexts, the study confirmed this hypothesis and identified as positive determinants of quantity of milk purchased the budget allocated to animal sourced food, the number of children below 4 years living in the household, the age of the household head and education level of the spouse. The quantity purchased from formal markets within the same period negatively affected the amount purchased from informal market.

Beyond the demographic drivers of quantity purchased, this study showed that the lower the income of the households, the less diversity of dairy products purchased. Other factors like household size and price of commodity – which is higher for industrially processed products – are factors that are associated with lower milk purchase. The price of informal dairy products negatively and significantly affected the quantity purchased.

Looking at the consumption patterns of children, this study shows that informal markets play a key role in nutritional requirements of children. However, the consumption is still below the recommended amounts of at least 2 cups of milk a day. The findings also show that majority do not consume as milk but as an ingredient in other food items, hence lowering the quantities of milk consumed by children. These are key areas for policy consideration when attempting to promote milk intake and support the development of the dairy sector in the country.

Given the clear association of income and milk intake, our results would call for the dairy sector, and food policies generally, to consider developing regulatory measures that are cost-oriented in order to minimize the price effect of commodities to low-income consumers, especially in urban set-ups where there is limited own production. Such measures include reducing or subsidizing inputs of production, providing financial credits along the nodes of the value chain that undertake processing, reducing tax of retail dairy products and promoting investment that improves general food safety and quality. This can potentially create price-resilient households hence shifting preference to other factors of demand.

4.1. Limitations of the study

The findings of this research should be interpreted accounting for its limitations. The selection criteria targeted households that purchased milk primarily from informal markets and have a child between 6 and 48 months. This limits the extent to which it is comparable to other studies and hence caution is required. Secondly, whereas the study was restricted to a specific region, it only provides an outlook of a peri-urban set up and the results apply in such

context. For broader policy, a bigger sample size and study setting would be required to capture as much variability as possible. Thirdly, this was a cross-sectional survey using a 7-day recall period for purchase and consumption patterns. This may differ over a longer period given that there are other external factors like taste, cultural beliefs and perceived nutritional value of food that affect purchase and consumption patterns in a household faced with a budget constraint. For example, seasonality affects milk availability and hence prices, price of other animal sourced food items, age of children and school schedules. Further research is therefore required to untangle more drivers of purchase and consumption that would guide governments and stakeholders on interventions aiming at promoting dairy sector as a pathway to food security and safety.

Data availability statement

The datasets presented in this article are not readily available because the raw data can only be provided in anonymized form in line with affiliate institutional data management policy. Requests to access the datasets should be directed to EM, e.muunda@cgiar.org.

Ethics statement

The studies involving human participants were reviewed and approved by the International Livestock Research Institute—Institutional Ethics Research Committee (ILRI-IREC2018-17). Permission to conduct the survey was also obtained from NACOSTI and the relevant local authorities. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EM: methodology, software, formal analysis, investigation, and writing—original draft. NM: conceptualization, methodology, review, visualization, supervision, and funding acquisition. EB: supervision and writing—review and editing. FW: data curation. SA: conceptualization, resources, writing—review and editing, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1084067/full#supplementary-material

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A glance into traditional pig slaughtering practices in Vietnam and opportunities for zoonotic disease prevention

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Introduction: African swine fever in Vietnam is contributing to existing concerns over zoonotic disease transmission from sick pigs to humans. While slaughterhouses are key sites of occupational hazards to workers and contamination of meat, the specific slaughtering practices contributing to zoonotic occupational and foodborne disease risks remain under-researched. Our objective is to identify and characterize aspects of pig slaughtering processes that contribute to such risks.

Methods: We draw on qualitative observations, photos, and videos from three mobile slaughterhouses and seven abattoirs in Hung Yen, Vietnam.

Results: Based on our analysis, areas likely leading to zoonotic disease risks include slaughtering procedures, personal hygiene of workers, equipment sanitation, and facility sanitation. Within the small-scale swine industry, slaughtering practices are long-standing and difficult to change.

Conclusion: Our study underscores the importance of hygiene training of workers, improvements to equipment and facilities, and awareness-building activities targeting consumers to reduce the burden of zoonotic disease risks in small-scale pig slaughter settings.

KEYWORDS

food safety, pigs, Vietnam, slaughterhouse, zoonotic disease, One Health

1. Introduction

Since 2019, the impacts of African swine fever—exacerbated by the COVID-19 pandemic—have been contributing to concerns over zoonotic disease transmission from sick pigs to humans in Vietnam (Nguyen-Thi et al., 2021). These concerns are developing against the backdrop of already high existing worries about food safety in Vietnam among consumers, where repeated episodes of unsafe food practices receive widespread media attention (Nguyen-Viet et al., 2017). In response, the Vietnamese government has focused efforts on centralizing swine husbandry, based on the assumption that large-scale and regulated production will not only enhance productivity but also disease control (Nguyen et al., 2021).

Despite efforts to centralize Vietnam's swine industry, most pork production continues to occur among an informally defined network of small-scale actors from production to retail (Nguyen Thi Thuy et al., 2020). For example, at the point of slaughter, facilities of all types exist, from household slaughterhouses to intermediate-sized abattoirs and centralized facilities, though household slaughterhouses dominate the domestic pork supply. There also exists mobile

slaughtering, particularly in rural areas, where farmers hire workers to slaughter pigs directly on farms. Importantly, no formal government oversight exists for defining, guiding, and monitoring small-scale pig slaughtering operations (Dang-Xuan et al., 2016; Pham and Dinh, 2020). Occupational exposure to sick pigs and consumption of contaminated pork are important risk factors for zoonotic diseases associated with swine such as bacteria (e.g., *Salmonella spp.*, *Streptococcus suis*), parasites (e.g., *Trichinella*), and viruses (e.g., swine influenza) (Van De et al., 2015; Takemae et al., 2017; Rayanakorn et al., 2018). While slaughterhouses are key sites for occupational hazards to workers and contamination of meat, the specific slaughtering practices contributing to zoonotic disease risks in Vietnam remain underexplored (Hoa et al., 2011; Ho et al., 2011; Nguyen et al., 2021). This paper aims to identify and characterize risky pig slaughtering practices, and in doing so, inform interventions to decrease zoonotic disease transmission and address food safety concerns.

2. Methods

2.1. Context

This research was conducted as part of PigRISK, a 5-year program (2012–2017) that aimed to reduce disease risks and improve food safety in smallholder pig value chains in Vietnam (Lam et al., 2016). The core objectives of PigRISK were: (1) To assess the impacts of pork-borne diseases on human health and the livestock sector and to identify opportunities for risk management; (2) To develop incentive-based innovations to improve the management of human and animal health risks in smallholder pig value chains; and, (3) To improve capacity to assess and manage risks in smallholder pig value chains by engaging stakeholders. This study contributes to the first objective of PigRISK by characterizing risky pig slaughtering practices.

PigRISK was guided by One Health, a conceptual framework considered helpful for tackling food safety issues in low- and middle-income countries (Grace, 2017). One Health calls for different disciplines to work together on complex challenges at the intersection of human, animal, and environmental health (Zinsstag et al., 2011; Nguyen-Viet et al., 2022). Responding to this call, PigRISK mobilized researchers from different fields and institutions including public health (Hanoi University of Public Health), agricultural economics (Vietnam National University of Agriculture), and veterinary epidemiology (International Livestock Research Institute). Our multidisciplinary research team also worked closely with program participants (local authorities and slaughterhouse workers) to shape the direction of PigRISK activities.

2.2. Study site

We selected small-scale slaughterhouses and mobile slaughter settings in Hung Yen Province, where the PigRISK research team had developed longstanding relationships with local authorities. Hung Yen is 47 km southeast of Hanoi, the capital city of Vietnam; its proximity to urban markets has contributed to the province experiencing rapid, unplanned, and demand-driven development for pork (Dang-Xuan et al., 2016). Hung Yen has a population of around 1.2 million people across 10 districts (Hung Yen Portal, 2021). It is characterized by primarily rural areas with small-scale (<10 pigs/day) and medium-scale (11–50 pigs/day) pork processing settings (Dang-Xuan et al., 2017). Hung Yen represents a more intensive pork production system compared to other provinces considering pork in other provinces is mostly supplied by small-scale producers (Nguyen-Viet et al., 2019). We focused on two districts in Hung Yen (Van Giang and Van Lam) which have representation from different scales of pork production. We selected slaughterhouses that met the criteria of (1) operating every day and (2) operating at either small- or medium-scale. These criteria applied to both abattoir and mobile slaughter settings. To obtain a glimpse into traditional pig slaughter practices, only a subset of slaughterhouse settings that met the selection criteria in the two districts were included; these slaughter settings were randomly selected.

2.3. Data collection and analysis

We applied an observational study design to identify and characterize risky pig slaughtering practices because of its potential to enable researchers to visualize and contextualize behaviors most people likely would not describe in an interview (Harvey, 2018). Specifically, we documented pig slaughtering practices primarily through field notes. We also took photos and recorded videos to provide richer data on such practices (Asan and Montague, 2014). The process involved visiting one to two slaughter settings in the early mornings (e.g., around 3 am). On average, two to three slaughter settings were visited per week over the course of four weeks in July 2013. Two researchers conducted observations and interacted with workers. Around two to three workers were present at each slaughter setting and were all engaged in our study. While we did not ask workers about their education and training, previous research suggested workers mainly acquired knowledge and experience of pork handling practices through "learning by doing" rather than a specific training program (Dang-Xuan et al., 2016).

Then, through regular discussions among our multidisciplinary team, we generated hypotheses for practices that could contribute to (1) human infection from occupational exposure and (2) the potential for contamination of pork products. To guide this process, we drew on standard procedures for slaughterhouse operations in developing countries (Mann et al., 1983; Heinz, 2008) while remaining open to other practices that might not be documented in written procedures. Specifically, we documented practices that contradicted what was recommended—or was not covered—in standard procedures. After sharing observations among the team, we drew from our own experiences working in food safety contexts to highlight potential risks and consulted the literature to corroborate our findings.

We conducted a thematic analysis to identify patterns or themes in the data (Braun et al., 2018). Specifically, we applied three levels of sequential deductive coding. First, we examined the flow of the pig slaughtering process (e.g., from livestock receiving to bleeding and transport). Secondly, we focused on contextual factors influencing the slaughtering process (e.g., facility, employee hygiene). Finally, we explored opportunities for zoonotic occupational and foodborne disease risks (e.g., from handling

FIGURE 1
Photos of the pork processing process at a mobile slaughter site. **(A)** Transport from holding pens to the restraint area at a household site. **(B)** Exsanguination at a household site. **(C)** Carcass dressing at a household site. **(D)** Transport to market from household site.

carcasses, from contamination of pork). NVivo© software (QSR International, Burlington, MA, USA) was used to facilitate the coding of fieldnotes and descriptions of photos and still-frames from video footage. As a way of assuring research quality for this study, we wrote reflective memos alongside our notes to document contextual information and reflections on data collection. We also held regular discussions among the authors to co-develop the themes.

3. Results

A total of seven abattoirs and three mobile slaughter sites were visited. Slaughter practices were largely consistent across and within abattoirs and mobile slaughter sites, from pig restraint to transport to markets. Workers processed pigs one at a time. Where variations were observed were in the number of pigs processed per night, the number of employees, and the physical layout of facilities. For example, at the abattoirs, 6–15 swine were processed per day at a fixed site, often requiring two to three workers to complete the entire slaughter process. At mobile slaughter sites, a similar number of pigs were slaughtered on farm sites by mobile butchers who often worked in pairs (e.g., husband-wife team) and with additional help from their clients. Farms provided water and space, while slaughter workers brought knives, hooks, and motorbikes.

In reviewing pig slaughtering practices and evaluating their potential for health risks, we developed four overarching themes: slaughter process, personnel hygiene, equipment sanitation, and facility sanitation.

3.1. Overview of the slaughter process

3.1.1. Transport from holding pens to the restraint area

In both abattoirs and mobile slaughter sites, pigs were first moved from holding pens to restraint areas with a pointed metal rod placed deep in the ventral inter-mandibular space. Pigs were then hoisted onto a metal restraint device or a concrete block that restricted them to lateral recumbency (Figure 1A). Of note, animals with signs of disease were not separated from healthy animals in the abattoir setting. Antemortem and post-mortem examinations of pigs and carcasses were also not consistently conducted. There were no protocols for the identification and disposal of sick animals or diseased tissues, resulting in the potential for infected products to enter the food supply.

3.1.2. Bleeding

Workers performed exsanguination by a puncture wound through the skin at the base of the neck to sever the carotid arteries and jugular veins, with blood collected in a metal bowl that contained anti-coagulation salts (Figure 1B). Before bleeding, pigs were not stunned, resulting in a violent procedure. In the peri-mortem phase, the animals were often thrashing, with reflexive movements and high intravascular pressure contributing to a projectile expulsion of blood from the laceration site.

3.1.3. Dressing

Carcasses were moved from the restraint device directly onto the floor and to the processing area where all dressing procedures

were performed (Figure 1C). Carcasses were not disinfected before further processing. These procedures included scalding and manual hair removal. The water temperature in abattoirs and household sites was not controlled, however, boiling water was used for scalding to facilitate hair removal by knife scraping. Then, workers proceeded with head removal, evisceration (gastrointestinal tract, urogenital tract, liver, respiratory tract, and heart), trimming (lymph nodes and kidneys), and lastly, splitting of the carcass along the spine. While organs were removed *en bloc*, and evisceration did not result in organ perforation or leads in gastrointestinal contents, the knives used were not disinfected between carcasses. Cold-water rinses were applied once organs were fully removed. Viscera was rinsed in the same water used for washing other parts of the carcass, representing another possible pathway toward contamination. Fabric cloths were then used to dry the internal and external surfaces. They were reused between carcasses without disinfection.

3.1.4. Transport to the market

Each carcass half was then weighed and transported to nearby markets by motorbike with two to three carcasses and heads per vehicle (Figure 1D). Carcasses were uncovered and un-chilled during transportation. The time for carcasses to reach the market varied from as soon as possible or after a couple of carcasses have been processed. Slaughterhouses operated from around 2 am until 6 am while markets opened from 5 am to 11 am.

FIGURE 2
Abattoir employee wearing a plastic overall (lower body only) to transport pork from the slaughterhouse to the market.

3.2. Overview of personnel hygiene

The use of personal protective equipment (PPE) is often considered necessary to minimize the transmission of diseases and contamination of products, which include goggles, face masks, aprons, overalls, gloves, and boots. Although abattoir employees and mobile butchers wore some PPE, no participants wore PPE that offered complete protection of dermal, ocular, or mucous membrane surfaces. Employees at abattoirs were not provided uniforms or guidelines for PPE. In both abattoirs and at mobile slaughtering sites, we observed that employees wore personal attire at work and did not change their garments throughout the working period. Hand, arm, or face protection was not worn by any person participating in slaughter activities at any of the observed sites. At the mobile slaughter sites, five out of the six butchers wore rubber boots, and one wore plastic open-toed slippers. Three of the six had complete leg coverage which included rubber boots and plastic overalls. Similar observations were noted in abattoirs; for example, one abattoir employee donned a plastic overall (lower body only) to transport carcasses to the market (Figure 2). And while most abattoirs had employees that were fully clothed, in one abattoir one employee did not have a shirt on.

3.3. Overview of equipment sanitation

In abattoir and mobile slaughtering sites, the equipment consisted of a knife (general-purpose, axe, or a large butcher knife) to process pigs, a metal bowl to collect blood, a concrete block (or metal device) to restrain pigs, and a kettle for dehairing. There were also waste buckets, food storage containers, and woven nylon bags for viscera storage and transport. To store blood, employees used plastic containers for the storage of blood (Figure 3A). Mobile butchers brought their equipment, while additional receptacles for storage and waste were provided by the households. A single knife was employed throughout the slaughter process. Equipment disinfection was not performed between the different stages of the pig slaughter process.

3.4. Overview of facility sanitation

In the abattoirs observed in this study, pigs arriving from different households or farms were held in available pens for several hours before slaughter. There were no biosafety measures in place to prevent the co-mingling of pigs from different origins or of different species. Abattoirs were in one-story buildings that contained (1) a holding area; (2) a restraining, slaughtering, and bleeding area; (3) a carcass and viscera processing area; and, (4) a weighing and transportation area. The roof was made of corrugated metal while the walls were comprised of brick. Inside, the interior floors were paved concrete. Slaughter processes occurred in separate, closely neighboring zones, with no functional barriers to physically delineate them likely due to space limitations. To move between zones, two workers lifted the carcass and moved them manually. In two of the sites, we observed live pigs wandering through the slaughter areas while carcasses were being processed, increasing the probability of meat contamination. In other sites, we observed inadequate physical separation of dogs from the pig slaughter processes which creates additional avenues for disease transmission. All abattoirs had indoor drainage systems with a drain in the floor feeding into roadside channels that emptied into rice paddies. The manual removal of waste was often necessary as drains were not located in gravity-dependent parts of the facilities and were not built to accommodate the high volume of liquid (e.g., blood, wastewater) and solid waste (e.g., hair, bones) (Figure 3B).

In households, mobile slaughter sites were established in the outdoor foyer or backyard of residences within the vicinity of swine pens. Areas used for slaughtering/bleeding, dressing, and transport were paved, and finished pork products were placed on nylon tarps

FIGURE 3
Abattoir setting. **(A)** Equipment storage in an abattoir. **(B)** Drainage system in an abattoir.

for clients. As within abattoirs, the slaughter process largely occurred within the same area. Wastewater and materials were swept directly into roadside channels that emptied into the rice paddies.

There were no hand washing stations for employees at abattoirs and slaughtering sites in the vicinity of the slaughtering and processing areas. However, workers had access to household bathrooms for handwashing, although we did not observe this practice performed throughout the slaughter process. There were no established sanitation protocols to guide the use of soap/detergents, the temperature of the water, and the frequency of cleaning or disinfection. Cleaning and disinfection were not performed between the slaughter of individual animals but only at the very end of the slaughter process.

4. Discussion

While infected pigs are considered an important source of human infections with zoonotic disease (Hoa et al., 2011), the specific routes of zoonotic transmission remain unclear. For example, while *S. suis* carriage among healthy pigs entering slaughter facilities is common, how infected pork could affect workers and contaminate uninfected pork remains unclear (Nguyen et al., 2021). Proposed routes of human infection include exposure through skin injuries, mucous membranes, inhalation, and ingestion of contaminated food, though some instances of swine-related human disease have also been reported without known pork exposure (Rayanakorn et al., 2019; Nguyen et al., 2021). Given that occupational exposure and ingestion of contaminated foods are the most likely sources of infection (Rayanakorn et al., 2018), this study aimed to identify gaps in pig slaughterhouse activities which could result in zoonotic occupational exposure for workers and foodborne disease risk for consumers.

4.1. Gaps in the slaughter process

4.1.1. Transport from holding pens to the restraint area

During slaughtering, there were several opportunities for human contact with live pig secretions and infected areas, increasing the possibility of disease transmission from swine to humans. For instance, *S. suis* has a particular predilection for the upper respiratory tract (specifically, the tonsils and nasopharynx) and the genital and gastrointestinal tracts in healthy pigs (Lun et al., 2007). Among diseased pigs, the bacteria have also been isolated in the brain, lung, heart, and joint tissues (Cheung et al., 2008). Workers across all pig slaughter settings did not wear gloves when working with various parts of the swine carcass, presenting opportunities for occupational disease risks for workers considering, for instance, *S. suis* can enter human skin through small scratches or wounds.

4.1.2. Bleeding

Knives were not disinfected between each animal slaughtered; the opening cut presented opportunities for infected knives to potentially contaminate the blood and various tissues and subsequently pork products. Additionally, given the absence of PPE used, particularly facial protection, the exsanguination process presented an opportunity for dermal, mucosal, and ocular exposure of workers to the blood of diseased pigs. Reuse of the blood collection pan and pooling of blood sources from different animals can further propagate pathogen transmission, especially as it is also sold as a food product in Vietnam.

4.1.3. Dressing

As with other studies characterizing pig slaughterhouse practices in Vietnam, all dressing procedures took place on the floor where the processing area was not disinfected (Yokozawa et al., 2016; Chau et al., 2017). Potential contact with fecal matter and debris from the pens and processing areas was an additional opportunity for contamination of the carcass, especially because bacteria such as *Salmonella spp.* and *S. suis* can remain viable in dust and feces (Lun et al., 2007). Indeed, slaughter areas located close to lairages without hygienic measures were identified as a significant risk factor for carcass contamination with *Salmonella spp.* (Dang-Xuan et al., 2019).

The recommended water temperature for scalding and hair removal is 60–62°C (Heinz, 2008). While boiling water temperature was used for scalding, it was likely inadequate for sanitization—with previous studies suggesting that hot-water decontamination requires 80°C for 12–15 s (Alban and Sørensen, 2010); this excessive heat for scalding, aggravated by knife scraping, has the potential to damage swine skin through protein coagulation, resulting in increased potential for pathogen colonization of deeper tissues.

Contaminated water might also have reached the swine's respiratory system during the scalding process, presenting another possible route of bacterial transmission (Marois et al., 2008). Additionally, the gastrointestinal and urogenital tracts were rinsed in the same water used for washing other parts of the carcass and equipment, another possible vector for contamination.

4.1.4. Transport to the market

Processed pork was brought directly to markets for sale without chilling considering many consumers in Vietnam prefer fresh, room-temperature pork. Consumption of undercooked pork may be particularly hazardous considering that pork products kept at high ambient temperatures can have high bacterial counts (Hoa et al., 2011).

4.2. Gaps in personal hygiene

All participants in the slaughter process had some degree of skin (arms or legs), ocular, and/or mucous membrane exposure throughout the entire process. These results are consistent with a previous study that found over two-thirds of workers at abattoirs visited in Southern and Central Vietnam never used PPE; the same study also found that over two-thirds did not know they could contract infections from healthy animals, and over a quarter did not know they could become infected through direct contact with diseased animals (Tu et al., 2019). Furthermore, in a study where researchers conducted sampling at slaughterhouses in Hanoi and Hung Yen, researchers observed that slaughterhouse workers were not wearing any PPE and suggested that this gap could pose a risk of influenza transmission from swine to humans (Baudon et al., 2018). Yet, complete coverage of hands and feet along with surfaces prone to micro-abrasions (including open wounds) are often considered necessary to prevent disease transmission (Hoa et al., 2011). Handwashing can also help to protect slaughterhouse workers against transmitted bacterial pathogens such as *Salmonella* spp. as well as protect the meat from contamination (Cook et al., 2017; Durmuşoglu et al., 2020). And given emerging evidence of the bio-aerosolization of pathogenic bacteria such as *S. suis* (Bonifait et al., 2014; Nguyen et al., 2021; Tang et al., 2021), facial shields or masks are increasingly necessary to both prevent potential inhalation of infected particles.

4.3. Gaps in equipment sanitation

During decapitation, cuts were made through the chain of pharyngeal and cervical lymph nodes as well as parts of the upper respiratory system where *S. suis* tends to reside (Lun et al., 2007). Infected knives could be a source of cross-contamination within and between carcasses as knives were not disinfected after instances of organ perforation (Dang-Xuan et al., 2018). Although guidance documents recommend a number of sterilizers for hand tools and knives be available (Mann et al., 1983; Heinz, 2008), the use of the same knives for different carcasses without sanitation between carcasses was observed across all abattoirs and mobile sites visited.

4.4. Gaps in facility sanitation

The separation of animals—such as pigs and dogs—was not often practical given space constraints, increasing the potential risk of disease transmission between animals. For example, beyond swine, *Salmonella* spp. and *S. suis* have been discovered in domestic animals such as dogs and cats (Wertheim et al., 2009; Wei et al., 2020). The presence of other animals on pig farms is also a risk factor for other diseases such as swine influenza and *Trichinella* infection by acting as "couriers" for spreading diseases within a farm (Takemae et al., 2016; Le et al., 2022a). Additionally, without a protocol for waste disposal, waste products often accumulate throughout a slaughter shift. The absence of adequate sanitation procedures for facilities could bacterial colonization within facilities (Dang-Xuan et al., 2016).

4.5. Opportunities and barriers

Our study identified potential entry points within slaughter processes to limit intra-carcass contamination, inter-carcass contamination, and direct occupational transmission of zoonoses associated with swine (Table 1). Currently, in small-scale slaughter settings, there is intimate contact between humans, live animals, and meat products; upgrades to facilities that provide better separation would be highly impactful but are not often possible in small-scale settings where profit margins are low and surplus income is insufficient to cover facility upgrades. Without external investments, more light-touch interventions would be promising.

Efforts to reduce disease transmission have previously focused on developing centralized systems for slaughtering (Lapar et al., 2012; Nguyen-Viet et al., 2017). However, focusing exclusively on centralized systems comes at a high cost to many rural families who depend on swine raising for a significant portion of their income along with consumers who prefer these pork products. Light-touch interventions that promote sanitary slaughter processes (e.g., ante-mortem evaluation of animals before slaughter) and personal hygiene (e.g., PPE providing coverage of primary routes of transmission) within smallholder slaughter settings will be important for supporting this industry and are generally seen as most feasible given the low cost and effort involved, followed by programs targeting equipment sanitation and facility sanitation. Such interventions must also consider principles of animal welfare including good housing and good health (Smith et al., 2021). Thus, continued investment in the education and training of employees will be crucial in working toward humane slaughter, a safe pork product, and a safe working environment. Additionally, considering some workers have existing food safety knowledge, training should be accompanied by improvements to the enabling environment (e.g., equipment, facilities) to facilitate knowledge application (Lam, 2022).

Importantly, there is a lack of regulation within small-scale slaughter settings. Nearly 10 years later after collecting data for this study in 2013, small-scale slaughtering practices remained largely the same, as noted by observations from the second phase of PigRISK (SafePORK: 2017–2022) (Hennessey et al., 2020; Lam et al., 2021). Strengthening regulations will be essential for supporting the sustainability of hygienic slaughtering practices. The creation of oversight committees for small-scale slaughterhouses is a promising way to ensure slaughter settings can maintain their businesses

TABLE 1 Opportunities and barriers to improving food safety in the areas of pork processing, personal hygiene, equipment sanitation, and facility sanitation in small-scale settings.

	Opportunities	Barriers
Slaughter process	• Formal ante-mortem evaluation of animals before slaughter and segregation of animals by origin could help prevent disease transmission • Cleaning of animals and prevention of their movement within the facility with physical barricades could help reduce disease spread • Training on animal welfare principles for reduced discomfort and pain (e.g., housing that prevents pigs from seeing/hearing the slaughter process; stunning before exsanguination) • Removal of organ systems should be performed *en bloc* using clean knives to reduce the potential of contaminating carcasses • Separation of areas in which viscera are processed from the remainder of the slaughtering can also prevent cross-contamination of products • Water used for carcass rinsing should not be reused for other carcasses or carcass parts to prevent cross-contamination	• High cost of stunning equipment with the investment that may not translate into tangible benefits • Perceptions that stunning could change the texture, temperature, taste, and appearance of pork products • Long-standing practices are difficult to change • Facility size constraints
Personal hygiene	• Personal protective equipment that provides coverage of primary routes of transmission can serve as significant barriers to disease transmission, especially in environments where disinfection and sanitation protocols are lacking • Clothing changes or disinfection should be performed between the slaughtering of each animal to prevent cross-contamination between carcasses	• Heat and humidity may create difficulties in covering all body parts; consideration should be given to priority areas (e.g., mucous membranes and breaks in the skin) • Low awareness of disease transmission • Lack of access to training resources Long-standing practices are difficult to change
Equipment sanitation	• Sanitary knife procedures such as switching to a two-knife system (e.g., separate bleeding and dressing knife) or disinfection of equipment between cuts can reduce intra-carcass contamination • Bed dressing (dressing procedures on an elevated surface) or suspended dressing techniques can decrease contact with contaminants from the previous process and ensure that waste products are channeled away from the carcass being processed • Carcasses should be allowed to air dry unless materials are single-use or sanitized between uses • Cleaning equipment (brooms, mops, rags) used throughout the dressing process should be discontinued unless adequately disinfected between uses	• Costs of purchasing suspended dressing or bed dressing equipment, disinfectants, and soaps • Long-standing practices difficult to change
Facility sanitation	• Flooring should consist of non-permeable materials, such as glazed ceramic or glazed porcelain, to prevent the retention of contaminants • Drainage systems should also be built in gravity-dependent positions to improve the disposal of liquid waste throughout the slaughtering process • In abattoir settings, the adoption of a production line system where animals (as opposed to people) are transitioned through the stages of processing, can prevent contamination through people and fomites • At mobile sites where a production line is not possible due to limited staff, workers should try to minimize movements to reduce the possibility of cross-contamination	• Logistical constraints to promoting sanitary operations include space limitations, access to equipment and resources, and development and execution of protocols • Complete renovation of facilities is not feasible but thoughtful utilization of space and alterations in human and animal movement can contribute to improving sanitation and biosecurity • Determination of adequate frequency of equipment/facility disinfection, consistent adherence to protocols, and monitoring of implemented changes are additional barriers

without risk to workers and the general population (Nguyen-Viet et al., 2017; Pham and Dinh, 2020). Committees should develop protocols for monitoring and enforcement with input from swine workers to ensure buy-in and practicality. It will also be essential to engage local authorities to shape these protocols and support monitoring and enforcement. Equally important to a supportive environment is the need to raise awareness of and demand for safer food among consumers (Le et al., 2022b). Applying the concept of One Health whereby multiple sectors and multiple disciplines collaborate could inform food safety strategies that also support animal and environmental health (Grace, 2017).

In addition to the specific entry points for food safety improvement presented in Table 1, we suggest several recommendations for different food safety actors. First, improvements need to be made to food safety practices to enhance worker safety and reduce the risk of meat contamination. Training programs for workers should raise awareness of risks which could lead to the adoption of better practices. Secondly, facility managers should be aware of guidelines for slaughterhouse and meat hygiene and encourage workers to adhere to such guidelines. Additionally, the lack of access to improved infrastructure is an important factor constraining the ability to substantially improve hygiene; promoting access to finance could support facility managers in improving equipment and facilities (Nguyen-Thi et al., 2021). Finally, local and national governments should consider implementing programs that raise awareness of and demand for food safety among consumers, which could drive good hygienic practices in not only food processing settings but also retail (Le et al., 2022a). Importantly, strategies that are holistic and attuned to the needs, priorities, and constraints of different food safety actors are key to the success of food safety programs.

We note a couple of limitations of our study. First, while our research focuses on observing behaviors as they were happening (rather than what participants say they do), our research did not explore motivations or rationales behind behaviors in a systematic way. Future studies should consider interviews alongside observations to enrichen the findings. Secondly, there may have been issues of observer bias, where participants adjusted behaviors when they know they are being observed. However, we expect this phenomenon to be minimal considering this research was part of a larger research program where existing relationships and trust have been established. Despite the study limitations, this observational study enhanced our understanding of small-scale pig processing and entry points for food safety improvements. Having the visits feel informal by keeping questions conversational was helpful in supporting a sense of comfort among participants. In addition to keeping conversations informal, we wrote memos quickly and expanded on them after the visits. Furthermore, we recorded photos and videos as participants were working which could then be analyzed later. These strategies helped to reduce the research burden on participants and could reduce barriers to long-term participation. Given the strengths of this approach, we suggest further integrating observational studies into One Health research to visualize and characterize behavioral risks to health.

5. Conclusion

While slaughterhouse pigs are an important reservoir for human infection from zoonotic diseases, the specific pig slaughtering practices contributing to disease transmission remain under-researched. This study identified and characterized elements of the pig slaughtering process that likely contribute to the spread of zoonotic pathogens—from infected pigs to uninfected pigs and humans—drawing on a case study of traditional pig slaughter settings in Vietnam. Areas potentially leading to zoonotic occupational and foodborne disease risks included slaughtering procedures, personal hygiene of workers, equipment sanitation, and facility sanitation. While small-scale slaughtering practices are long-standing and difficult to change, light-touch interventions that target safe handling practices show promise. Our study underscores the importance of hygiene training of workers—supported by an enabling environment—in helping to reduce the burden of zoonotic disease risks.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All slaughterhouse owners and/or workers verbally provided their informed consent to participate in this study and to be photo- and video-documented. This work received research ethics approval from the Institution Research Board of the Hanoi University of Public Health as part of the PigRISK project (IRB no. 148/2012/YTCC-HD3).

Author contributions

Conceptualization, data collection, analysis, and writing: NT. Conceptualization and revision: NN, JG, SD-X, and HN-V. Analysis and revision: SL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Impact of perception and assessment of consumers on willingness to pay for upgraded fresh pork: An experimental study in Vietnam

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Traditional pork shops play an essential role in delivering pork, the most popular food in Vietnam, to consumers. Studies have shown the need for investment in training and equipment to improve the safety of pork sold at traditional shops. However, it remains unclear how consumers perceived improvement to the hygiene in pork shops and if they are ready to pay premium prices for safer products. This study used an experimental approach to determine consumers' perception and assessment of improved pork shops and their willingness to pay (WTP) for pork products. A total of 152 respondents in two provinces in Vietnam joined in a Becker–DeGroot–Marschak (BDM) mechanism experiment to collect data on WTP for pork from typical and upgraded pork shops. A questionnaire was used to record consumers' perceptions and assessments of the pork shops and products. Overall, consumers were willing to pay 20% more for upgraded fresh pork than for what is currently available on the market. Consumers trusted in the effectiveness of the upgraded intervention and the quality of pork at the pork shop, which increased their WTP for the upgraded pork. Concerns about contaminated pork had a negative impact on the WTP for typical pork, while the high frequency of pork consumption and the existence of elderly family members led to higher WTP for both products. The findings indicate the potential economic benefit of upgrading pork shops, which would be an important driver to motivate sellers to improve food safety.

KEYWORDS
food safety, willingness-to-pay, Becker-DeGroot-Marschak mechanism, market intervention, experimental auction

Introduction

Rapid economic development in Vietnam has led to increased meat consumption in recent decades (Hansen, 2018). Nguyen et al. (2014) reported that consumers were highly concerned about the safety of meat, especially pork, which is the most popular meat in Vietnamese cuisine. There also seems to be cause for concern as many studies found a

high prevalence of microbial contamination in pork in all types of retail establishments (Nhung et al., 2018; Dang-Xuan et al., 2019; Ngo et al., 2021). Modern retail is considered the key solution to improve the safety of pork (Wertheim-Heck et al., 2015; The World Bank, 2017), but the cost of improving food safety in this retail is significantly high (Ortega and Tschirley, 2017; Karanja et al., 2022). In addition, many consumers still prefer and trust traditional value chains (Maruyama and Trung, 2010; Unger et al., 2019; Wertheim-Heck and Raneri, 2020). Therefore, traditional pork retail still plays an important role and should be improved. In addition, there is a change in Vietnam's food safety policies, which shifts responsibility from the authorities to the food producers (Pham and Dinh, 2020) who need to be motivated to upgrade their facilities and practices. The most important motivation proposed is to emphasize the potential profit from the consumers' willingness to pay (WTP) for safe products. Therefore, it is suggested to investigate consumers' WTP and relevant factors to support investors such as governments, funders, or private sectors in estimating the benefit and sustainability of food safety programs.

Many studies have indicated a high demand from Vietnamese consumers for safe products and emphasized the credence of food quality as a critical factor that drives consumers to pay a higher price (Mergenthaler et al., 2009; Ifft et al., 2012; My et al., 2018; Ha et al., 2019; Tran et al., 2022). Labeling is a popular tool to deliver product attributes and increase consumer trust in food products (Ares et al., 2013; Fernqvist and Ekelund, 2014; Le et al., 2020). However, the habit of Vietnamese consumers relying on sensory evaluation (e.g., touching or smelling) to assess the quality of fresh food products (Cadilhon et al., 2002; Maruyama and Trung, 2010) makes it difficult to apply food packaging and labeling to retailed pork at traditional shops.

In the Vietnamese context, previous studies have measured the WTP of consumers for safe pork products through stated-preference surveys (Khai et al., 2018; Thi Nguyen et al., 2019), but this method tends to overestimate the WTP due to the absence of market discipline (Murphy et al., 2003; Lusk and Shogren, 2007). Moreover, the different attributes between safe and conventional pork in previous studies were explained vaguely to participants by citing national standards or suppliers' definitions without any sensory experiment on the products. Consequently, the elicited values from these surveys might be inconsistent. Thus, this study aimed to (1) measure the WTP of consumers for fresh pork from typical and upgraded pork shops by using an experimental methodology, (2) investigate the perception and assessment of consumers about food safety practices and pork shops, and (3) explore the influence of food safety perception, knowledge, and risk message on the WTP of consumers.

Methodology

Conceptual framework

To assess the influence of relevant determinants of the WTP, we develop a framework as presented in Figure 1. The WTP can be affected by internal and external factors. In our framework, we have grouped these factors into (1) consumer characteristics, (2) product assessment, and (3) environmental factors.

Many studies found an impact of demographic characteristics on consumers' decisions. At the household level, the number and attributes of family members would motivate the buyer to purchase safe food products (Zheng et al., 2018; Chege et al., 2019; Kytö et al., 2019; Neill and Holcomb, 2019). At the individual level, the age, education, and income of the buyer have contradictory effects on their WTP. While a higher level of education or income led to higher WTP (Angulo and Gil, 2007; Mergenthaler et al., 2009; Li et al., 2016; Zheng et al., 2018; Chege et al., 2019; Riccioli et al., 2020; My et al., 2021), the older consumers had a lower WTP for premium food products (Yu et al., 2014, 2018). In addition, the food consumption habit of the consumers also significantly increased the probability to purchase the food (Kytö et al., 2019). Angulo and Gil (2007) found that the level of beef consumption is a key factor that influences consumers' WTP for beef products, while Yu et al. (2018) and Zheng et al. (2018) found similar results for salmon and vegetable products. Researchers also reported the positive impact of risk perception about food-borne diseases on the WTP (Angulo and Gil, 2007; Mergenthaler et al., 2009; Yu et al., 2018; Neill and Holcomb, 2019), while the enhancement in food safety knowledge might correspond to increasing WTP for safe vegetables (Mergenthaler et al., 2009).

Furthermore, consumers' exposure to information about the food product's characteristics can significantly affect their WTP. For example, many studies showed that the product description on the label strongly motivated consumers to pay more for food (Meenakshi et al., 2012; Zhang et al., 2012; Jin et al., 2017; Chege et al., 2019; Liu et al., 2019; Katt and Meixner, 2020; Riccioli et al., 2020). Important information that enhances consumers' WTP in many studies was the certification of the food products (Owusu-Sekyere et al., 2014; Ortega and Tschirley, 2017; Wang and Tsai, 2019), even if it is not a government certificate (Liu et al., 2019). Therefore, some alternative methods to make consumers distinguish between the different attributes among products should be considered so that they may reveal their true WTP. Nonetheless, providing consumers with messages about food safety risks right before they make their decision might be a critical point that affects WTP. Britwum et al. (2019) found that the message about reported cases of disease due to microbial contamination in food motivated consumers to pay more for safe food, while Bruner et al. (2014) reported a reduction in WTP for traditional food products due to the information about the estimated risk of the new food products. In contrast, the experiment by Hayes et al. showed that consumers were not affected by providing the figure about the probability of food-borne diseases (Hayes et al., 1995).

Finally, consumers' assessment of food products and food stores was identified as important determinants of their WTP for food. Owusu-Sekyere et al. (2014) indicated the hygienic condition surrounding the shop significantly affected consumers' WTP for the food product, while Zheng et al. (2018) found that a high assessment of the food by consumers motivated them to purchase the product.

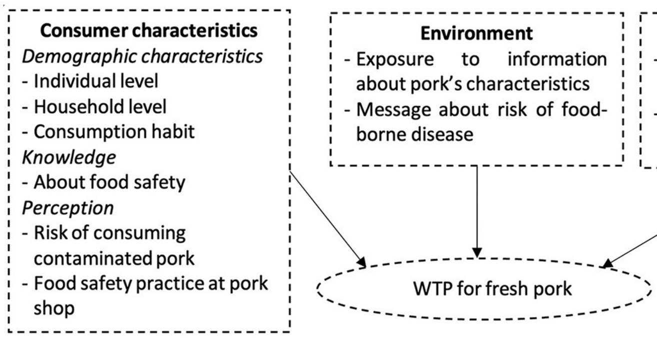

FIGURE 1
Conceptual framework.

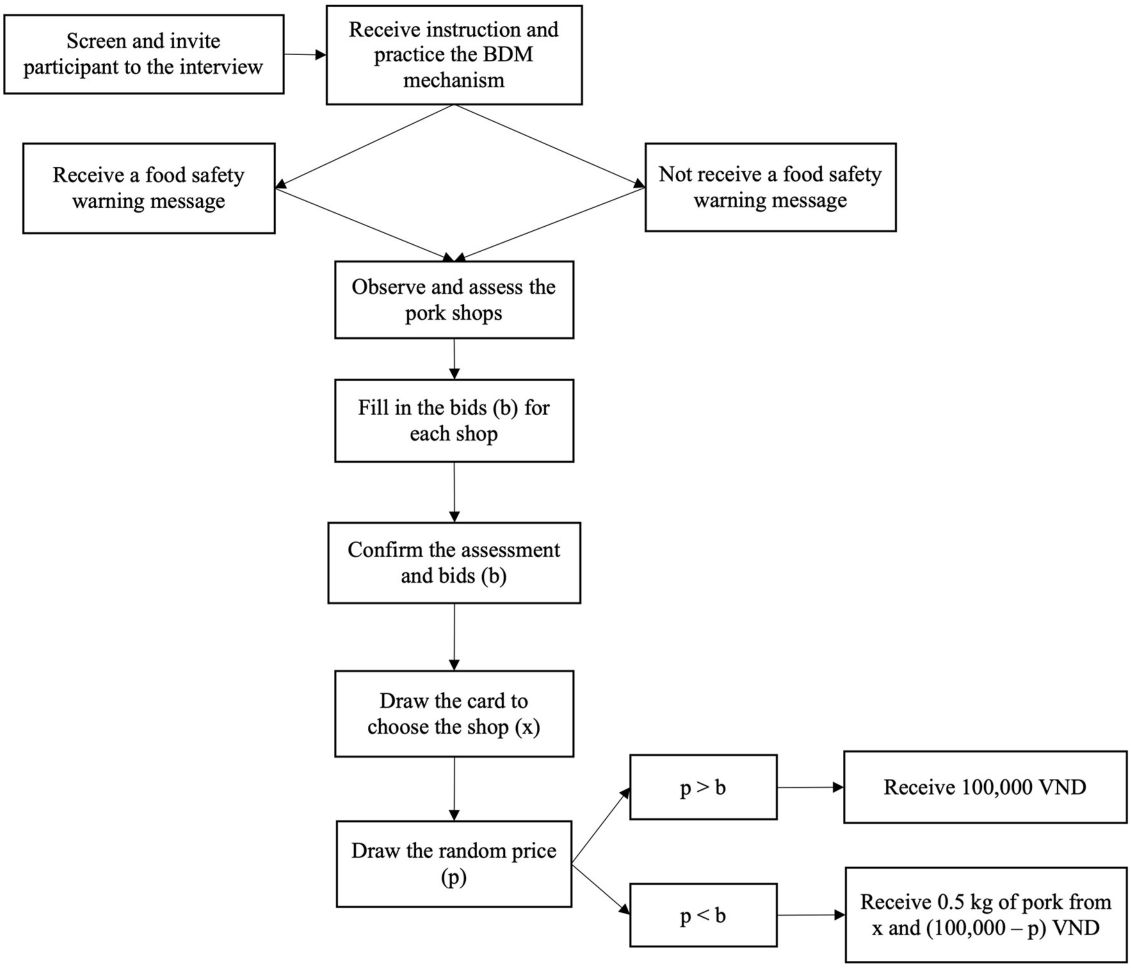

FIGURE 2
Illustration of the Becker–DeGroot–Marschak (BDM) mechanism.

Experiment design

In this study, conducted between October and November 2021, we used a Becker–DeGroot–Marschak (BDM) mechanism with a full bidding approach to measure the WTP of consumers for raw pork at traditional pork shops in Vietnam. This experiment design creates a market environment where participants can incorporate market feedback and reveal their value for the product *via* a bid (Lusk and Shogren, 2007). In other words, the participants will use real money to purchase the product from a set-up market, which can improve the reliability of the study (Koschate-Fischer and Schandelmeier, 2014), especially when compared with survey

TABLE 1 Characteristics and preferences of participants.

Characteristics	Mean	SD
Age	51.9	11.8
Food expenditure (thousand VND/day)	102	68
Household size	3.98	1.71
Number of children	0.35	0.6
Number of elderlies	0.73	0.8
Number of pork dish/week	6.44	5.43
Amount of pork/shopping (kg)	1.08	1.05
Education (number of schooling years)	9.22	2.67
Gender	**Frequency**	**Percentage**
Male	15	9.9
Female	137	90.1
Occupation		
Small-scale vendor	57	37.5
Farmer	44	28.9
Taking care of household	25	16.4
Other	26	17.1
The most consumed type of pork		
Bacon	99	65.1
Shoulder	33	21.7
Others	20	12.2

methods where participants tend to hide their true behavior (Kytö et al., 2019). In addition, Jaffee et al. (2019) indicated that the precision of consumers' WTP could be improved by presenting alternative products and letting them make decisions under their usual budget constraints.

Study location

This study was part of a larger project (SafePORK) aiming to improve food safety at traditional markets focusing on pork (ACIAR, 2016) through food safety interventions at different levels, including upgrading pork shops. The experiments were implemented at three traditional markets where some pork shops had been upgraded as part of the interventions. Two markets were located in Thai Nguyen province and one in Hung Yen province in the North of Vietnam. Traditional markets represent the most popular retail channel in Vietnam for distributing fresh food such as animal-source food, vegetables, or fish (Nga et al., 2014; Unger et al., 2019). In each market, two pork shops were set up for the experiments, as described in the following section.

TABLE 2 Participants' experiences with pork-borne diseases and preventing measures.

Items	Frequency	Percentage
Experience with pork-borne disease symptoms ever		
Stomachache	24	15.8
Diarrheal	16	10.5
Nausea	12	7.9
Vomit	7	4.6
Buy pork from hygienic shop		
Never	7	4.6
Sometimes	18	11.8
Always	127	83.6
Frequently wash hand during pork handling		
Never	4	2.6
Sometimes	9	5.9
Always	139	91.4
Eat well-cooked pork only		
Never	2	1.3
Sometimes	3	2
Always	147	96.7
Separate cooked pork and raw food in process and storage		
Never	5	3.3
Sometimes	3	2
Always	144	94.7
Use different tools for cooked pork and raw food		
Never	6	3.9
Sometimes	12	7.9
Always	134	91.4
Concerns about eating contaminated pork		
Not worried	2	1.3
A bit worried	8	5.3
Worried	36	23.7
Very worried	38	25
Extremely worried	68	44.7
Slaughtering on grid improves food safety		
No	5	3.3
Yes	87	57.2
Don't know	60	39.5
Food safety knowledge score	Mean: 7.64	SD: 1.57

TABLE 3 Participants' perception and assessment about food safety at pork shop.

Item	Min	Max	Median	Mean	1st quartile	3rd quartile	Data source
Perception about food safety at pork shop	6	34	26	25.3	24	29	Survey
Overall assessment about food safety of pork shop							Experiment
Typical shop	2	10	8	7.3	7	8	
Upgraded shop	5	10	10	9.6	9	10	

TABLE 4 Correlation (Spearman's rank correlation rho) between perception, knowledge and difference in shop assessment.

Variables	Difference in pork shop assessment	Perception about food safety at pork shop
Perception about food safety at pork shop	−0.05	-
Food safety knowledge	−0.01	0.16*

*$p < 0.05$.

Participant selection

On the day prior to the experiment, the research team came to the selected markets to recruit participants from potential consumers. One out of every three consumers who visited the market was asked to participate in the study. Upon their consent, the respondents who intended to buy pork on the following day would be interviewed using a structured questionnaire. Once completed, they received a coupon and were invited to attend the experiment on the following day. In total, 152 consumers (Nhai market—Hung Yen, $n = 52$; Dong Quang—Thai Nguyen, $n = 50$, and Dan market—Thai Nguyen, $n = 50$) were recruited, finished the interview, and participated in the experiment.

Procedure

On the experiment day, two pork shops were set up at the selected markets. Each shop was supplied with 25 kg of pork shoulder sourced from the same slaughterhouse and delivered on the morning of the experiment day. The experiment was held on a day when the market was closed, to limit the interference of the market operation. According to previous studies (Nguyen-Viet et al., 2019; Thi Nguyen et al., 2019), pork shoulder is the most popular choice for Vietnamese consumers, so it was chosen as the product for the experiment. The experiment included two types of traditional pork shops: upgraded and typical pork shops. An upgraded pork shop had taken part in the food safety intervention from the SafePORK project. Both shops were equipped with fundamental tools for traditional pork shops, with a set of tools (cutting board, knives, scale, cloth to wipe hands, and other tools) and protection clothing (apron and mask) for the seller. The upgraded shop was provided with a disinfection package (sprayer, disinfection liquid for cleaning surfaces, and hand sanitation gel) and a poster to motivate the seller to frequently clean hands, surfaces, and tools, as well as to introduce recommended food safety practices to the consumers at a pork shop. In addition, the typical shop used paperboard to display pork on the granite table, while the upgraded shop displayed pork directly on the granite surface. The pork at both types of shops was supplied from the same slaughterhouse on the experiment day to make sure that the quality of pork was affected by the seller and the shop's facilities only.

The Becker–DeGroot–Marschak (BDM) mechanism (Becker et al., 1964; Lusk and Shogren, 2007), using a full bidding approach, was selected for this study. In this type of experiment, the participants compete against a random price by giving their full bid for 0.5 kg of shoulder pork. If the bid is higher than the random price, the consumer purchases the pork at the random price; if the bid is lower, the consumer does not purchase. To avoid demand reduction effects, only one type of pork shop was allowed for each individual buyer during the game (Lusk and Shogren, 2007). The BDM mechanism (Figure 2) had four main steps for each individual participants as follows:

- Step 1: On the first day, the participants were informed about the project and gave informed consent to participate in the study. Each participant filled in the questionnaire and was given a coupon equal to 100,000 Vietnam dong (VND) (∼US$4.50). This coupon could be exchanged for a half kilogram of pork shoulder or an amount of money depending on the result of the experiment. The amount of money was not revealed to prevent participants from deliberately making high bids so that they would lose the game and receive the money instead of the pork.
- Step 2: Selected participants were instructed about the BDM process. To get familiar with the process, participants later practiced three rounds of the BDM mechanism with cakes and candy. Before the actual game was conducted with fresh pork, every second participant received a paper with the food safety warning message "On average, one out of five Vietnamese persons suffered salmonellosis (such as diarrhea and vomiting) due to consumption of typical pork from a traditional shop". Then, the participants moved to observe both shops and assess their food safety condition, by giving a point on a scale from 0 (worst) to 10 (best). Subsequently, they offered a bid for pork in both shops.
- Step 3: Following this, the participants were asked to re-confirm their assessment and bids described earlier.
- Step 4: The enumerator randomly drew a piece of paper to select either an upgraded (A) or typical (B) shop. Based on the result of selecting the shop, another drawing step took place to define the price of pork. If the drawn price was higher than the participants' bid, this individual lost the game and got VND 100,000 (value of the coupon). Otherwise, participants

won the game, and they had to buy the pork at the drawn price and then received the remaining money deducted from VND 100,000.

The random price of pork shoulder was 40,000 VND per half a kilogram, while the random price was generated following uniform distribution and not shared with the participants. The range of the random price was 10,000–80,000 VND to cover the potential values and not limit the winning opportunity of the participants with reasonably high valuations (Lusk and Shogren, 2007). The mechanism is illustrated in Figure 2.

Questionnaire

The research team developed a structured questionnaire that covered the potential variables in the conceptual framework and consisted of six parts: demographic information, pork preference, experience of food-borne diseases, perceptions about food safety, practices to prevent pork-borne diseases, and knowledge about pork safety. The demographic part included age, gender, occupation, education, and household characteristics (such as food expenditure and household size). The part assessing consumers' preference for pork covered their pork consumption habits (such as frequency, amount, or type of pork), while the experience of food-borne diseases focused on some common food poisoning symptoms (including stomachache, diarrhea, nausea, and vomiting).

The perception component comprised two parts: food safety practices at pork shops and the risk of pork-borne diseases. The first part included beliefs (measured by a five-level ordinal scale) in seven practices (that promote food safety) at the upgraded pork shop and one practice (that reduces food safety) at the typical one. The questions and results of this component are presented in Appendix A.

Practice to reduce the effect of pork-borne diseases was measured by asking the participants about the frequency and effectiveness of five practices on a five-level ordinal scale. The knowledge about pork safety was assessed by ordinal questions (yes/no/do not know) adapted from da Cunha et al. (2019), with 12 questions in total. The questions and answers on food safety knowledge are presented in Appendix B.

The internal consistency of the questions assessing perception about food safety at the pork shop (eight questions), practice to prevent pork-borne diseases (10 questions), and food safety knowledge (12 questions) was tested using Cronbach's alpha, with the results 0.903, 0.724, and 0.809, respectively, showing high internal consistency and adequate reliability of the questions (di lorio, 2005).

Data analysis

Interview data and experiment results were entered in Microsoft Excel. Descriptive analysis was applied to describe the characteristics of variables, while the mean WTP and pork shop assessment between different groups were compared using Wilcoxon signed rank test.

To assess the perception of consumers about the food safety practice of pork sellers, we calculated the overall score by adding up the score of each of the eight practices. The seven practices that promote food safety were graded from 1 to 5 points per question, while poor practice was graded from−5 up to−1 point. The overall perception score ranged from 2 to 34 points.

Regarding food safety knowledge, each response was marked 0 (for an incorrect answer) or one (for a correct answer) and then summed up to make the total score (ranging from 0 to 12 points). The Spearman rank correlation test was used to determine the relationship between the perception about food safety at pork shops, assessment of pork shops, food safety knowledge, and attitude about the risk of pork contamination.

Univariable analyses were implemented first to identify variables to include in the multivariable models. Variables were included if they had a p-value of ≤ 0.1 in univariable analyses. For the regression models, the dependent variables were the WTP (1,000 VND) for pork from the typical and upgraded shop and the difference in WTP for 0.5 kg between the two products. The linear quantile regression with the market variable as a random effect was implemented for all three models at 10, 50, and 90% quantile for the bids using the lqmm package in R (Geraci, 2014).

Ethical clearance

This study was reviewed and approved by the Institute Review Board at the Hanoi University of Public Health (No. 110/2018/YTCC-HD3). Verbal informed consent was obtained from each participant before conducting the interview.

TABLE 5 The willingness to pay of consumers for each type of pork.

Price of pork (thousand VND)	Min	Max	Median	Mean	1st quartile	3rd quartile
Upgraded shop (1)	25	70	40*	39	35	40
Typical shop (2)	20	60	30*	32.5	30	35
Difference in price	−5	30	5	6.5**	3	10

*Significantly different ($p < 0.01$) with Wilcoxon signed rank test.
**Significantly different from 0 ($p < 0.01$) with t-test.

TABLE 6 Linear quantile mixed model coefficient estimates.

Variables	Pork from upgraded shop			Pork from typical shop			Difference in WTP		
	10% quantile	50% quantile	90% quantile	10% quantile	50% quantile	90% quantile	10% quantile	50% quantile	90% quantile
Number of elderlies	1,590.54***	1,582.69***	1,645.47***	1,121.51***	1,057.46***	1,169.96***	505.66	450.68	471.99
Number of pork dishes/week	144.20	274.14**	181.27***	63.03	172.66***	19.01	−146.70	25.0	166.44
Amount of pork/shopping	999.79*	977.72*	1,038.81*	1,172.15***	1,128.86***	1,291.89***	−190.20	−139.36	−100.02
Food safety knowledge	−45.46	62.06	154.23	−123.20	−71.82	157.51	−60.75	64.67	131.64
Perception about risk of consuming contaminated pork	−645.49	−635.29	−639.43	−924.37***	−860.28***	−806.14***	203.57	210.19	249.18
Perception about food safety practice at pork shop	−323.75***	−110.80	192.88	−344.14*	−91.53	129.18	−87.78	35.78	142.59*
Difference in shop assessment	1,151.00**	1,178.96**	1,282.48**	−1,302.63**	−1,318.23***	−1,203.18**	2,287.42***	2,570.24***	2,556.84***
Risk message	−1,208.40	−936.10	−895.30	−857.11	−887.67	−790.94	−61.73	−26.732	−58.53
Variance of random effect (ICC)	0.40 (0.000)	0.89 (0.000)	1.11 (0.000)	0.00 (0.000)	0.25 (0.000)	0.00 (0.000)	0.00 (0.000)	0.20 (0.000)	1.23 (0.000)

***, **, *Represent significance at 0.01, 0.05, and 0.1 levels, respectively.

Result

Sociodemographic characteristics

Table 1 describes the characteristics of the participants. The average respondent was of middle age (51.9 years old on average), with 9.22 years of education, and most were female (90.1%). Many participants were small-scale vendors (37.5%) and rice farmers (28.9%), followed by those who took care of the household (16.4%). The mean participants' household size was 3.98 with many of them not having any children or being elderly (73 and 50.7%, respectively). In other words, most of the household members were working-age adults. Furthermore, on average, they had 6.44 pork dishes per week and purchase 1.08 kg of pork per shopping time. The most purchased pork type was bacon (65.1%), followed by the shoulder (21.7%).

Experiences with food-borne disease, food safety practice, and knowledge

Table 2 presents the experiences of participants with food-borne disease and their food safety practice and knowledge. The participants reported that they rarely suffered from common food poisoning symptoms. The most regular symptoms among the respondents and their families were stomachache (15.8%), followed by diarrhea (10.5%), nausea (7.9%), and vomiting (4.6%). In addition, the respondents reported that they regularly implemented food safety practices at home, especially eating well-cooked pork only (96.7%) and separating raw pork and cooked food (94.7%). Furthermore, the participants' average knowledge score was 10.96 (out of a maximum score of 12), with a standard deviation of 2.03. Pork-borne diseases seemed to be a concern for respondents since more than 90% worried about eating contaminated pork while more than half of them (57.2%) believed that slaughtering on a grid, instead of the floor, can improve the safety of pork.

Perception and assessment about food safety practice at pork shops

Overall, participants gave positive feedback on the intervention packages at the upgraded pork shops. More than 80% of respondents believed that the suggested practices at upgraded shops would improve the safety of pork. In contrast, for the poor practice, which was carried out at the typical shop (placing the pork on the wooden table or carton board), half of the respondents believed this practice would improve the safety of pork, while only little more than one-third believed it would reduce the safety of pork. The details are presented in Appendix A. In addition, in the experiment, the participants evaluated the overall food safety condition of the upgraded shop (9.3/10) significantly higher than the typical one (7.6/10) ($p < 0.05$). The details are presented in Table 3.

The independence of variables was tested between pork shop assessment, food safety perception, and knowledge. The result showed a weak association between factors except for perception

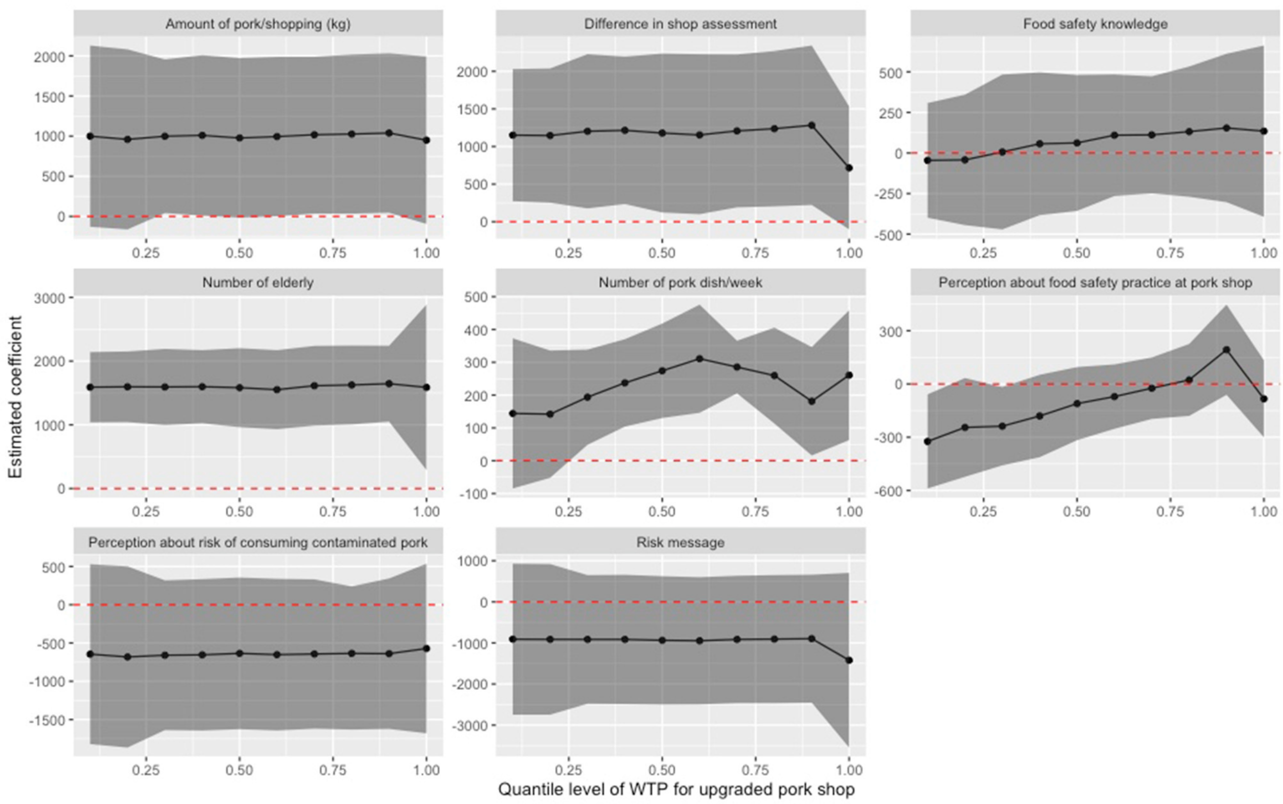

FIGURE 3
Estimated coefficients (black lines) and 95% confidence intervals (gray areas) at different quantiles of consumers' willingness to pay (WTP) for pork from upgraded pork shops.

about food safety and concern about eating contaminated pork. The detailed correlation is presented in Table 4.

Willingness to pay and associated factors

The experiment showed that the typical pork received the mean bid at 32,500 VND per 0.5 kg, while the figure for upgraded pork was 39,000 VND per 0.5 kg. Thus, the respondents were willing to pay a premium of ∼6,500 VND per 0.5 kg (or 20%) for the upgraded pork compared with the typical one. The detailed result is presented in Table 5.

Table 6 describes the relationship between relevant factors to the bids and the difference in the bids of the two pork types. Overall, the pork consumption habits and household characteristics had a strong impact on the WTP for both pork products while the risk message had no effect. On the other hand, the difference in food safety assessment between the two shops was the only indicator that significantly affected the deviation of the WTP for each product. In addition, the market cluster effect did not cause a significant impact on the WTP. The tendency of each coefficient for each variable across quantile levels is presented in Figures 3–5.

For the upgraded pork, the number of pork dishes per week, the number of elderlies in the household, the difference in assessment between the two shops, and the perception about food safety practice significantly increased the consumers' WTP while the perception about food safety at pork shops show a negative impact. However, the number of pork dishes per week is not significant in the 25th percentile of WTP, whereas the perception score only affects in 25th percentile of WTP (Figure 3). Finally, the amount of pork in each shopping time, the participants' knowledge and perception about contaminated pork, and the risk message did not have any relationship with their WTP in any quantile.

The WTP for pork from typical shops was significantly affected by most variables except the knowledge score, the perception about food safety at pork shops, and the risk message. In detail, the consumption habit (the amount of pork in each shopping and the number of pork dishes per week) and the number of elderlies in the household caused a positive impact on WTP in all quantiles (Figure 4). On the contrary, concern about eating contaminated pork and the shop assessment difference had a negative effect, while the perception about pork shop practices only affects a low percentile of WTP (10th percentile).

The difference in shop assessment, the number of pork dishes per week, and the perception score are the only factors that affect the difference in WTP between the two types of pork (Figure 5). Although both variables significantly increased the difference, the first had an impact on all percentile of WTP while the other two only have an impact on the 60th percentile or higher.

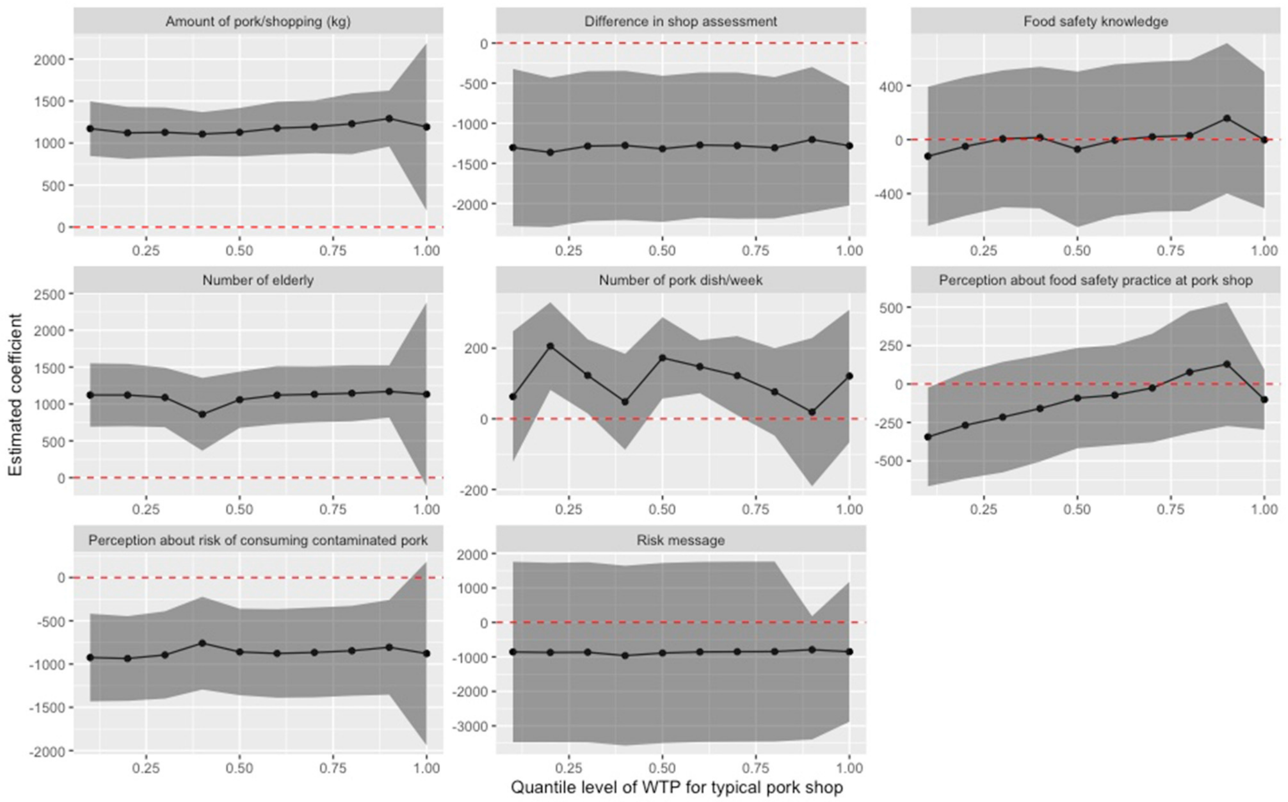

FIGURE 4
Estimated coefficients (black lines) and 95% confidence intervals (gray areas) at different quantiles of consumers' willingness to pay (WTP) for pork from typical shops.

Discussion

This is the first study to use an experimental economics approach to investigate the WTP of consumers for raw pork. The BDM mechanism creates a market environment that could motivate consumers to reveal their true behavior toward the research group. Overall, the consumers highly rated the upgraded pork shops and tended to pay a premium (20% higher) for those products. This figure is much lower than the finding of Khai et al. (2018) and Thi Nguyen et al. (2019) who found an increased WTP of 81.2 and 224.2%, respectively, for fresh pork products, but it was nearly similar to the result of 15% higher WTP for fresh chicken products by Ifft et al. (2012). A potential reason for this difference is the impact of the COVID-19 pandemic, which strongly affected food consumption not only during the time of the study (Eftimov et al., 2020) but also the studies by Thi Nguyen et al. and Khai et al. were conducted totally in urban areas where people may have higher income. Our study and the one by Ifft et al. may have estimates closer to the true WTP of consumers for food products due to the BDM mechanism, while non-market methods, such as those used in the study of Thi Nguyen et al. and Khai et al., might overestimate the consumers' WTP (Lusk and Shogren, 2007; Jaffee et al., 2019). However, the number is still low compared with other low-value products, such as rice at 33% (My et al., 2021) or Chinese mustard at 60% (Mergenthaler et al., 2009). This may indicate that pork is already more expensive, and the customers are not able to pay too much for it.

This study also consolidated the correlation between consumers' perception and their assessment about food safety at pork shops. In summary, consumers perceived that the hygiene packages at the upgraded pork shop were effective in improving food safety, which created a gap in their assessment of the two shops. In consequence, this different assessment motivated the consumers to pay more for the pork at upgraded shops and reduced their WTP for pork at the typical shops. This could be because the consumers are inclined to position the pork from the upgraded shop in a different segment rather than the typical one, which corresponds to the difference in price. This finding is consistent with the result from previous research (Angulo and Gil, 2007; Ortega and Tschirley, 2017; Zheng et al., 2018; Wang and Tsai, 2019; Riccioli et al., 2020) that the belief in the product's quality significantly increases the WTP. To create this effect, the consumers should be informed through some visible indicators such as a certificate, label (Ortega and Tschirley, 2017; Neill and Holcomb, 2019; Wang and Tsai, 2019), or appropriate risk message (Hayes et al., 1995; Bruner et al., 2014; Yu et al., 2018; Britwum and Yiannaka, 2019). Since it was not feasible to deliver this information *via* food package or label in our study, we communicated *via* posters, tools, and direct comparison between the two shops.

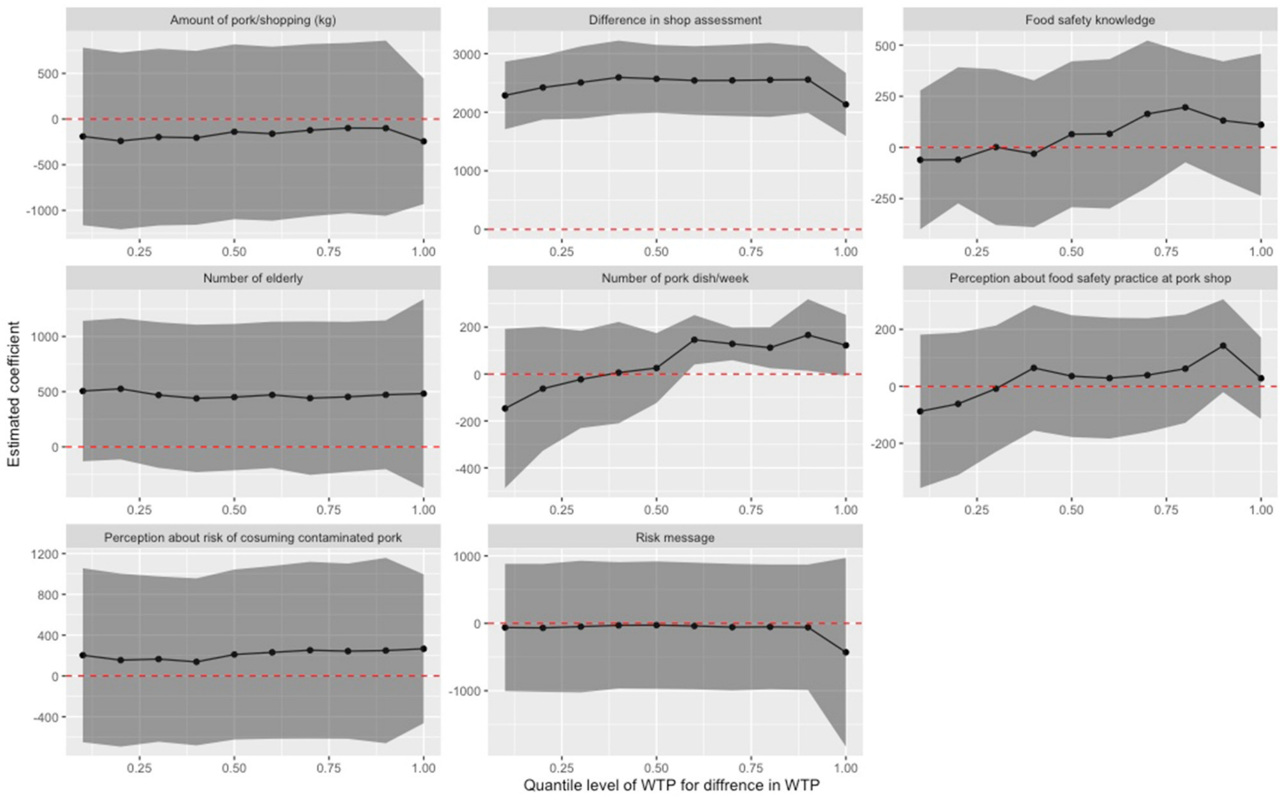

FIGURE 5
Estimated coefficients (black lines) and 95% confidence intervals (gray areas) at different quantiles of difference in consumers' willingness to pay (WTP) for pork products from the two shops.

Furthermore, the respondents had good knowledge of food safety as well as reported regularly maintaining good practice in pork safety, but the regression results showed no effect of knowledge on the WTP of either pork product. In contrast, Khai et al. (2018) identified pork safety knowledge as a positive driver of consumers' WTP to safe pork. In addition, we found that concern about the risk of eating contaminated pork had a negative impact on the WTP for the pork from typical shops, which is confirmed in previous studies (Hayes et al., 1995; Yu et al., 2018). Similarly, the message about the risk of consuming pork did not affect the consumers' decision. This was explained by Hayes et al. (1995) that the consumers trust their prior perceptions more than new information about the odds of illness, and this belief is not biased by the researcher (Bruner et al., 2014). It is reasonable since the study population reported a low prevalence of food poisoning symptoms (4.6% for vomiting and 15.8% for stomachache), especially those related to pork consumption. This low figure may be the consequence of frequently applying food safety practices or bias in recalling information from memory (Prince, 2012; Lightle, 2016) and difficulty in diagnosing gastrointestinal disease (Culligan et al., 2009). It has earlier been shown that consumers in low- and middle-income countries do not always choose safe food even though they are concerned about food safety issues (Liguori et al., 2022).

In addition, the frequency of pork consumption and household characteristics are undoubtedly factors that increase consumers' WTP. We found that high pork consumption is associated with high WTP for both two products, which is different from the results of Thi Nguyen et al. who indicated the negative impact of pork consumption on WTP (Thi Nguyen et al., 2019). The difference in the study population and study design might explain this contrast. In addition, the experimental approach motivates the participants to reveal their true intention better than the hypothetical choice experiment (Noussair et al., 2004; Vecchio and Borrello, 2019). In addition, we found that the consumer tends to pay more for pork products if there is an elderly member in the household. This can be interpreted that the popularity of pork dishes in Vietnamese daily meals motivates them to pay more to reduce the risk of exposing the elderly, a vulnerable group to food-borne diseases through contaminated pork. This finding is consistent with the findings from Dang-Xuan et al. (2017) and Khai et al. (2018). However, we did not find a relationship between children, another vulnerable group, with the WTP while Neill and Holcomb found a significant effect (Neill and Holcomb, 2019).

Finally, the consumers seemed to be familiar with the technique of slaughtering on grids that we applied to improve food safety at the slaughter stage. It is a good signal for the higher price of pork products from upgraded shops since consumers often show a higher acceptance of new technology (Bruner et al., 2014; Britwum and Yiannaka, 2019).

Limitation

This study did not cover participants in urban areas where supermarkets and convenience stores are strong competitors to traditional pork shops. Further studies should be implemented to find additional information about this group. Another limitation is the limited choice of pork type in the study, which may not completely reflect consumers' preferences. However, this design helped us to reduce bias in consumers' decisions due to the demand reduction effect. Finally, the convenience sampling procedure with a small sample size in this study may cause bias in the estimate of the regression model.

Conclusion

Our study confirmed the potential profit from pork provided by upgraded pork shops among traditional pork retailers in Vietnam. Along with the popularity of pork in Vietnam and the increasing trend in pork consumption, this is a significant driver to encourage small-scale pork producers to invest in and maintain the food safety condition of their establishments. These are important signals to the consumer about the food safety of the product. However, further studies to analyze the cost-benefit need to be implemented to assess the sustainability of the investment.

Moreover, this study corroborated consumers' concerns toward pork safety, but this was not the driver to motivate them to purchase a safer product. Instead, they classified the products into different categories according to their characteristics (including food safety attributes) and then positioned them with different prices. In other words, the typical pork shops still have their own consumers, even though they may prefer buying from the upgraded pork shop. Hence, along with market mechanisms, other impacts from relevant stakeholders are required to considerably improve the safety of pork.

Finally, consumers showed concrete knowledge and regular practice in food safety that was not affected by a simple risk message. Therefore, an appropriate communication strategy is required to effectively enhance their perception about the risk of pork-borne diseases, especially for the vulnerable group in the household. This would be the key to consolidate the sustainability of local efforts to reduce the burden of pork-borne diseases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institute Review Board at the Hanoi University of Public Health (No. 110/2018/YTCC-HD3). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

HHTN, FU, HNV, SDX, DG, and PPD contributed to conception and design of the study. PNH and HLT organized the database. HHTN performed the data analysis and wrote the first draft of the manuscript. MM and JL wrote the discussion and introduction sections of the manuscript. SDX and FU wrote the methodology section. HHTN and TTHL wrote the result section. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1055877/full#supplementary-material

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Quantitative assessment of aflatoxin exposure and hepatocellular carcinoma (HCC) risk associated with consumption of select Nigerian staple foods

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Aflatoxin contamination of staple grains and legumes has been linked to hepatocellular carcinoma (HCC) and other adverse health outcomes, constituting a substantial public health concern globally. Low-resource food environments in sub-Saharan Africa are often under-regulated and are particularly vulnerable to adverse health and nutrition outcomes associated with aflatoxin exposure. This study identifies levels of HCC risk in the northern Nigerian adult population, leveraging a systematic review of available evidence on aflatoxin contamination in Nigerian maize, groundnut, rice, cowpea, and soybean. Estimated dietary intake (EDI) was computed using publicly available dietary consumption data and a probabilistic quantitative risk assessment was conducted to determine the relative risk of HCC associated with consumption of selected aflatoxin-contaminated commodities. In total, 41 eligible studies reporting aflatoxin contamination were used to model the distribution of aflatoxin concentrations in Nigerian commodities. EDIs for maize, groundnut, rice, and cowpea exceeded the provisional maximum tolerable daily intake (PMTDI) level of 1 kgbw-1 day-1, with maize yielding the highest mean EDI (36.7 kgbw-1 day-1). The quantitative risk assessment estimated that 1.77, 0.44, 0.43, 0.15, and 0.01 HCC cases per year/100,000 population were attributable to aflatoxin exposure through maize, groundnut, rice, cowpea, and soybean, respectively. Sensitivity analysis revealed that aflatoxin concentration, dietary consumption levels, consumption frequency, and other variables have differing relative contributions to HCC risk across commodities. These findings constitute a novel multi-study risk assessment approach in the Nigerian context and substantiate existing evidence suggesting that there is reason for public health concern regarding aflatoxin exposure in the Nigerian population.

KEYWORDS

aflatoxin, risk assessment, exposure, LMIC, mycotoxins, food safety, Nigeria, Africa

1. Introduction

Traditional food markets—also known as informal markets and comprising "wet" or public markets, small shops, vendors and other non-modern retail—are critical for ensuring access to safe, nutritious diets in many low- and middle-income countries (LMIC) (Wertheim-Heck et al., 2019). Food safety regulatory systems in many LMIC markets, formal and informal, are often insufficient for preventing exposures to contaminants that can jeopardize community health and offset the nutritive value of food. However, most of the poor obtain food from traditional markets so these are of especial interest (Grace, 2015). One such contaminant, aflatoxin, is widespread

in informal grain value chains in LMICs and constitutes a food safety concern of burgeoning importance both for public health and economic prosperity in traditional market settings. Aflatoxin is a potent carcinogen, implicated in hepatocellular carcinoma (HCC) in humans, and, in some contexts, a leading risk factor for HCC (Liu and Wu, 2010). In addition to its carcinogenic properties, aflatoxin is associated with growth impairment in children (Khlangwiset et al., 2011) and may be an important driver of environmental enteropathy, resulting in compromised gut integrity and limited nutrient adsorption (Smith et al., 2012), although evidence for contribution to stunting is less strong (Hoffmann et al., 2018). The risk of dietary aflatoxin exposure is particularly pronounced in many sub-Saharan African food systems, where obsolete monitoring infrastructure, poverty, and weak beaurocratic structures coincide with high reliance on aflatoxin-susceptible staples, such as maize and groundnuts.

In the context of Northern Nigeria, the breadth and magnitude of aflatoxin exposure risk associated with foods sold in traditional markets are poorly understood, particularly for the most vulnerable consumers, limiting the capacity to identify and mobilize mitigation opportunities. Foods acquired at traditional markets have distinct characteristics compared to those produced on farms and consumed locally. Foods sold at these markets are usually subjected to various layers of informal and formal quality control, such as sorting, pest management, or visual inspection by supply chain intermediaries, which can in some circumstances measurably reduce the aflatoxin burden in food; for example, it was found that the prevalence of mycotoxins in traders' maize was lower than at other nodes of the value chain, even after a period of storage (Liverpool-Tasie et al., 2019). In addition, the consumer has direct control of quantity when purchasing food, enabling them to purchase quantities that they know will not spoil before consumption. On the other hand, with respect to purchased foods, the consumer has little control over pre-market nodes of the value chain, such as production, storage, and transport, which can all be critical control points for aflatoxin management (Massomo, 2020). Given that aflatoxin contamination is often not visibly identifiable without specialized technology, even grain that appears safe in the marketplace may be contaminated.

Northern Nigeria's traditional food markets cater to diverse populations, and supply communities with numerous food commodities. As in the rest of the country, maize and rice—both susceptible to aflatoxin contamination—are important staple grains in the region. Maize is commonly ground to flour and consumed as a stiff porridge (*tuwo masara*) or similar preparations. Rice is consumed either cooked as-is or ground to flour and consumed as cakes (*masa*). High-protein legumes such as cowpea and soybean are an important part of the local diet, but there has historically been little evidence on aflatoxin contamination in this crop. A steamed cowpea pudding, *moimoi*, is commonly consumed in the region. Soybean is commonly consumed as a curdled soymilk "cheese" or tofu-like product (*awara*) in the region. Groundnut, a major source of dietary aflatoxin globally, is an important crop in the region with production centered in the northern states of Niger, Kano, Zamfara, Kebbi, and others, contributing measurably to the Nigerian economy (Usman et al., 2013). Apart from whole groundnuts as a snack food, groundnut press cake (*kulikuli*)—a by-product of traditional or mechanical oil extrusion—is often shaped into various forms and consumed as a fried snack. Many of these commodities are available as raw ingredients or as ready-to-eat prepared foods sold at the marketplace.

Despite a substantial body of survey evidence regarding aflatoxin contamination in Nigerian food commodities, including in marketplace environments, there has hitherto not been any comprehensive risk assessment that has focused on this context. The aim of this study, implemented as part of the United States Agency for International Development (USAID) Feed the Future Initiative program, EatSafe: Evidence and Action Toward Safe, Nutritious Food (EatSafe), is to elucidate the extent of aflatoxin exposure risk associated with commodities commonly purchased from traditional food markets in Nigeria. At the start, a systematic review was conducted of aflatoxin contamination in five aflatoxin-susceptible local commodities, namely maize, groundnut, rice, cowpea, and soybean, reported in surveys from Nigeria. We used these aflatoxin occurrence data, in conjunction with secondary data on local dietary consumption, to estimate exposure and risk of HCC using a probabilistic quantitative risk assessment methodology. Based on these findings, the relative risk associated with local consumption of each commodity was determined and key recommendations identified for aflatoxin management in northern Nigerian traditional food markets.

2. Materials and methods

2.1. Literature review of aflatoxin contamination and prevalence in Nigerian commodities

A systematic review of peer-reviewed literature was conducted in Web of Science to ascertain reported data on aflatoxin contamination in Nigerian maize, groundnut, cowpea, soybean, rice, and other food products derived therefrom. Each search included three keywords, (1) "aflatoxin," (2) the commodity name, and (3) the geographic focus, "Nigeria." Articles were first assessed for relevance based on a title and abstract screening, followed by full text review of relevant articles. Eligibility for inclusion in the study was assessed using the following inclusion criteria: (1) publication date 1980—present, (2) aflatoxin, either total aflatoxin or aflatoxin B1 (AFB1) concentration determined for raw commodity (not cooked or prepared), (3) samples are reasonably intended for human consumption (not for livestock feed, etc.), (4) fungal growth is naturally-occurring, not artificially inoculated, (5) aflatoxin is detected in at least one sample (studies with 0% detection retained for modeling prevalence, but excluded from the contamination model), (6) reported means reflect batches of several distinct samples, not replicates of the same pooled sample(s), (7) the number of total samples analyzed is reported, and (8) an estimate of batch variance (standard deviation, range, etc.) is reported. Additional studies were identified by the authors *via* non-systematic searches in Google Scholar using similar keywords.

Data extracted from eligible studies included: mean, standard deviation, median, and range of aflatoxin concentration (ng/g); study year; country; sub-national division; commodity type; sample source (household, market, or prepared food); metabolite (total aflatoxin or aflatoxin B1); total number of samples; number of positive samples or detection rate; limit of detection. Individual batch means reported within studies were extracted separately, when possible. Risk modeling was done based on the aflatoxin B1 (AFB1) metabolite, in

accordance with the known carcinogenic potency associated with the hepato-carcinogenic effects of AFB1 in conjunction with hepatitis B infection (JECFA, 1999). Whenever available, reported estimates of AFB1 concentration were extracted from the included studies. To be as conservative as possible (i.e., to prevent under-estimation of exposure) in risk assessment when only total aflatoxin concentration was reported, the reported total aflatoxin levels were treated as AFB1 in the subsequent risk assessment. When only the concentration range (minimum and maximum) was reported, the midrange (e.g., min+max/2) was used for analysis in place of the mean, as previously reported (Andrade and Caldas, 2015).

The spatial distributions of available aflatoxin contamination data for each commodity were visualized using GIS mapping. Batch-wise estimates of mean aflatoxin concentration were grouped by state of sample origin. Studies wherein multiple (>2) states/regions were pooled, and therefore geographic origin indistinguishable, were omitted from GIS analysis. If a study's reported batches encompassed two states but state origins were not reported on a batchwise basis, the study was classified as the majority-sampled state for GIS mapping if >2/3 of batches were from that state; otherwise, the study was omitted from the maps. Maps were generated with the 'ggspatial' package in R using open-access GIS datasets (Global Administrative Areas, 2022).

2.2. Risk modeling approach

A quantitative risk assessment model was developed to estimate exposure to AFB1 associated with consumption of staple foods in Nigeria, as well as the risk of HCC, a key adverse health outcome associated with aflatoxin dietary exposure. The model considers a "market to table" scenario to assess absolute and relative exposure associated with different food commodities and does not aim to include pre-harvest or supply chain risk factors upstream of retail. The model takes as key input the distribution of aflatoxin prevalence and concentration in the product at the point of purchase, and follows portions of each food through home handling, preparation, and consumption. Parameters included in the model and their distributions are summarized in Table 1 and described in the following sections. The model was programmed and executed in the R software environment (R Core Team, 2021). A second-order Monte-Carlo approach was used to account for both variability and uncertainty (using 1,000 iterations in each dimension) in input parameters and implemented using the "mc2d" package in R. The model included several key assumptions: (1) no fungal growth or additional toxin accumulation occurs during storage, before consumption; (2) aflatoxin concentration is stable at normal cooking temperatures, i.e., no concentration reduction occurs during cooking (Kabak and Var, 2008); (3) no detoxification procedure is implemented by the consumer, i.e., no "inactivation" of the toxin is accounted for.

The exposure model was based on Estimated Daily Intake (EDI) of aflatoxin attributable to per-capita consumption of each commodity. EDI was estimated for the study population using an equation adapted from that described previously (Udovicki et al., 2021):

$$EDI\ (ng\ kg^{-1} bw\ day^{-1}) = \frac{C \times S \times i \times f_{cons}}{BW} \quad (1)$$

Where C = concentration of aflatoxin per kg sample (ng/g), S = g consumed per person per day on a day the product is consumed, i = the frequency of contamination among portions, f_{cons} = the frequency of maize consumption in the population, and BW = body weight (kg). An exposure model was constructed for each commodity using the probability distributions of C, S, i, f_{cons}, and BW, following the EDI equation described above, and incorporated into a Monte-Carlo simulation model for risk assessment. Exposure model input parameters for each commodity are summarized in Table 1.

2.3. Probability distributions of input parameters

2.3.1. Aflatoxin prevalence in samples (i)

To account for the fact that not all samples (and, thus, not all portions consumed) are contaminated with detectable levels of aflatoxin, the probability of consuming a contaminated portion (i) was approximated by aflatoxin prevalence, or detection rate, in the model. The prevalence data reported by the literature considered were fit to a beta distribution and uncertainty around the parameter estimates was modeled using parametric bootstrapping (1,000 iterations) using the 'bootdist' function in the 'fitdistrplus' R package (Delignette-Muller and Dutang, 2015). In addition to its representation of the probability of consuming a contaminated portion, the 'i' parameter was also used to correct for possible overestimation of exposure attributable to the tendency in the literature to report means only for contaminated samples (i.e., excluding non-detects). Due to the small number of studies reporting detection rate for soybean, it was not possible to fit a beta distribution to the data; instead, the mean detection rate reported in the available literature (7.3%; see reference in Table 1) was used in the exposure model.

2.3.2. Aflatoxin concentration in samples (C)

To select an appropriate probability distribution to describe the observed aflatoxin concentrations (C) reported by the included studies, for samples where aflatoxin was detected, the empirical distribution of study means was examined, and a series of goodness-of-fit tests were performed. Concentration estimate data were assumed to be derived using assays of the same sensitivity and precision; in other words, the initial mass of samples collected and analyzed was ignored. Given the expected skewedness of aflatoxin data, three probability distributions suitable for skewed data were considered: gamma, Weibull, and log-normal, in addition to the normal distribution. Q–Q plots, P–P plots, and cumulative distribution functions (CDFs) were examined for model fit. Three Goodness-of-Fit statistics (namely the Kolmogorov-Smirnov statistic, the Cramer-von Mises statistic, and the Anderson-Darling statistic) were estimated for each of the probability distributions and compared (Supplementary material S1). As a final test of Goodness-of-Fit across the candidate distributions, uncertainty in the parameters of the fitted distributions was estimated using parametric bootstrapping (1,000 iterations). To ensure adequate fit of the selected distributions, Q–Q plots and P–P plots were examined for the final distributions before proceeding with exposure assessment and risk analysis (see Supplementary material S2). For use in quantitative risk

TABLE 1 Summary of exposure model input parameters for each commodity.

Variable	Sym.	Value or distribution[a]	Source
Maize			
Aflatoxin concentration (ng/g)	C	∼*TWeibull (l = 93.6, k = 0.75, 0, 1874)*	Included studies: (Adebajo et al., 1994; Atawodi et al., 1994; Bankole and Mabekoje, 2007; Atehnkeng et al., 2008; Ayejuyo et al., 2011; Onilude et al., 2012; Perrone et al., 2014; Bandyopadhyay et al., 2019; Keta J. et al., 2019; Keta J. N. et al., 2019; Oyeka et al., 2019; Ayeni et al., 2020; Mbaawuaga et al., 2020; Onyedum et al., 2020; Shehu et al., 2020; Ekpakpale et al., 2021; Ezekiel et al., 2021)
Contamination frequency (%)	i	∼ *Beta (a = 1.11, b = 0.54)*	
Consumption Frequency (portions/day)	f_{cons}	∼ *Empirical ({days consumed per week}, {proportion of population}) =* ∼ *Empirical ({0, 1/7, 1}, {0.104, 0.150, 0.746})*	(Olayiwola et al., 2012)
Mass conversion rate (g/g)	m	$\frac{1\ g\ maize}{5.15\ g\ tuwo\ masara}$	(Fadupin, 2009)
Consumption Level (g/portion)	S	∼ *TN (200*m, 86.6*m, 150*m, 300*m)*	(Sanusi and Olurin, 2014)
Mean adult body weight (kg)	BW	∼ *N (63.7, 10.28)*	(Akinpelu et al., 2015)
Groundnut			
Aflatoxin concentration (ng/g)	C	∼*TlnN (m = 2.72, s = 1.47, 0, 1281)*	Included studies: (Bankole et al., 2005; Jimoh and Kolapo, 2008; Odoemelam and Osu, 2009; Ayejuyo et al., 2011; Afolabi et al., 2015; Oyedele et al., 2017; Adetunji et al., 2018; Ekhuemelo and Abu, 2019; Odeniyi et al., 2019; Vabi et al., 2020; Ezekiel et al., 2021; Adefolalu et al., 2022)
Contamination frequency (%)	i	∼ *Beta (a = 3.31, b = 0.61)*	
Consumption Frequency (portions/day)	f_{cons}	∼ *Empirical ({days consumed per week}, {proportion of population}) =* ∼ *Empirical ({0, 1/30, 1/7, 3/7, 1}, {0.100, 0.64, 0.0407, 0.0358, 0.0331})*	(Maziya-Dixon, 2004; Ezekiel et al., 2013)
Groundnut oil yield (for mass conversion	δ	∼ *TN (0.283, 0.0865, 0, 1)*	(Nkafamiya et al., 2010)
Mass conversion rate (g/g)	m	$\frac{1}{1-\delta}$	
Consumption Level (g/portion)	S	∼ *TN (121.45*m, 60.59*m, 30*m, 300*m)*	(Sanusi and Olurin, 2014)[b]
Mean adult body weight (kg)	BW		(Akinpelu et al., 2015)
Rice			
Aflatoxin concentration (ng/g)	C	∼*TN (m = 52.1, s = 36.7, 0, 372)*	Included studies: (Ibeh et al., 1991; Ayejuyo et al., 2011; Makun et al., 2011, 2014; Adejumo et al., 2013; Olorunmowaju et al., 2014; Rofiat et al., 2015; Awuchi et al., 2020; Onyedum et al., 2020; Ekpakpale et al., 2021; Ezekiel et al., 2021; Wartu, 2021)
Contamination frequency (%)	i	∼ *Beta (a = 1.28, b = 0.39)*	
Consumption Frequency (portions/day)	f_{cons}	∼ *Empirical ({days consumed per week}, {proportion of population}) =* ∼ *Empirical ({0, 2/7, 4/7, 1}, {0.837, 0.0709, 0.0511, 0.0378})*	(Maziya-Dixon, 2004)
Mass conversion rate (g/g)	m	$\frac{1\ g\ rice}{3\ g\ cooked\ rice}$	Assumed
Consumption Level (g/portion)	S	∼ *TN (420*m, 182.23*m, 100*m, 1,000*m)*	(Sanusi and Olurin, 2014)
Mean adult body weight (kg)	BW	∼ *N (63.7, 10.28)*	(Akinpelu et al., 2015)
Cowpea			
Aflatoxin concentration (ng/g)	C	∼*TlnN (m = 3.29, s = 1.38, 0, 200)* truncation upper bound (200 ng/g) is the highest batch maximum reported	Included studies: (Houssou et al., 2009; Afolabi et al., 2019; Awuchi et al., 2020; Ogungbemile et al., 2020; Ezekiel et al., 2021)
Contamination frequency (%)	i	∼ *Empirical ({1, 0}, {0.367, (1-0.367)})*	
Consumption Frequency (portions/day)	f_{cons}	∼ *Empirical ({days consumed per week}, {proportion of population}) =* ∼ *Emp ({1/7, 3/7, 5/7, 1}, {0.474, 0.363, 0.009, 0.153})*	(Odogwu et al., 2021)
Mass conversion rate (g/g)	m		(Akuso and Kiin-Kabari, 2012)

(Continued)

TABLE 1 (Continued)

Variable	Sym.	Value or distribution[a]	Source
Consumption Level (g/portion)	S	~ *TN (198.3*m, 122.4*m, 50*m, 780*m)*	(Sanusi and Olurin, 2014)
Mean adult body weight (kg)	BW	~ *N (63.7, 10.28)*	(Akinpelu et al., 2015)
Soybean			
Aflatoxin concentration (ng/g)	C	~ *TlnN (1.88, 0.91, 0, 4)*	(Ogunsanwo et al., 1989; Atawodi et al., 1994; Fapohunda et al., 2018) (concentration); (Niyibituronsa et al., 2019) (truncation upper bound)
Contamination frequency (%)	i	~ *Empirical ({1, 0}, {0.073, (1-0.073)})*	(Fapohunda et al., 2018)
Consumption Frequency (portions/day)	f_{cons}	~ *Empirical ({days consumed per week}, {proportion of population}) = ~ Emp ({0, 1/7, 2/7 3/7, 1}, {0.1875, 0.1667, 0.2083, 0.239})*	(Adewale, 2005)
Mass conversion rate (g/g)	m	$\frac{1\ g\ soyben}{1.927\ g\ awara}$	(Noh et al., 2005)
Consumption Level (g/portion)	S	~*TN (94*m, 44*m, 0, ∞)*	ILRI consumption data
Mean adult body weight (kg)	BW	~ *N (63.7, 10.28)*	(Akinpelu et al., 2015)

[a]Distribution shorthands N = "normal", lnN = "log normal", T = Truncated. Aflatoxin concentration and contamination frequency parameters represent bootstraps on observed values reported in included studies.
[b]No distribution for *kulikuli* portion size was identified; the values used are for *akara*, another legume-based local snackfood with assumed similar portion size.

assessment, a distribution simulating variability conditional on the uncertainty bootstraps was generated using the 'mcstoc' function in the 'mc2d' R package. The upper bound used for truncation of the distribution was the maximum batch aflatoxin concentration reported for each commodity in the included studies.

Given that there were very few ($n = 1$) observations of detectable aflatoxin in soybean batches in the included studies, it was not possible to compute Goodness-of-Fit statistics for the candidate distributions, for this commodity. Instead, a log-normal distribution (truncated between 0 and the highest observed sample maximum) was assumed and fit to the available data. As the one included study reporting detectable aflatoxin in Nigeria reported a sample maximum of only 2 ng/g, a higher (i.e., more conservative) value of 4 ng/g, reported in a Rwandan study (Niyibituronsa et al., 2019) was used as the upper bound of the truncated distribution. Similarly, as there were scant records of aflatoxin contamination in Nigerian cowpea samples, one record from a neighboring country (Benin) that was identified in the systematic literature review was included in the dataset (Houssou et al., 2009).

2.3.3. Commodity consumption (f_{cons} and S)

Two food consumption parameters were included as inputs in the exposure assessment model: (1) the frequency of consumption events (f_{cons}), and (2) the quantity of product consumed per consumption event (S). For all commodities, f_{cons} and S were derived from previously reported consumption data for key prepared food consumed in the study region. Local foods selected for the estimation included *tuwo masara* (maize), *kulikuli* (groundnut), cooked rice, *moi-moi* (cowpea), and *awara* (soybean), each of which is a common local preparation of the focus commodity. Empirical distributions were fit to f_{cons} data (consumption events/week) for each commodity, based on reported values in the literature (Table 2). To account for the fact that aflatoxin data were reported for raw (unprepared) commodities, mass conversion rates (m) were applied to portion sizes to determine the equivalent of raw commodity consumed per portion (Table 2). Consumption amount S was assumed normally-distributed, and means (±SD) from prior studies were used to fit a probability distribution truncated at the lower bound of 0 (e.g., no negative consumption).

2.3.4. Adult body weight

Previously reported estimates of adult body weight (men, women, and the general population) for a Nigerian population (Akinpelu et al., 2015) were used to fit a probability distribution of this parameter for the exposure assessment. A mean (±SD) adult body weight of 63.17 (±10.28) kilograms (Akinpelu et al., 2015) was used for exposure assessment. Adult body weight was assumed to follow a normal distribution as per the approach previously described by Sirma et al. (2019).

2.3.5. Risk characterization: HCC

The risk of aflatoxin B1-associated hepatocellular carcinoma (HCC) attributable to consumption of each commodity was estimated based on probability distributions of exposure (i.e., EDI), the constituent aspects thereof, and the carcinogenic potency of aflatoxin. The carcinogenic potency of aflatoxin, defined as the probability of HCC ("r") per 100,000-individual population was estimated using the equation (Udovicki et al., 2021):

$$r = \left(0.3\ \times HBsAG^{+}\right) + \left(0.01\ \times \left(1 -\ HBsAG^{+}\right)\right)$$

It has been demonstrated that the probability of HCC attributable to aflatoxin exposure is greater for individuals with Hepatitis B surface antigen (HBsAG) positivity by a factor of 30. A HBsAG positivity rate of 13.2% has been reported for the Nigerian population (Garba et al., 2021). Thus, the carcinogenic potency was estimated as: (0.3 × 0.132) + [0.01 × (1–0.132)] = 0.04828. The risk of disease (HCC cases/year per 100,000 population) was estimated by multiplying the EDI by the carcinogenic potency (Udovicki et al., 2021), using the equation:

$$HCC\ Risk = EDI \times r$$

TABLE 2 Summary of batch-wise aflatoxin concentrations reported in reviewed literature.

	Maize $N^* = 40^a$	Groundnut $N = 26^a$	Rice $N = 36^a$	Cowpea $N = 5^a$	Soybean $N = 3^a$
Metabolite					
AFB1	18 (45%)	17 (65%)	27 (75%)	4 (80%)	2 (67%)
Total aflatoxin	22 (55%)	9 (35%)	9 (25%)	1 (20%)	1 (33%)
% Positive samples	68.8 (27.4, 0.0–100.0)	84.5 (17.8, 39.3–100.0)	74.3 (29.3, 0.0–100.0)	31.1 (46.1, 3.7–100.0)	7.3 (12.7, 0.0–22.0)
(Missing)	8	10	1	1	0
Mean concentration (ng/g)[b]	104.4 (154.1, 0.0–611.8)	78.8 (219.2, 3.4–939.0)	46.6 (37.8, 0.0–157.3)	53.5 (54.6, 3.6–122.0)	0.6 (1.1, 0.0–1.9)

*N = number of batches reported across included studies.
[a] n (%); Mean (SD, Min-Max range of batch means).
[b] All samples of both metabolites were averaged together. Observations of total aflatoxin were treated as AFB1 for the risk assessment to prevent under-estimation of risk, as described in the Methods section.

Sensitivity analyses were performed for each commodity-wise risk model in order to determine the relative contributions of the input parameters to the level of HCC risk. Spearman's rank-order correlation coefficient (ρ) was computed and tornado graphs generated for each model. This coefficient takes values between -1 and 1, where values nearer to 1 are more highly correlated with the outcome (HCC risk). Mean and median values of Spearman's ρ were computed and assessed together with 95% uncertainty intervals around the estimates.

3. Results

3.1. Occurrence of aflatoxin in Nigerian food products

Results of the systematic literature review are presented in Figure 1. The search strategy yielded 285 studies from the Web of Science search. An additional 14 studies were identified by the authors using Google Scholar and other sources, yielding a total of 300 studies, eligible for title and abstract screening. Among these records, 244 were found to be irrelevant and were excluded from full-text review. The remaining 55 records were subjected to full-text screening for eligibility against the inclusion and exclusion criteria. A total of 41 studies satisfied all criteria and were included in the analysis, including 18, 12, 12, 5, and 3 studies each for maize, groundnut, rice, cowpea, and soybean; five of the included studies reported on more than one of the target commodities (see Table 1).

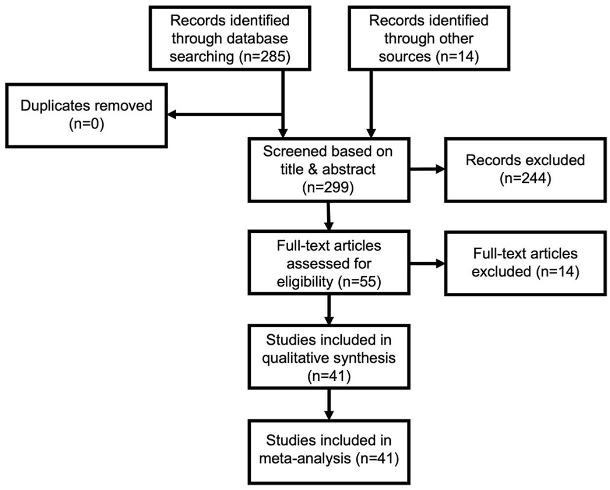

FIGURE 1
PRISMA flow diagram illustrating the systematic literature review process.

The included studies reported 40, 26, 36, 5, and 3 estimates of batch-wise aflatoxin contamination in Nigerian maize, groundnuts, rice, cowpea, and soybean, respectively, including 4 batches in which aflatoxin was not detected. A batch is here defined as a mean representing several (>2) distinct samples, not replicate measures of the same pooled sample. Aflatoxin contamination data reported in the included studies are summarized in Table 2. Of the 41 studies included in the analysis, 16 (39%) reported only total aflatoxin, while the remaining studies included estimates of AFB1. Among the batches included in the study, AFB1 estimates were available for 62%, while the remaining 38% of batches yielded estimates of total aflatoxins only. Prevalence data (% positive samples reported within a given batch) were available for 81.8% of reported batches, where "positive" is here used interchangeably with "detected". Prevalence was highest in groundnut (84.5% ± 17.8) and rice (74.3% ± 29.3) batches, and lowest in soybean (7.3% ± 12.7). However, very few observations available for soybeans and cowpea make results for these two commodities not generalizable.

Maize and groundnut were observed to have the highest mean aflatoxin contamination levels (106.4 ± 154.1 and 78.8 ± 219.2 ng/g, respectively) among the considered commodities. Mean AFB1 concentrations exceeded Nigerian regulatory legal limit of 2 ng/g AFB1 in maize and 20 ng/g total aflatoxin in groundnuts, respectively (Chilaka et al., 2022). Aflatoxin is presently not regulated in Nigeria for rice, cowpea, and soybean, but concentrations observed in rice and cowpea exceeded all limits for aflatoxins in regulated commodities. Groundnut yielded the highest batch-wise maximum aflatoxin concentration, with a concentration of 939 ng/g in the most contaminated batch. The mean values for all batches with at least one contaminated sample are shown in Figure 2.

The aflatoxin data identified in the systematic review were not representative of the entire Nigerian context, but rather were concentrated in certain regions (Figure 3). Of the individual batches

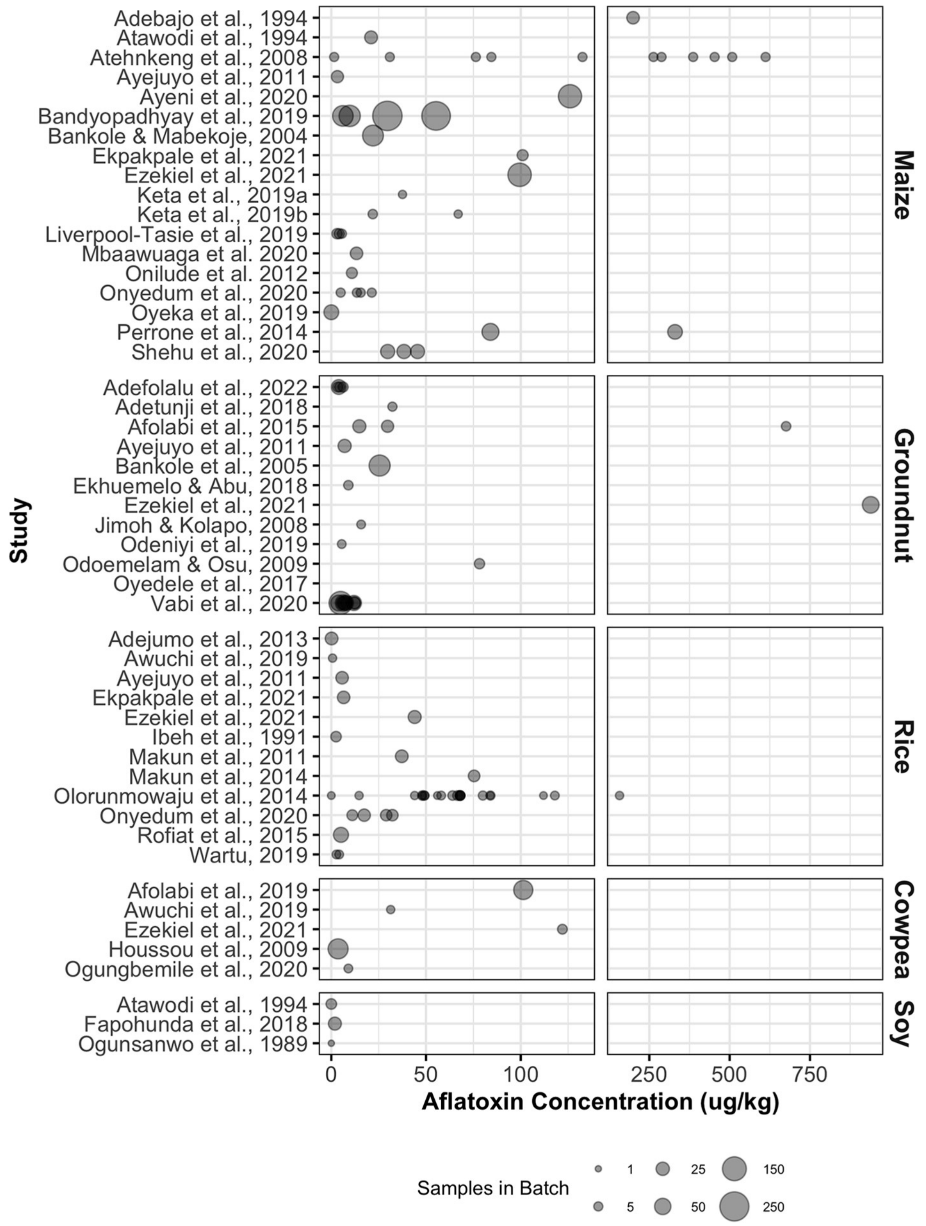

FIGURE 2
Summary of mean aflatoxin concentrations reported for batches of raw commodity samples in the included studies. Point size corresponds to the number of samples within each batch.

reported, the geographical origins were specified at State-level for 70%, while the remaining 30% of batches were geographically specified only at the regional level. By far, the greatest sampling effort at State-level was Kaduna State, which yielded 20% of the batches included in this study. Other states with relatively high sampling efforts included Niger State (8%), Kebbi State (7%), Oyo State (6%), and Lagos (5%). There was a conspicuous lack of available data reporting aflatoxin concentrations in the northeastern and southern regions of the country. The geographical breadth of sampling effort across commodities corresponded to the overall abundance of each commodity in the dataset; the geographical distributions of maize and groundnut batches (the most abundant reported commodities) were greater than those of the other commodities.

3.2. Exposure levels and risk assessment

The probability distribution of the EDI was estimated for each commodity based on the distributions of aflatoxin concentrations, commodity consumption, and adult body weight. There is no tolerable daily intake (TDI) level for aflatoxin, because it has been classified as a Class 1 carcinogen, and thus exposure is considered unsafe at any dose (van de Perre et al., 2015; Saha Turna and Wu, 2022). However, a proposed provisional maximum tolerable daily intake level (PMTDI) of 1 ng/kg bw/day has been used as a reference value for judging risk associated with aflatoxin exposures (Kuiper-Goodman, 1998; Magrine et al., 2011). The commodity with the highest estimated EDI in the exposure model was maize, with a value of 36.7 (95% CI: [22.7–54.8]) ng/kg bw/day, substantially higher than

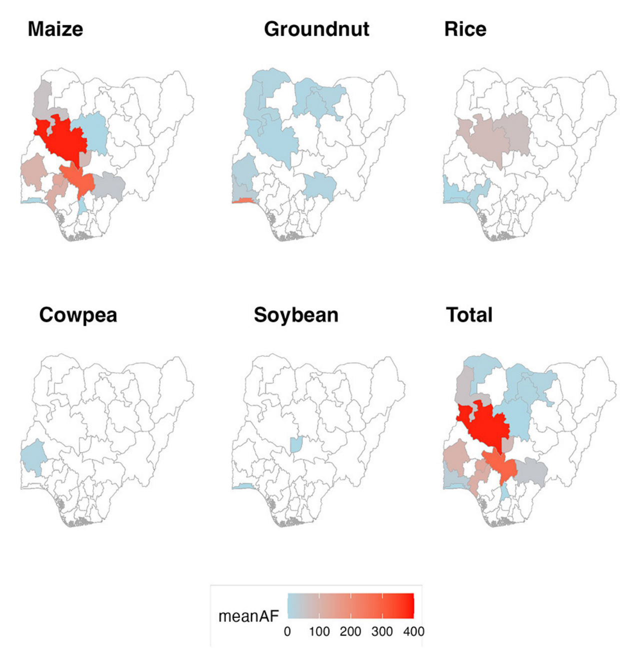

FIGURE 3
Geospatial overview of aflatoxin contamination reported in reviewed literature, reflecting sampling effort and detected levels.

the PMTDI level. The estimate of EDI in the groundnut, rice and cowpea models also exceeded the PMTDI level, but more modestly, with estimates of 9.12 (95% CI: [4.41–19.21]), 8.85 (95% CI: [6.91–11.09]), and 3.14 (95% CI: [1.25–7.22]) ng/kg bw/day, respectively. Dietary aflatoxin exposure attributable to soybean consumption in the study population is much less than the other commodities, with an EDI of 0.28 (95% CI: [0.24–0.32]) ng/kg bw/day, below the PMTDI level. The mean risk of HCC attributable to consumption of maize, groundnut, rice, cowpea, and soybean in the target population was estimated as 1.77 (95% CI: [1.10–2.65]), 0.44 (95% CI: [0.21–0.93]), 0.43 (95% CI: [0.33–0.54]), 0.15 (95% CI: [0.06–0.35]), and 0.01 (95% CI: [0.01–0.02]) HCC cases per year/100,000 population, respectively.

Sensitivity analysis of the relative impacts of variability in the model's input parameters on HCC risk indicated that variability in aflatoxin concentration (C) in a consumed portion has the greatest impact on HCC risk attributable to maize consumption (Spearman's $\rho = 0.64$), followed closely by variability in the frequency of maize consumption ($\rho = 0.58$). Variability in *S*, *i*, and *BW* had comparatively low impact on risk in the maize model. In the rice model, the frequency of rice consumption (f_{cons}) had a very large impact on the level of HCC risk ($\rho = 1.00$), whereas estimates for the other parameters did not exceed 0.1. In both the cowpea and soybean models of HCC risk, the most impactful parameter on risk level was the frequency of contamination (*i*), for which Spearman's ρ was estimated at 0.97 and 1.00 for cowpea and soybean, respectively, indicating a near-perfect correlation. While this suggests that contamination frequency may be a contributory factor, it should be acknowledged that the estimated exposure attributable to these commodities was relatively low, and that the limited data available for these commodities may have obscured contributions from other factors in the sensitivity analysis.

4. Discussion

This study presents novel evidence that dietary consumption of several key commodities found in Nigerian traditional food marketplaces, particularly in the northern regions, may lead to aflatoxin exposures sufficient to cause adverse health outcomes. HCC incidence rate in Nigeria, from all causes, was previously estimated as 2.6 cases per 100,000 individuals (Kedar Mukthinuthalapati et al., 2021); thus, our findings suggest that a substantial proportion of total HCC incidence in the region may be attributable to dietary aflatoxin exposure *via* the consumption of the considered commodities, particularly maize and groundnut.

The estimates presented here are above the chosen PMTDI levels but below prior risk estimates for aflatoxin-associated HCC attributable to single-commodity consumption for infants and children in Nigeria (Adetunji et al., 2017). The relative ranking of these products observed in this study is consistent with existing evidence that cereals (maize and groundnuts in particular) are more susceptible to aflatoxin accumulation than pulses. For both maize and rice in the study area, the higher estimated risk levels are reflective of both high consumption levels and high contamination levels, whereas cowpea and soybean, despite moderate consumption levels in the population, harbor low concentrations of aflatoxin and therefore pose lower risk of aflatoxin-associated HCC. Despite having aflatoxin concentrations comparable to those of maize samples, the HCC risk associated with groundnut consumption is relatively lower, owing to low overall levels of groundnut consumption in the target population.

Moreover, while the present estimate of maize-associated HCC risk is very similar to what was estimated for aflatoxin exposure *via* groundnuts in a Nigerian population (1.38 HCC cases year^{-1} 100,000 pop^{-1}) (Adetunji et al., 2018), the estimate of risk associated with groundnut consumption in the present study was substantially lower. The discrepancy in risk associated with groundnut consumption between the present study and the study conducted by Adetunji et al. could be attributable to the fact that point estimates, rather than probability distributions, of groundnut consumption and aflatoxin concentration were used in their exposure assessment, and that detection rates were not accounted for. This illustrates the potential utility of an exposure modeling approach that accounts for population-specific probability distributions in exposure-related variables. Another Nigerian study (Garba et al., 2021) estimated AFB1-associated HCC risk levels much higher (3.06 cases per 100,000 population) than what was observed in this study; this illustrates the importance of taking a multi-commodity approach in risk assessment.

The risk estimates presented here are on par with other estimates in Nigeria, but they are higher than what was observed in a European population with lower maize consumption and contamination levels (Udovicki et al., 2021). This is consistent with the observation that our EDI estimates for maize and groundnut alone far exceed the average upper bound exposure level for adults reported by EFSA [EFSA Panel on Contaminants in the Food Chain (CONTAM), 2020]. In many sub-Saharan African food system contexts, optimal conditions for fungal growth and aflatoxin accumulation are often coupled with poor food monitoring and regulatory systems. In Nigeria, regulation and enforcement of food safety policy and standards is impeded by outdated infrastructure and insufficient expertise (Okoruwa and Onuigbo-Chatta, 2021).

While the present study offers important insights into the exposure and health risks associated with aflatoxin contamination of key foods in Nigerian traditional food marketplaces, several data gaps and limitations need to be considered. First, the models presented in this study were populated using secondary data sources, which necessitates some assumptions about their applicability to the target population. For instance, data from different Nigerian states were pooled together, unweighted—hence implicitly weighted by sampling effort in reported literature; estimates should therefore be considered representative of current evidence in the country, but not of specific regions or states or of the country as a whole. The systematic review methodology used to compile comprehensive regional aflatoxin contamination data was sufficient for commodities with well-established aflatoxin literature (i.e., maize, groundnut, and rice), but was much more limited for cowpea and soybean, which have scarcer published evidence on aflatoxin contamination levels, in all sub-Saharan Africa. Further risk assessments using primary data from more susceptible commodities, and applying context-specific sampling, would contribute invaluably to the knowledge base around aflatoxin exposure risk in specific regions, as the basis for targeted interventions.

Second, the heterogeneity in reporting formats for aflatoxin prevalence and concentrations hinders the characterization of concentration distributions in meta-analysis. An approach based on batch means was implemented here, capturing the variability across batches, while deprioritizing within-batch variability due to data limitations. In addition, some assumptions were made on consumption amounts and body weight, in the absence of more precise national consumption data. Overall, data were sufficient to support a sound preliminary assessment, but estimates are less accurate than assessments conducted using primary data. Following agreed-upon best reporting practices for aflatoxin detection rate, limits of detection, and concentrations would improve estimate accuracy. However, the level of detail included in this study—if applicable to the specific local context—is sufficient to inform intervention decisions and to highlight critical data gaps that prevent such decisions (e.g., here for cowpea or soybean and derived foods).

Third, this study focused specifically on risk of HCC, which is just one of several possible health outcomes of interest (Gong et al., 2016). HCC was selected based on its severity and its relatively well-defined dose-response relationship with aflatoxin. However, aflatoxin-associated malnutrition and growth impairment outcomes are increasingly a focus of the research community and are critically in need of comprehensive risk assessment as studies to date have not conclusively shown a causal linkage (Turner et al., 2007; Leroy et al., 2018) and the biological mechanisms have not been elucidated (Tessema et al., 2021). As these outcomes often arise as a result of several environmental factors in combination (Moore et al., 2001), it is challenging (and ethically dubious) to articulate dose-response relationships for these outcomes (Turner, 2013).

Our findings indicate substantial reason for concern regarding the risk of aflatoxin exposure in Nigeria, but this is set against a backdrop of promising management efforts in the country that have potential for further scalability and contextualization. For example, Nigeria has been a pioneer in Africa in the development and use of biocontrol agents to combat aflatoxin-producing fungi (Bandyopadhyay et al., 2019; Ortega-Beltran and Bandyopadhyay, 2021). Additionally, value chain approaches to mycotoxin management have been initiated in the country. For example, one recent study found that aflatoxin-safe labeling programs influence willingness to pay for actors across the value chain and may incentivize consumption of certified aflatoxin-safe products (Sanou et al., 2021). These efforts and others illustrate that there are practical control strategies in place that could leverage risk assessments to ensure that intervention is appropriately directed to populations with the greatest need.

Overall, this study provides a comprehensive quantitative synthesis of published evidence (as of 2022) on aflatoxin contamination in key staple commodities in Nigeria, national-level exposure and risk estimates based on such data, and a novel approach leveraging secondary data that can be adapted by other researchers to conduct similar assessments. Findings point to a non-negligible potential HCC risk associated with maize and groundnut, in alignment with previous estimates that highlight the specific contributions of maize and groundnut to HCC risk. Clear data gaps exist for cowpea and soybean, preventing a meaningful risk assessment; however, because of moderate consumption and less implication in aflatoxin exposure, cowpea and soybean are of less concern than maize and groundnut. The heterogeneity in aflatoxin levels, and hence risk estimates, across the country warrants attention if using these findings to inform localized intervention decisions; complementing country-scale findings with local primary data is encouraged.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AW and EL designed the study and developed the methodology. AW synthesized input data, coded, and run the model. EL oversaw all study steps and provided guidance. FM, LT, and DG provided input on study approach, input data, and result interpretation. AW led manuscript writing and graphics, with inputs and review from EL. All authors reviewed the final draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1128540/full#supplementary-material

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Describing capability, opportunity, and motivation for food safety practices among actors in the Cambodian informal vegetable market

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Introduction: Several Cambodian initiatives seek to improve nutritional outcomes *via* increased production and consumption of nutrient-dense foods, including vegetables. However, food safety gaps in informal markets, where most vegetables are purchased, allow for the transmission of foodborne pathogens and threaten the positive nutritional outcomes associated with vegetable consumption.

Methods: This study describes a tool used to measure perceptions of Cambodians involved with informal vegetable markets regarding their capabilities, opportunities, and motivations to implement food safety practices. The quantitative tool could also be used to assess capability, opportunity, and motivation to adopt a behavior in a wide range of development contexts. To these ends, a questionnaire assessing these perceptions was developed using the Capability, Opportunity, Motivation-Behavior (COM-B) model of behavior and the Theoretical Domains Framework (TDF).

Results: The questionnaire was piloted with vegetable vendors in Phnom Penh ($N = 55$), revised, and subsequently implemented in the provinces of Battambang and Siem Reap with vegetable producers, distributors, and vendors ($N = 181$). Confirmatory factor analysis resulted in a nine-factor model corresponding to TDF constructs with a comparative fit index of 0.91, a Tucker-Lewis index of 0.89, and a root mean square error of ∼0.05. Further analysis indicated that vegetable vendors and distributors typically had significantly higher ($p < 0.05$) levels of perceived motivation and capability to implement the target food safety practice (washing surfaces that come in contact with vegetables with soap and water every day) compared to their perceived opportunity to do so. Among farmers, however, levels of perceived motivation were significantly higher ($p < 0.05$) than levels of perceived opportunity and capability. In addition, vendors in Battambang had significantly higher ($p < 0.05$) levels of perceived capability, opportunity, and motivation to implement the target food safety practice in comparison to farmers in either province. Vendors in Battambang had significantly higher ($p < 0.05$) levels of perceived opportunity and motivation than vendors in Siem Reap.

Conclusions: These data suggest that efforts to bolster vegetable vendors' and distributors' perceived opportunity and vegetable farmers' perceived opportunity and capability to implement food safety practices could increase the likelihood of adoption of the target food safety practice.

KEYWORDS

Cambodia, food safety, capability, opportunity, motivation

Introduction

Cambodia has seen rapid economic growth and significant reductions in poverty over the past several decades, to the extent that the World Bank changed its classification of Cambodia from a low income to a lower-middle income economy in 2015 (World Bank Group, 2017). Cambodia's population has also grown in recent years and is currently estimated to be ~16.7 million (National Institute of Statistics and Ministry of Planning, 2020a; Central Intelligence Agency, 2022). Over 60% of the Cambodian population lives in rural areas, and, though the number of individuals primarily employed in the agricultural sector has declined, between 50% (National Institute of Statistics and Ministry of Planning, 2020b) and 80% (National Institute of Statistics and Ministry of Planning, 2020a) of the employed rural population between the ages of 15 and 64 still works primarily in production agriculture (National Institute of Statistics and Ministry of Planning, 2020a).

Despite recent economic improvements, malnutrition remains a problem throughout Cambodia and is of particular concern in rural areas, in households in which the mother has little or no formal education, and in households with incomes in the lowest quintile (National Institute of Statistics, Ministry of Health, and ICF, 2022). The most recent national health survey performed in Cambodia found that 9.6% of surveyed children under the age of 5 experienced wasting and 21.9% were stunted (National Institute of Statistics, Ministry of Health, and ICF, 2022); these incidence rates are considered medium and high, respectively, based on the prevalence thresholds developed by the World Health Organization and UNICEF (de Onis et al., 2018). Both the Cambodian government and international development organizations have taken steps to address these nutritional challenges by implementing various nutrition-focused policies and programs, including those designed to increase local production and consumption of vegetables (Ministry of Agriculture, Forestry and Fisheries, the European Union, and the United States Agency for International Development, 2015; Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification, 2020; Save the Children, 2020).

Currently, much of the fresh produce sold in Cambodia is sold in the informal vegetable market (Sokhen et al., 2004). The informal vegetable market in Cambodia is similar to informal markets found in countries throughout the world in that it is largely unregulated in terms of governmental monitoring or fee collection (e.g., taxing) and exists in parallel with the formal vegetable market. In Cambodia, the informal vegetable market is composed of a loose network of vegetable farmers, collectors, distributors, and vendors (Sokhen et al., 2004; Desiree et al., 2020). There are typically few to no food safety standards enforced on the farms where vegetables are produced or in the open-air wet markets where vegetables are sold, and poor sanitary conditions are common in both environments (Sokhen et al., 2004; Desiree et al., 2020). In addition, previous research found that raw vegetables and vegetable contact surfaces in open-air wet markets in Cambodia are commonly contaminated with bacterial pathogens (Phoeurk et al., 2019; Desiree et al., 2021; Schwan et al., 2021). These findings indicate that current food safety practices in these markets are not sufficient to mitigate the foodborne disease risks associated with the consumption of raw vegetables. Some of the key food safety gaps in the informal vegetable market supply chain noted by Desiree et al. (2020) included improper manure composting and application, use of poor quality water to irrigate or wash vegetables, failure to regularly sanitize vehicles prior to vegetable transport, scarcity of cold storage, lack of improved sanitation (e.g., toilets, handwashing stations), and the comingling of fresh produce with raw animal-sourced foods, among others. Chemical contamination of food is also of concern in Cambodia, and both chemical and microbial contamination have been linked with foodborne disease outbreaks in recent years (Thompson et al., 2021).

Fresh fruits and vegetables present a particular food safety concern because these foods are often eaten raw and are not typically subjected to high cooking temperatures or other kill steps that reduce or eliminate pathogenic bacteria. Consuming food contaminated with pathogens can cause a variety of foodborne illnesses, of which diarrheal diseases are the most common (Havelaar et al., 2015). Diarrhea is associated with dehydration, malnutrition, decreased economic stability due to lost productivity and healthcare costs, and, in some cases, death (National Institute of Diabetes and Digestive Kidney Diseases, 2016; World Health Organization, 2017; Niyibitegeka et al., 2021). Diarrheal diseases are particularly significant for children's health: among children under 5 years of age, diarrhea is a leading cause of mortality and malnutrition worldwide (GBD 2015 Mortality and Causes of Death Collaborators, 2016; World Health Organization, 2017). In Cambodia specifically, the WHO estimates that 7.4 and 15% of all deaths among children under 5 (0–4 years of age and 1–59 months of age, respectively) are due to diarrhea (World Health Organization, 2018). Furthermore, in a survey performed in 2010, ~15% of all Cambodian children under 5 years of age were reported to have had diarrhea within the past 2 weeks (National Institute of Statistics, Directorate General for Health, and ICF Macro, 2011). While the proportion of diarrheal disease that is caused by foodborne pathogens is unknown, even conservative estimates would suggest that the diarrheagenic diseases caused by microbial contamination of food have significant negative impacts on overall health outcomes (Grace, 2015).

Considering that substantial food safety gaps exist in informal produce markets in Cambodia and that several studies have confirmed the presence of pathogens on vegetables in these markets (Sokhen et al., 2004; Phoeurk et al., 2019; Desiree et al., 2020, 2021; Schwan et al., 2021), it is clear that consuming fresh vegetables puts Cambodians at risk of contracting foodborne illnesses such as diarrhea. Given that the intestinal disruptions caused by foodborne illnesses often result in nutrient malabsorption, it then follows that foodborne illness contracted due to consuming vegetables may negate the nutritional benefits of fresh vegetable consumption. Thus, in order to effectively improve the nutritional status of Cambodian communities, food safety concerns must be addressed alongside nutritional concerns.

Much of the knowledge and technologies needed to mitigate the food safety concerns present in the Cambodian vegetable market, and in many food markets throughout the world, already exists (Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, 2015). However, poor food safety is a persistent issue in many nations. Lack of access to resources, insufficient training, regulatory limitations, and the need for significant behavioral change at both individual and community levels may all contribute to implementation gaps between food safety knowledge and food safety practice. Facilitating voluntary behavior change can be especially challenging, even when the change needed appears straightforward to the development organization or government entity promoting it. For this reason, techniques that allow program implementers to more effectively enable behavior change are key to both improving food safety and to facilitating positive behavior change in a variety of development contexts. Using behavior theory to inform program design may be one such technique. Behavior theories are theoretical models intended to explain and predict behavior by identifying and defining the factors that influence behavior and explaining how these factors relate to each other (Kerlinger, 1986, as cited in Glanz et al., 2015, p. 26). By applying a specific behavior theory or theories to their projects, program designers and implementers could position themselves in a clear, operationalizable framework within which they may identify and define potential determinants of behavior. These frameworks may then be used to inform the creation of assessment tools that measure behavioral determinants within particular audiences, thereby increasing program designers' and implementers' capacity to recognize and address audience-specific barriers to and facilitators of behavior change.

Conceptual framework

The Capability, Opportunity, Motivation-Behavior (COM-B) model of behavior (Michie et al., 2011) and the Theoretical Domains Framework (TDF; Michie et al., 2011; Cane et al., 2012) were used in combination as the conceptual framework of this research. Taken together, the COM-B model and the TDF provided a specific perspective by which to identify and define the potential determinants of food safety practice adoption and the relationships between these determinants. The TDF aligns with the COM-B model but provides, in many cases, higher resolution with the three main constructs of COM-B (capability, opportunity, and motivation).

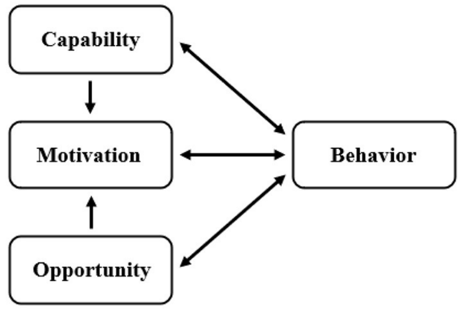

FIGURE 1
The COM-B model of behavior (adapted from Michie et al., 2011).

The COM-B model of behavior proposed by Michie et al. (2021; Figure 1) posits that the combination of an individual's capability, opportunity, and motivation for a behavior determines whether or not the individual will perform that behavior. Capability is defined as the "psychological and physical capacity" to perform a behavior, while opportunity is defined as "all the factors that lie outside the individual that make the behavior possible or prompt it" (Michie et al., 2011). Finally, motivation encompasses all of the mental processes that "energize and direct" behaviors (Michie et al., 2011). In the COM-B model, Michie et al. (2011) suggest that capability and opportunity have the potential to affect motivation and that all three of these behavioral constructs may both influence and be influenced by behavior (Figure 1). The TDF, a synthesis of over 30 distinct behavior theories, further defines and operationalizes capability, opportunity, and motivation by subcategorizing each of these behavioral constructs into measurable domains (Figure 2; Michie et al., 2011; Cane et al., 2012).

Originally, the COM-B model was designed to serve as an improved theory of behavior and to aid in the creation of context-specific interventions that effectively promote behavior change (Michie et al., 2011). In the research presented herein, determinants of food safety behavior change were explored in the context of informal vegetable markets in Cambodia. The results of this research were ultimately intended to inform the design of food safety engagement programs that facilitate the adoption of food safety practices. Thus, the COM-B model seemed well-suited to the purposes of this study. The use of this model in conjunction with the TDF was particularly fitting, as the combination of these frameworks enabled the design of a theoretically grounded quantitative questionnaire that may be used to quantify and assess perceptions of capability, opportunity, and motivation for specific behaviors both among Cambodians involved with informal vegetable markets and in other development contexts.

Research objectives

Informed by the COM-B model of behavior and the TDF, the primary purpose of this research was to develop a validated

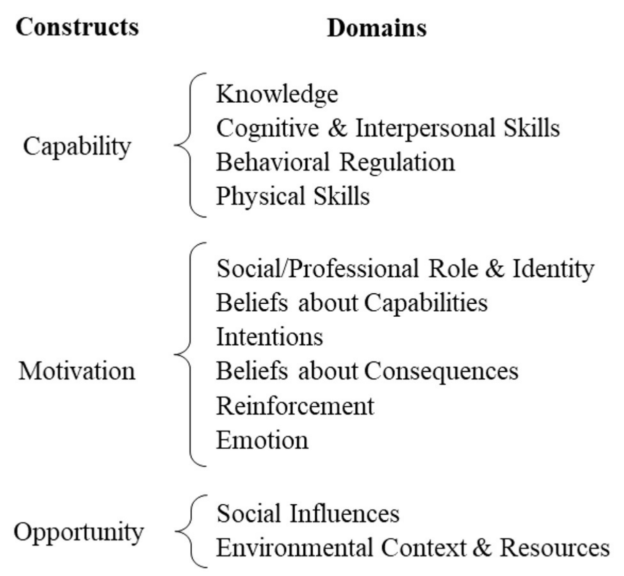

FIGURE 2
The behavioral constructs (capability, opportunity, and motivation) and within-construct domains of the COM-B model and TDF used to create the questionnaire (Michie et al., 2011; Cane et al., 2012). To note, the TDF domains optimism, goals, and memory, attention, and decision processes were excluded from the questionnaire. Optimism and goals are in the motivation construct, while memory, attention, and decision processes is in the capability construct.

instrument that explores, describes, and quantifies the perceptions of Cambodians involved with informal vegetable markets regarding their own capabilities, opportunities, and motivations to implement food safety practices. Specifically, the results of the research were intended to provide quantitative data on the levels of these three behavioral components within each group and to identify which, if any, of these components may be acting as a limiting factor for behavior change within a group. This quantitative data could then be used to tailor food safety programs to specific audiences, with the ultimate goal of increasing both the implementation of food safety practices and the positive outcomes expected to accompany these practices. Additionally, we aimed to create the instrument in a manner that would allow measurement of target audiences' perceptions of their own capability, opportunity, and motivation for specific behaviors across a variety of development contexts, both within and outside of the realm of food safety. Such a questionnaire could facilitate the incorporation of behavior theory into program design and provide development practitioners with quantitative data that could be used to inform and improve the design and implementation of development programs in many fields.

Methods

Ethical approval

All research described here was reviewed by the Institutional Review Board of Purdue University (West Lafayette, IN) and by the Royal University of Agriculture (Phnom Penh, Cambodia) and deemed exempt (IRB-2020-383). No participants received any form of remuneration for participating in the study.

Questionnaire overview

A quantitative questionnaire was designed for the primary purpose of measuring the perceptions of Cambodians involved with informal vegetable markets regarding their own capabilities, opportunities, and motivations to implement a specific food safety practice. However, the questionnaire was designed in such a way so as to be easily adaptable to different target audiences, practices, and geographic locations in order to facilitate its use in other development contexts. The content and structure of the questionnaire was based on the COM-B model of behavior and the TDF in order to ground the proposed research in validated theory. The questionnaire assessed participants' perceptions of the three behavioral constructs identified as the primary determinants of behavior in these models (i.e., capability, opportunity, and motivation) through quantitative questions based on 12 construct-specific domains drawn from the TDF (Figure 2). These domains refined the definitions of capability, opportunity, and motivation, thereby facilitating a comprehensive and precise evaluation of each construct (Figure 2). To note, three TDF domains (i.e., optimism, goals, and memory, attention, and decision processes) were excluded from the questionnaire due to limited applicability to the target behavior or excessive overlap with other domains.

Questionnaire validation

An initial draft of the questionnaire consisting of 68 content (i.e., non-demographic) questions was reviewed for content and face validity. This review was intended to ensure that (1) questions were aligned with the appropriate behavioral construct and construct-specific domain, and that (2) the questionnaire adequately addressed all relevant aspects of the three behavioral constructs with minimal redundancy. Subsequently, a revised version of the questionnaire containing 45 content questions was reviewed by Cambodian university students and Cambodian members of the research team to ensure that all questions were understood easily, understood in the intended way, and culturally appropriate. Following additional revisions, the questionnaire was translated into Khmer by Cambodian members of the research team. Back translation was then performed by an outside translation service to confirm that questions had retained their meaning during the initial translation process. Finally, the research protocol was submitted for ethical approval and the questionnaire was digitized using KoboToolbox (Kobo Inc, 2023) to facilitate field implementation and delivery. The digitized version of this questionnaire served as the pilot questionnaire and consisted of 39 content questions.

Quantitative survey research course

Concurrent with the first steps of questionnaire creation, several members of the research team hosted an online course on quantitative survey research for Cambodian undergraduate students. The course met for ∼1.5 h twice a week for 5 weeks and covered topics including survey question creation, reliability and validity assessments, survey implementation, and data analysis. The goal of this course was to build capacity for future Cambodian-led survey research by training Cambodian students in rigorous quantitative survey research and providing them with an avenue for obtaining certification to conduct human subjects research through the Collaborative Institutional Training Initiative Program (The CITI Program, n.d.). In total, 77 students from the Cambodian Institute of Technology and the Royal University of Agriculture completed the course. The students involved in this course reviewed the quantitative questionnaire developed for this study to ensure that the questionnaire was both easy to understand and culturally sensitive. Several students also received additional training regarding survey implementation and served as survey enumerators during data collection.

Pilot study and questionnaire revision

The pilot study was limited to vegetable vendors in informal markets in Phnom Penh, Cambodia in September 2021 and was intended to assess questionnaire reliability and validity. Vegetable vendors were selected for participation using availability sampling techniques with total sample numbers determined by availability and the questionnaire was administered in-person by Cambodian members of the research team (Daniel, 2012). Survey enumerators read each question and its corresponding answer scale aloud in Khmer to each participant so that participants would have a clear and consistent understanding of the questionnaire's content regardless of literacy level. The food safety practice chosen was "washing surfaces that come in contact with vegetables with soap and water." Two rounds of data collection were performed using the pilot questionnaire. In total, 55 vegetable vendors were approached using availability sampling for participation in the pilot study. Excluding individuals who were approached but declined ($n = 2$) to participate and respondents who answered <50% of the questions on the questionnaire, these selection and sampling methods led to 50 sets of complete responses in the data set. To note, all participants were 18 years of age or older and consented to participate in this research.

Responses collected during the pilot study were evaluated to assess the reliability and validity of the questionnaire using correlation plots, heat maps, and confirmatory factor analysis (CFA) created and performed *via* the corrplot (corrplot package), heatmap (stats package), and cfa (lavaan package) functions of R in RStudio Team (2020). Correlation plots and heat maps enabled visualization of the correlation structure of the data and filtering of questions that did not align with other questions in the same behavioral construct, that correlated negatively with many other questions, or that correlated strongly with questions across multiple constructs. CFA loadings and fit indices were used to evaluate the strength of individual questions and whether or not the theoretical models that informed the design of the questionnaire (i.e., the COM-B model of behavior in conjunction with the TDF) adequately fit the observed data. Based on these analyses and on feedback from survey enumerators regarding their observations of participants' confusion over certain questions, several questions were either removed or revised to improve clarity and alignment with the appropriate construct.

Data collection in the provinces

The final questionnaire consisted of 30 questions surrounding a single food safety practice (i.e., washing surfaces that come in contact with vegetables with soap and water every day). All but two questions asked respondents to indicate their level of agreement with a particular statement (e.g., I would wash the surfaces that come in contact with harvested vegetables with soap and water every day if it helped me to sell my vegetables to new buyers") using a 7-point Likert scale (1 = Strongly disagree; 2 = Disagree; 3 = Somewhat disagree; 4 = Neither agree or disagree; 5 = Somewhat agree; 6 = Agree; and 7 = Strongly agree). The questionnaire was implemented with vegetable farmers, vegetable distributors, and vegetable vendors involved in the informal vegetable market in the provinces of Battambang and Siem Reap during November of 2021. These participant groups were specifically targeted for inclusion in this research as they represented the primary groups involved in the informal vegetable market in Cambodia (Sokhen et al., 2004; Desiree et al., 2020). The provinces of Battambang and Siem Reap were chosen as the geographic focus of the final data collection events because both of these provinces are a part of the current USAID Feed the Future Zone of Influence in Cambodia (United States Agency for International Development, 2014).

Survey enumerators identified potential participants in Battambang and Siem Reap using availability sampling techniques (Daniel, 2012), which dictated the total number of samples collected. A total of 196 individuals were approached for participation in the study. Both men and women were surveyed, but the distribution of responses was not equal across genders because certain participant groups were composed almost entirely of only one gender (e.g., most Cambodian vegetable vendors are female). As such, due to significant differences in samples sizes between male and female respondents throughout the study making statistical analysis inappropriate, data were not disaggregated by gender. All respondents were at least 18 years of age and voluntarily agreed to complete the questionnaire. Excluding individuals who were approached but declined ($n = 5$) to participate and respondents who answered <50% of the questions on the questionnaire, these selection and sampling methods led to 181 sets of complete responses in the data set. Specifically, complete responses were collected from vegetable farmers ($n = 27$), vegetable distributors ($n = 37$), and vegetable vendors ($n = 26$) in Battambang, and from vegetable farmers ($n = 30$) and vegetable vendors ($n = 61$) in Siem Reap. No vegetable distributors were surveyed in Siem Reap as there is no large vegetable distribution center in that province. As before, the questionnaire was delivered in-person in Khmer by native

Khmer-speaking Cambodian national enumerators. Enumerators read each question aloud in Khmer and repeated the corresponding answer scale aloud in Khmer for each question.

Data analysis

Following data collection in Battambang and Siem Reap, response data were cleaned (i.e., questionnaires that were < 50% complete were excluded) and formatted for analysis in RStudio using the mice (mice package); corrplot (corrplot package), heatmap (stats package), and cfa (lavaan package) functions of R (RStudio Team, 2020). The mice function was employed using the predictive means matching method to generate values for missing data points assuming missing values were missing at random (MAR). Correlation plots and heat maps were created to allow visualization of the correlation structure of the data. CFA was also performed in order to assess the level of fit between the theoretical model and the data set. Given that the COM-B model includes three behavioral constructs, initial iterations of CFA were based on a three-factor model. During additional iterations of CFA, questions were further divided based on visual analyses of correlation plots, the structure of the COM-B model and the TDF, and model fit statistics (i.e., the comparative fit index, Tucker-Lewis index, and root mean square error of approximation). To note, the questionnaire included one free response question regarding who, if anyone, required participants to wash vegetable contact surfaces with soap and water every day. Responses to this question were not included in these statistical analyses due to their qualitative nature.

After performing CFA, an ANOVA shell as adapted by Stroup (2013) was used to formalize description of the data generation process and subsequently specify the statistical model to be used for data analysis. A general linear mixed model was fitted to questionnaire responses defined in a 1–7 Likert scale assuming a normal approximation. The linear predictor included the fixed effects of functional group (defined by five levels reflecting the appropriate combinations of participant role and location, i.e., farmers in Siem Reap, farmers in Battambang, vendors in Siem Reap, vendors in Battambang, and distributors in Battambang) and behavioral construct (i.e., capability, opportunity, and motivation) as well as their two-way interactions. Random effects fitted into the model consisted of person nested within functional group and its cross product with behavioral construct in order to properly identify the experimental units for each of the treatment factors, respectively, as well as recognize subsampling in the data collection process. Doing so ensured proper calibration of tests and estimation of degrees of freedom for inference. Model assumptions were evaluated using externally studentized residuals and were considered to be reasonably met.

The statistical model was fitted using the GLIMMIX procedure of SAS® data analysis software (SAS Version 9.4. SAS Institute Inc., Cary, NC, 2023). Variance components were estimated using restricted maximum likelihood, and a Kenward-Rogers approach was used to estimate degrees of freedom and to make corrections to estimated standard errors. Estimated least square means and their corresponding standard errors were calculated at the appropriate level of inference in the factorial treatment structure. Relevant pairwise comparisons were also conducted using a Bonferroni adjustment to avoid inflation of the Type I error rate due to multiple comparisons. Adjusted p-values were used for statistical inference and differences were considered significant at a level of $p < 0.05$.

Results

Pilot study and questionnaire validation/revision

Due to inconsistency in response trends between survey enumerators in the initial data collection event for the pilot study ($N = 30$ participants), additional steps were taken to ensure that the questionnaire was understood clearly and consistently by all enumerators and respondents. These steps included showing respondents a visual representation of the answer scale as well as reading the scale aloud after each question. Inter-enumerator response consistency improved in the second data collection event ($N = 20$ participants), with comparatively less variation in respondents' answers across different enumerators for most questions. For this reason, the correlation plot used to inform the revisions process was created using response data from the second data collection event only.

The pilot questionnaire was revised based on both enumerator feedback and visual evaluations of correlation within and across behavioral constructs. Initial discussions with enumerators led to the exclusion of three capability construct questions that respondents perceived as confusing or illogical. Survey enumerators also noted that some respondents did not understand the purpose of the first two questions on the pilot questionnaire, which asked if and by whom respondents were required to perform the target behavior, making these questions seem strange and slightly off-putting to some respondents. With this in mind, these two questions were relocated to the end of the questionnaire in order to avoid confusing respondents at the outset of the questionnaire. In addition, nine questions that were considered to be outside of the scope of the research or non-essential were removed in order to shorten the questionnaire and minimize respondent fatigue. Specifically, four motivation construct questions that concerned vegetable quality, two questions that referred to participants' "businesses" (one opportunity and one motivation construct question), and three opportunity construct questions that were deemed non-essential were removed in order to focus and shorten the questionnaire.

The correlation plot of response data from the second data collection event of the pilot study indicated that questions in the motivation and capability constructs generally correlated positively with other questions in the same construct. Questions in the opportunity construct, however, tended to correlate positively within their respective TDF domains but not across domains within the same construct. In addition, several questions were found to correlate positively across multiple constructs (Figure 3). Though some positive correlation across different behavioral constructs was expected based on the relationships and interdependencies between capability, opportunity, and motivation described in the COM-B model, two questions in the motivation construct that correlated positively across multiple constructs were re-evaluated for clarity. One question was removed, while the other was re-assigned to a

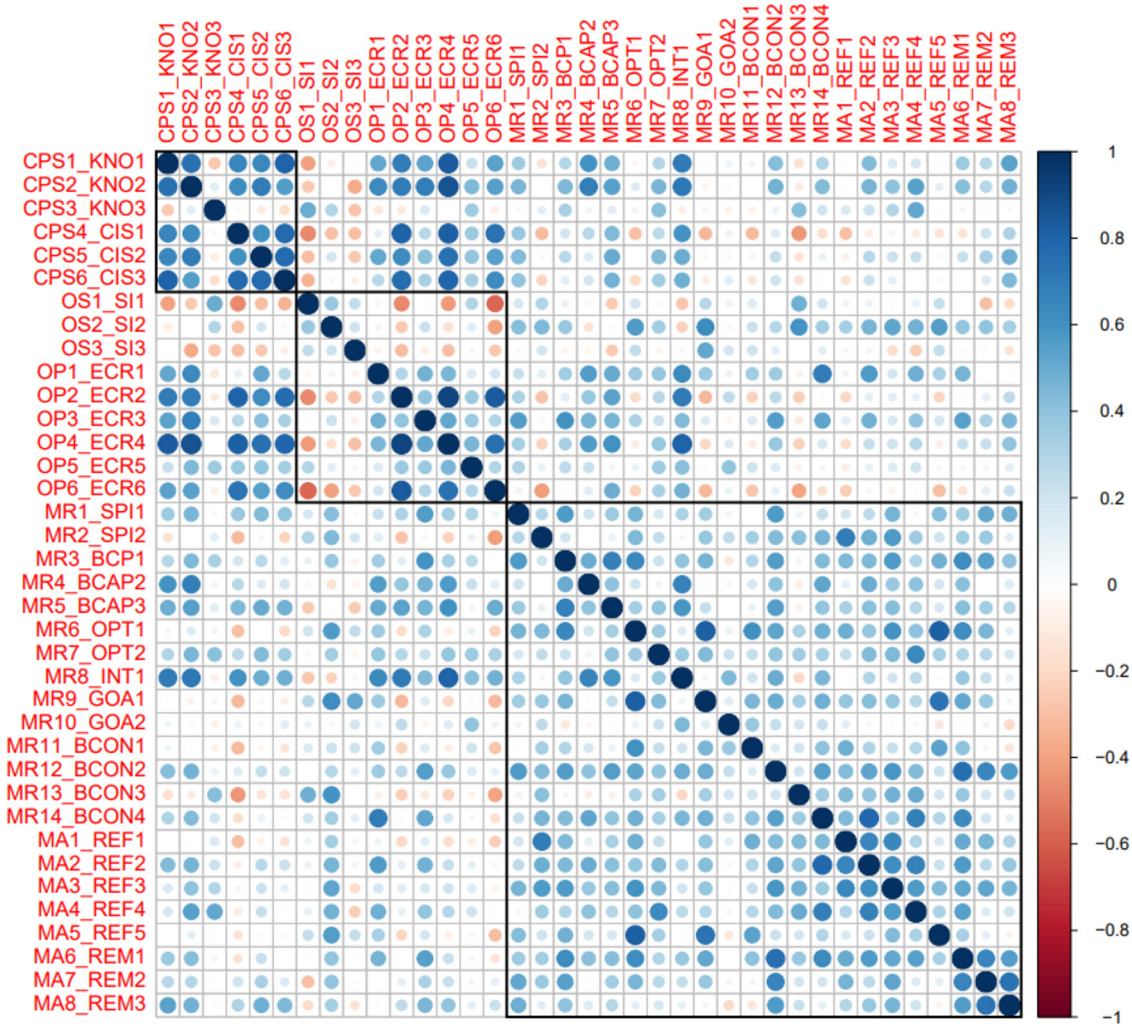

FIGURE 3
Correlation plot of response data collected from 20 vegetable vendors in Phnom Penh during the second data collection event of the pilot study. Question codes (e.g., CPS1_KNO1) indicate the COM-B construct and TDF domain in which the question falls. Black squares indicate questions in the same behavioral construct.

different TDF domain within the motivation construct to better reflect the question's central focus. In addition, one question in the capability construct appeared to correlate weakly with the other capability construct questions. This question was re-assigned to the opportunity construct in order to further refine the TDF domain groupings.

After the removal of all of the aforementioned questions, one additional question in the motivation construct was also re-assigned to a different TDF domain in order to consolidate two single-question domains in the motivation construct (i.e., goals and intentions, see Figure 2). Three questions in the motivation construct were also reworded slightly to improve their alignment with the appropriate TDF domain. Finally, four questions were added in order to support a thorough evaluation of the domains and constructs of the TDF and COM-B model. Two of these questions were added to the capability construct, one to the motivation construct, and one to the opportunity construct. Additionally, the target behavior, "washing surfaces that come in contact with vegetables with soap and water" was amended to "washing surfaces that come in contact with vegetables with soap and water every day" in order to reflect the repetitive, ongoing nature of the target behavior. CFA was then performed to assess the validity of the pilot questionnaire.

Taken together, these revisions resulted in a final questionnaire composed of 30 content (i.e., non-demographic) questions, of which 28 had a 7-point agree/disagree Likert answer scale. Of the two remaining questions, one had a binary yes/no answer scale and the other was a free response question. The final questionnaire had five questions in the capability construct, seven questions in the opportunity construct, and 17 questions in the motivation construct on the final questionnaire. The disparity in the number of questions contained in each construct was due to differences in the number of components required to comprehensively assess each construct as reflected in the TDF model. The free response question was not categorized into a construct. The final version of the questionnaire has been provided under Supplementary material.

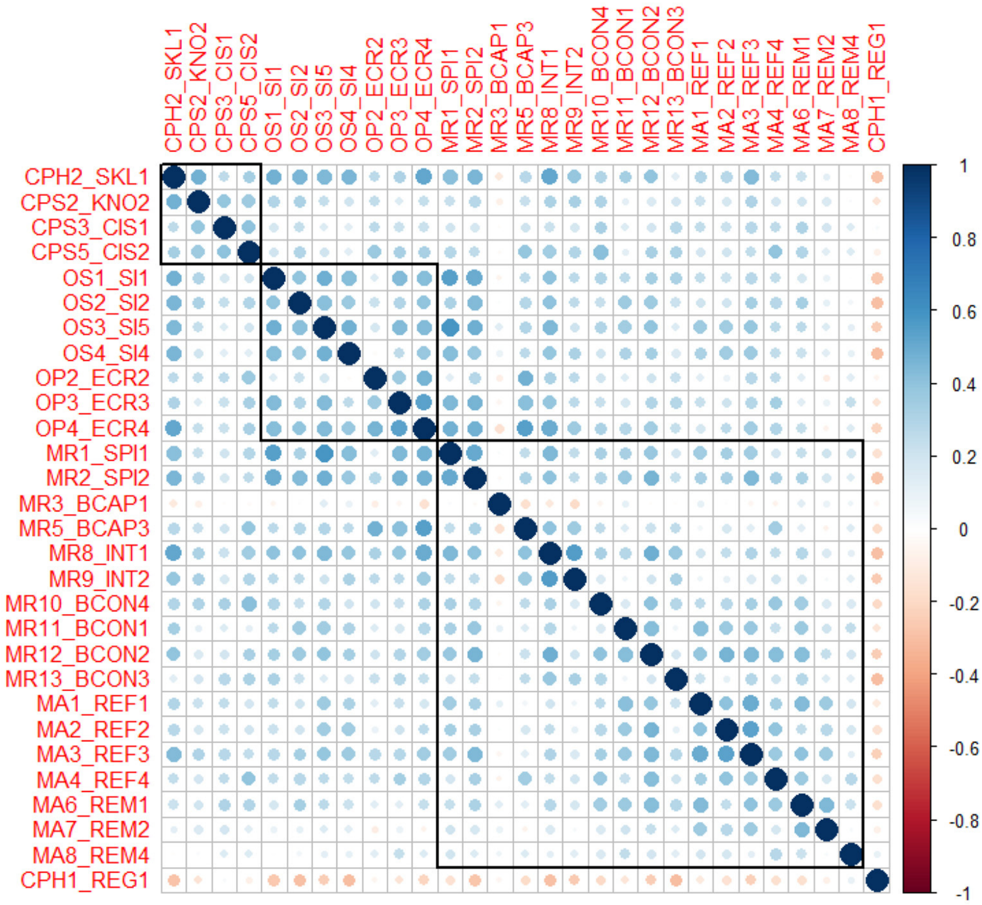

FIGURE 4
Correlation plot of response data collected from vegetable farmers, vendors and distributors in Battambang and from vegetable farmers and vendor in Siem Reap (data from $n = 181$ participants in total). Question codes (e.g., CPS1_KNO1) indicate the COM-B construct and TDF domain in which the question falls. Black squares indicate questions in the same behavioral construct. To note, though not located in the same square, CPH1_REG1 was also included in the group of questions comprising the capability construct (upper lefthand square).

Data collection in the provinces

Visual analyses of correlation plots of response data (Figure 4), qualitative analyses of question suitability, and CFA resulted in the exclusion of data from three questions from the final factor analysis model. One question in the capability construct was excluded because it correlated negatively with virtually all other questions, loaded negatively onto the CFA model (estimated loading of -0.17 ± 0.04 in the first iteration of CFA), had a binary yes/no answer scale rather than a 7-point agree/disagree Likert scale, and was a demographic question. Another question in the capability construct was removed because it was identified as a measurement of the target behavior itself rather than a measurement of participants' perceptions of their capability, opportunity, or motivation for that behavior. Finally, a question in the motivation construct was excluded because it correlated weakly with nearly all other questions, loaded poorly onto the CFA model (estimated loading of 0.085 ± 0.13 in the second iteration of CFA) and involved a double negative that may have made the question difficult to understand clearly and consistently. To note, the correlation plots created using this data set indicated that many questions positively correlated with questions in multiple constructs. As previously noted, this cross-construct correlation was expected to some degree due to the inter-relatedness of capability, opportunity, and motivation within the COM-B model.

CFA was performed iteratively, beginning with a model with three factors. These three factors corresponded to the capability, opportunity, and motivation constructs of the COM-B model and consisted of five, seven, and 17 questions, respectively. Following the removal of the three questions described in the previous paragraph, CFA concluded with a nine-factor model. These nine factors were comprised of nine groups of questions that corresponded to the capability construct ($n = 3$ questions) and to the social/professional role and identity ($n = 2$ questions), beliefs about capabilities ($n = 1$ question), intentions ($n = 2$ questions), beliefs about consequences ($n = 4$ questions), reinforcement ($n = 4$ questions), emotion ($n = 3$ questions), social influences ($n = 4$ questions), environmental context, and resources ($n = 3$ questions) TDF domains. Questions in the capability construct were not divided by their TDF domains during the final iteration of CFA due to the limited number of questions within the construct and because the correlation plot supported grouping these questions by

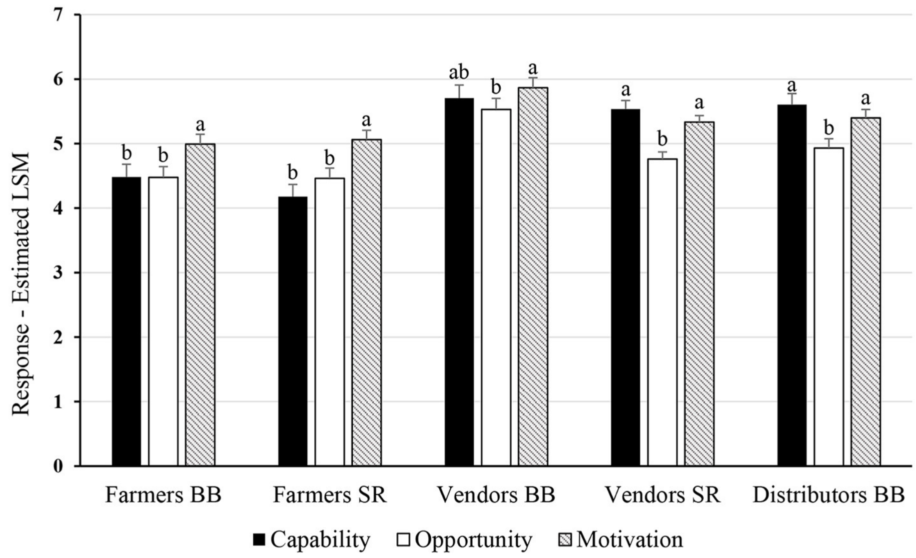

FIGURE 5
Estimated mean responses (and corresponding SE) to the sets of questions comprising the behavioral constructs of capability, opportunity, and motivation for each functional group. Different letters indicate significant differences ($p < 0.05$) between behavioral constructs within a functional group.

construct, among other considerations. The final nine-factor model had a comparative fit index of 0.91, a Tucker-Lewis index of 0.89, and a root mean square error of ∼0.05.

Following these assessments of reliability and validity, differences in levels of perceived capability, opportunity, and motivation within each functional group were investigated (Figure 5), as supported by a significant two-way interaction ($p < 0.001$). To note, the estimated differences in questionnaire responses reported here are meaningful only in relation to one another, not necessarily as absolute values. Farmers in Battambang and Siem Reap reported higher levels of perceived motivation for washing vegetable contact surfaces with soap and water every day than of perceived opportunity ($p < 0.001$ and $p < 0.001$ for Battambang and Siem Reap, respectively) or capability for the same behavior ($p = 0.009$ and $p < 0.001$ for Battambang and Siem Reap, respectively; Figure 5). Among farmers in Battambang, perceived motivation for washing vegetable contact surfaces with soap and water every day was estimated to be 11.6% higher than perceived opportunity and ∼11.4% higher than perceived capability. For farmers in Siem Reap, perceived motivation was ∼13.5 and 21.2% higher than perceived opportunity and capability, respectively. By contrast, levels of perceived capability and opportunity were not found to be significantly different from each other among farmers in either location (Figure 5). Vendors in Battambang also reported significantly higher ($p = 0.04$, 6.1% higher) levels of perceived motivation than perceived opportunity (Figure 5). In this group, levels of perceived capability for the target behavior were intermediate and were not observed to be significantly different ($p > 0.99$ in both cases) from levels of either motivation or opportunity (Figure 5).

Among both vendors in Siem Reap and distributors in Battambang, levels of perceived capability and motivation were observed to be significantly higher ($p < 0.001$, vendors; $p < 0.001$ and $p = 0.001$, respectively, distributors) than levels of perceived opportunity (Figure 5). Specifically, vendors in Siem Reap reported levels of perceived capability and motivation for the target behavior that were estimated to be 16.3 and 12.1% higher, respectively, than the level of perceived opportunity for the behavior; similarly, for distributors, estimates of perceived capability and motivation were 13.7 and 9.5% higher than perceived opportunity, respectively. In both of these functional groups, levels of perceived capability and motivation were not found to significantly differ from each other (Figure 5). To note, levels of perceived motivation for washing vegetable contact surfaces with soap and water every day were higher ($p < 0.05$; ranging from 6.1 to 13.5% higher) than levels of perceived opportunity for this behavior across all functional groups (Figure 5).

In order to further explore the interaction between functional group and behavioral construct, levels of perceived capability, opportunity, and motivation were compared across functional groups (Figure 6). Farmers in both provinces were found to have reported lower ($p \leq 0.001$) levels of capability in comparison to distributors and both groups of vendors (Figure 6). Levels of perceived capability for the target behavior were estimated to be 23.5–36.6% higher among distributors and vendors compared to farmers. No other significant differences in levels of perceived capability were observed across functional groups ($p > 0.99$ in all cases; Figure 6). Analyses of response estimates for perceived opportunity and motivation indicated that vendors in Battambang had significantly higher levels of perceived opportunity ($p < 0.002$) and motivation ($p < 0.05$) for washing vegetable contact surfaces with soap and water every day compared to vendors in Siem Reap and both groups of farmers (Figure 6). In particular, levels of perceived opportunity and motivation were estimated to be 23.5–23.9% and 15.9–17.5% higher, respectively, among vendors in Battambang than among farmers, and ∼16.2 and 10.0% higher,

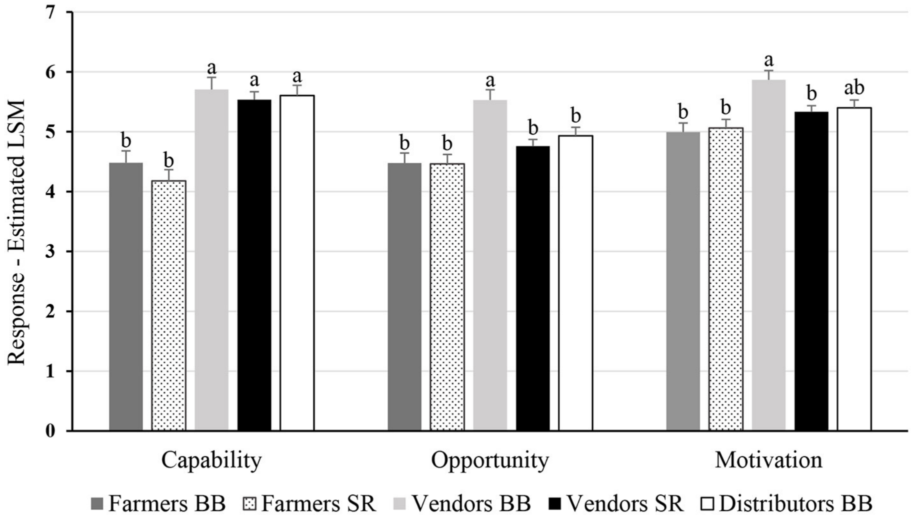

FIGURE 6
Estimated mean responses (and corresponding SE) to the sets of questions comprising the behavioral constructs of capability, opportunity, and motivation for each functional group. Different letters indicate significant differences ($p < 0.05$) between functional groups within a behavioral construct.

respectively, among vendors in Battambang than among vendors in Siem Reap. Levels of perceived opportunity and motivation among distributors in Battambang were not found to significantly differ ($p > 0.05$) from those of any other group (Figure 6).

Discussion

The primary objective of this research was to explore and describe perceptions of capability, opportunity, and motivation for a specific food safety practice among Cambodians involved with informal vegetable markets in order to inform future food safety engagement programs in Cambodia. Toward this end, we aimed to develop a quantitative questionnaire to assess the likelihood of food safety practice adoption based on the COM-B model of behavior (Michie et al., 2011) and the TDF (Michie et al., 2011; Cane et al., 2012). To assess capability, opportunity, and motivation to implement different food safety practices. For the purposes of developing the questionnaire, we chose a single food safety practice (i.e., washing vegetable contact surfaces with soap and water every day); however, the questionnaire was designed such that any practice of interest can be easily interchanged (e.g., washing hands before handling vegetables, separating vegetables from animal sourced foods, etc.).

Developed in 2011 and 2012, respectively, the COM-B model of behavior and the TDF are relatively new behavior theories (Michie et al., 2011; Cane et al., 2012). Since their creation, however, these models have been used in a variety of fields and have been applied most often in research regarding the promotion of health-related behaviors (e.g., dental hygiene, sexual health, weight management, exercise, hand hygiene, smoking cessation, etc.; Cowdell and Dyson, 2019; Buchanan et al., 2021; Greene and Wilson, 2022). The COM-B model and TDF have also been applied, albeit to a much more limited extent, to research on food safety behavior change.

To our knowledge, all of the existing literature in this area focuses on the retail or consumer level (Courtney, 2017; Ipsos MORI, 2017, 2021; Thiavalappil et al., 2018; Wodnik et al., 2018; Yavelak, 2019). Furthermore, we are aware of only five Cambodian development programs to which the COM-B model has been applied in some way (Keats et al., 2018; Bartholomew et al., 2019). In all such programs, the COM-B model was applied following program implementation as a tool to evaluate the interventions and impacts of the program itself rather than being used as a basis for assessing capability, opportunity, and motivation among the target audience in order to inform program design. Thus, it is a clear that capabilities, opportunities, and motivations for food safety practices among food producers and distributors in Cambodia have been significantly underexplored. The research described in this study was intended to address this gap, with the understanding that theoretically grounded, quantitative assessments of these potential determinants of behavior could equip program designers to develop food safety engagement programs that more effectively facilitate the adoption of positive food safety practices.

The results of this research indicated that the perceptions of Cambodians involved with informal vegetable markets regarding their own capabilities, opportunities, and motivations for washing surfaces that come in contact with vegetables with soap and water every day are specific to each combination of functional group (i.e., the combination of a participant's geographic location and role in the informal vegetable market) and COM-B construct (i.e., capability, opportunity, and motivation). Based on these findings, it appears that future food safety engagement programs for vendors and distributors would benefit from addressing the relatively low levels of perceived opportunity for the target behavior observed in these groups, while programs for farmers would be better served by focusing on both perceived opportunity and capability. Additionally, as levels of perceived capability, opportunity, and motivation were found to be higher among vendors in Battambang than among farmers in both Siem Reap and Battambang, there may

be a greater opportunity for increasing the adoption of the target food safety practice among vendors than among farmers.

The Behavior Change Wheel put forward by Michie et al. (2011) at the same time as the COM-B model provides additional guidance for program design by linking the individual components of capability, opportunity, and motivation with specific intervention types. Based on the Behavior Change Wheel and the nature of the target behavior, environmental restructuring-based interventions may be well-suited to addressing perceived deficiencies in opportunity. This type of intervention aims to alter the social or physical context of the audience and could include efforts to make the physical infrastructure of informal vegetable markets more conducive to positive food safety practices. Education- and training-based interventions, on the other hand, may be more appropriate for addressing deficiencies in perceived capability (Michie et al., 2011). Education-based interventions are intended to improve an audience's understanding of the target behavior and could include educational programs that present information about food safety and food safety practices. Training-based interventions involve sharing techniques for performing the target behavior; in this context, hands-on demonstrations of food safety practices could be considered training-based interventions. While bearing in mind that the specific context of the program may make some intervention types more appropriate and feasible than others, program designers could use these recommendations to guide the selection of food safety practices and the development of engagement programs that promote the adoption of such practices (Michie et al., 2011). For vegetable vendors and distributors in particular, for instance, incorporating environmental restructuring-based interventions into engagement programs may be most useful for encouraging the implementation of the target behavior. Of interest are the differences in responses between several of the respondent groups. These differences may result from differences in awareness (e.g., previous food safety education programs in one region but not the other), proximity to agriculture-focused learning institutions or extension services, or even greater interfaces with end-users (e.g., producers vs. vendors). However, hypotheses as to factors influencing these differences remain conjecture at this time and may be the foci of future studies.

The results of statistical analyses investigating the alignment of the theoretical models used to design the questionnaire (i.e., the COM-B model and the TDF) with the response data indicated the theoretical models adequately fit the response data (Lin, 2021). It was therefore concluded that the questionnaire was a valid instrument for assessing Cambodians involved with informal vegetable markets' perceptions of their own capabilities, opportunities, and motivations to perform a specific food safety practice. As the questionnaire was intentionally designed to be easily adapted for use with different audiences and target behaviors and in various geographic locations, the validation of the questionnaire also served to fulfill the second objective of this research, namely, developing a quantitative questionnaire that could be used to assess capability, opportunity, and motivation in a wide range of development contexts.

This research is intended to provide program designers with a starting point for developing food safety engagement programs in Cambodia. However, we acknowledge that the transferability and generalizability of the results in this study are limited. Even within Cambodia, extrapolation regarding the capabilities, opportunities, and motivations of Cambodians involved with informal vegetable markets in other provinces should be done with caution as differences in education, resources, or local norms, among other factors, may impact determinants of behavioral. Research that uses the questionnaire developed in this study to measure Cambodians' capabilities, opportunities, and motivations for other food safety practices (e.g., washing hands after handling money, properly composting manure, etc.) is also needed in order to provide insight into the relative levels of these behavioral components across different food safety practices. With such information, program designers may be able to identify existing or develop new food safety practices and interventions for which Cambodians report higher overall levels of capability, opportunity, and motivation, thereby enabling more informed decisions regarding which practices and interventions are most suitable for inclusion in food safety engagement programs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Purdue University Institutional Review Board and Royal University of Agriculture Research Council. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SM: responsible for questionnaire design, testing, revision, data analysis, and manuscript preparation. KO and MC: responsible for questionnaire design, testing, revision, and data collection. NB: responsible for data analysis and manuscript preparation. JV and LH: responsible for experimental design and manuscript preparation. PE: responsible for experimental design, questionnaire design, testing, revision, data analysis, and manuscript preparation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Exploring the composition and structure of milk and meat value chains, food safety risks and governance in the Addis Ababa and Oromia regions of Ethiopia

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Demand for animal-source foods (ASF) is increasing globally, driven by population growth and changing dietary preferences. In global south countries, low compliance with good agricultural practices (GAPs) and food safety standards in the production of ASF is a major public health concern due to the high prevalence of foodborne diseases. This study examines the composition and structure of milk and meat value chains and explores food safety risks and governance in the Addis Ababa and Oromia regions of Ethiopia. Stakeholder discussions, key informant interviews and participant observation were undertaken to collect data on milk and meat value chain actors' perceptions of opportunities and constraints to improving access to safe, high-quality milk and meat products. The results reveal low compliance with rules and standards by milk and meat value chain actors which could compromise food safety and quality and expose consumers to public health risks. There was stricter enforcement of GAPs and food safety standards in the case of milk and meat products destined for export compared to products sold in the local market. The main barriers to compliance with food safety regulations were actors' low knowledge, small profit margins, absence of critical food safety infrastructure such as electricity and road and low access to capital to invest in the recommended equipment such as aluminum containers, coolers and fridges. This paper concludes there is a need for targeted efforts to support the adoption of low-cost technologies that could mitigate food safety risks. Additionally, there is a need for improved communication and tailored training for value chain actors that reflect local social, cultural and economic context to incentivise compliance with rules governing food safety and quality.

KEYWORDS

food quality, informal value chains, zoonoses, dairy value chain, meat value chain, food handling practices, food safety measures

1. Introduction

Animal-source foods (ASF), including meat and dairy products, are an important part of diets globally (Roesel and Grace, 2014). ASF are, however, highly perishable and easily contaminated and can serve as a conduit for the transmission of foodborne pathogens (Garedew et al., 2012; Cavalerie et al., 2021). Food safety compliance gaps are a major public health concern in developed and emerging economies due to the risks associated with the consumption of contaminated foods, such as zoonotic foodborne diseases (Fung et al., 2018; Hoffmann et al., 2019). Food safety risks are endemic in Africa, with millions of people becoming ill from foodborne diseases every year (Roesel and Grace, 2014; Fung et al., 2018; Hoffmann et al., 2019). There is, thus, an imperative to improve the adoption of food safety measures at the farm level and value chains from farm to table (Dongol et al., 2017).

The majority of ASF are produced by smallholder farmers and traded in formal and informal value chains; production and trade of ASF constitute an important source of livelihood in developing countries (Roesel and Grace, 2014; Zavala Nacul and Revoredo-Giha, 2022). Informal value chains involve small-scale actors that are not often registered or licensed to operate (Dongol et al., 2017; Zavala Nacul and Revoredo-Giha, 2022), and the majority of ASF food products are prepared and handled by these actors who are often inexperienced in implementing food safety protocols and complying with food safety standards and regulations (Limon, 2021).

Governance structures in value chains have been extensively studied based on vertical coordination and integration (Trienekens, 2011; Kilelu et al., 2017). A continuum of governance structures exist, based on the complexity of transactions and power dynamics between value chain actors and ranging from spot market to hierarchy governance (Indrawan et al., 2018; Hoang et al., 2021). In between the spot market and hierarchy governance, there are other governance structures like modular, relational and captive depending on value chain organization, actor relationship, and linkages with changes in markets and competition (Trienekens and Willems, 2007; Gibbon and Ponte, 2008; Trienekens, 2011; Kilelu et al., 2017). In the spot market governance structure, value chain actors exchange goods with price as the main determinant of the final transaction (Indrawan et al., 2018; Abel et al., 2019; Hoang et al., 2021). In the hierarchy governance structure, the value chain is complex including vertical integration of activities whereby products move between various stages of production, processing and distribution as a result of managerial decisions rather than the influence of prices (Gibbon and Ponte, 2008; Trienekens, 2011). Within a given value chains, there can be several governance structures existing as a consequence of the relationships and interaction between different value chain actors (Indrawan et al., 2018; Abel et al., 2019).

The degree of power asymmetry between a buyer and a supplier decreases as value chain governance structures move from hierarchy to spot market (Trienekens, 2011; Indrawan et al., 2018). Powerful value chain actors with access to resources influence the behavior of less powerful actors by enforcing private standards and rules of engagement to reduce the perceived risk of producer failure (Gereffi et al., 2005; Gibbon and Ponte, 2008; Trienekens, 2011). Governance structures are determined by the complexity of information and knowledge transfer required to execute a business transaction (Gereffi et al., 2005). The literature on food safety governance structures in agro-food markets in emerging markets is extensive, however, they have focused mostly on global value chains (GVCs) (Gereffi et al., 2005; Trienekens, 2011). There is a paucity of studies that have focused on food safety governance in informal markets in emerging economies in the global south (Trienekens, 2011; Indrawan et al., 2018; Nyokabi et al., 2018b).

This study takes Ethiopia as a case study country to examine the composition and structure of milk and meat value chains and explore food safety risks and governance. There are several reasons for this choice. Ethiopia has the largest national livestock herd in Africa and over 70% of the human population is directly engaged in the agricultural sector (Deneke et al., 2022). The prevalence of foodborne diseases particularly zoonotic diseases—such as bovine tuberculosis (bTB), brucellosis, anthrax, campylobacteriosis, meningitis, typhoid fever, and gastroenteritis—that can be spread through inhalation and ingestion of pathogens is high in ASF value chains in Ethiopia (Lemma et al., 2018; Alelign et al., 2019; Solomon et al., 2019; Deneke et al., 2022). Production and trade of ASF play an important role in the livelihoods of smallholder farmers and other actors along the Ethiopian livestock value chain (Lemma et al., 2018; Deneke et al., 2022). Moreover, although consumers are aware of possible zoonotic risks, arguably risky ASF consumption habits are still common in Ethiopia, such as the consumption of uninspected raw milk and raw meat (Deneke et al., 2022). Consumers' awareness of pasteurization and its benefits is low (Deneke et al., 2022). Previous studies have reported low compliance with food safety regulations and standards and unhygienic food handling practices of food handlers have been shown to cause food contamination in Ethiopia (Lemma et al., 2018; Amenu et al., 2019; Deneke et al., 2022).

2. Methodology

This study was part of the Ethiopia Control of Bovine Tuberculosis Strategies (ETHICOBOTS) project and was conducted in urban and peri-urban areas in the Addis Ababa (Bole, Kolfte, Ketema, and Kaliti sub-cities) and Oromia regions (Sendafa, Sebeta, Debre Zeit, and Holeta town) of Ethiopia (see Figure 1). These areas were chosen due to their high population levels and consumer base, and the importance of milk and meat markets for surrounding rural and peri-urban farming systems. The urban area hosts slaughterhouses, milk processing companies, butcheries and eateries, supermarkets and informal retailing shops.

Study participants were chosen through a purposive and snowball sampling approach and included previous participants of the ETHICOBOTS project. The inclusion criteria included: (1) experience working in either milk or meat value chain; (2) willingness to freely participate in the interviews; and (3) residence within the study region. The selected participants included public health officers, milk traders, farmers, butchery owners, slaughterhouse workers, veterinarians, artisanal processors, commercial processing companies, animal transporters, milk transporters and factory managers. The research had ethical

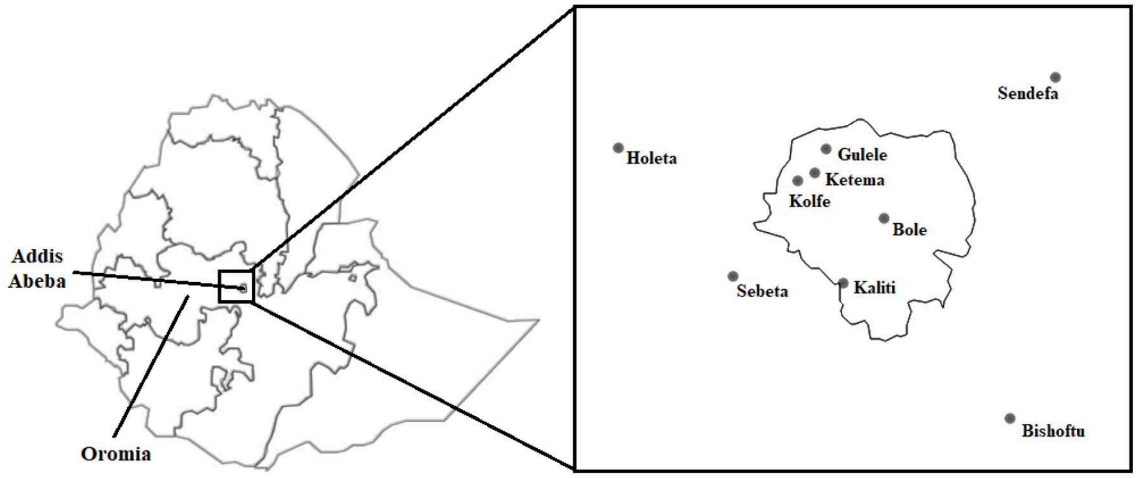

FIGURE 1
Map of the study area (Authors' own).

clearance from the University College London Research Ethics Committee (UCL-REC) (approval number 19867/001) and the Armauer Hansen Research Institute (AHRI) and ALERT hospital AHRI/ALERT Ethics Review Committee (AAERC) approval (protocol number PO-(46/14).

Four roundtable discussions were held with key stakeholders in the value chains, which included 30 farmers and 30 actors from meat and milk value chains (see Table 1). The discussion topics were based on food safety, food quality governance and zoonoses risk-specific literature on Ethiopia (Lemma et al., 2018; Alelign et al., 2019; Amenu et al., 2019; Solomon et al., 2019; Deneke et al., 2022). These roundtable discussions were led by the first author and explored farmers' and actors' knowledge of food safety standards and regulations, the level of compliance, the status of enforcement, and challenges to complying with rules and opportunities that exist to increase compliance with food safety standards and regulations. Data were also collected through semi-structured interviews with 53 key informants working in the meat and milk value chains (see Table 1). The roundtable discussions and key informant interviews were conducted in the local languages of Amharic and Afaan-Oromo and were recorded with participants' prior consent. Additional data were collected through participant observations conducted at milk and meat retail business premises, animal markets, slaughterhouses, milk bulking and transport, milk processing companies, and feedlots. Observations were recorded as notes and pictures with the participants' prior consent.

The recorded discussions, key informant interviews, and observation notes were transcribed verbatim. Transcription of the data into English was undertaken by two trained research assistants with a good command of the local languages—Amharic and Afaan-Oromo—and English. Transcripts were checked for consistency against the recordings to ensure that meaning or concepts were not lost in translation.

Cognisant of the difference in power between the researcher and the participants, the thematic analysis took a reflexive approach to ensure the data analysis was trustworthy and credible (Braun and Clarke, 2006, 2022; Kassan et al., 2020; Byrne, 2022). We undertook thematic analysis, as has been described by Green et al. (2007), which included an immersive reading of the data for familiarization and to understand the content. This was followed by coding, creating categories, and the identification of themes. Emerging themes were identified and added as appropriate during the analysis. Verbatim quotes from the recorded discussions and key informant interviews were identified and used to support the important findings and themes. NVIVO software was used for the thematic analysis.

3. Results

The value chain mapping exercise involved identifying the key informal or formal value chain actors, their function, key activities, participation and relationships and linkages between dairy and meat value chain actors.

3.1. Dairy value chain structure and governance in selected regions of Ethiopia

Figure 2, Table 2 present the key actors, their functions and activities, and their participation in the formal and informal milk value chains in Addis Ababa city and the Oromia regions. Milk value chain actors included input suppliers (agro-vets), farmers and farmer groups, middlemen, processors, transporters, middlemen brokers, wholesalers, retail traders and supermarkets. The majority of milk was produced by smallholder dairy farms in urban and peri-urban areas. Farmers procured inputs from the private market which comprised agro-vets, non-governmental organizations (NGOs) and local feed retail traders. Extension services and animal health services were primarily provided by government agencies. Supporting actors such as NGOs, church-based organizations, academia and multinational development agencies helped farmers

TABLE 1 Summary of research participants.

Exercise	Actor(s)	Number of participants
Two roundtable stakeholder discussions	Farmers	30
Two roundtable stakeholder discussions	Milk and meat value chain actors	30
Key informant interviews	Farmers	17
Key informant interviews	Veterinarians	13
Key informant interviews	Processor	1
Key informant interviews	Milk cooperative	1
Key informant interviews	Public health officers	2
Key informant interviews	Meat transporter	2
Key informant interviews	Livestock transporter	2
Key informant interviews	Researchers	3
Key informant interviews	Milk bar owners	3
Key informant interviews	Abattoir workers	2
Key informant interviews	Butcheries and eateries	7
Total		113
Participant observation sites		Number of sites visited
Butchery		4
Animal markets		5
Milk bulking Holeta		2
Milk processing company		1
Milk bars		5
Feedlots		2
Abattoir		2
Milk processing company		1
Milk cooperative		1
Farms		10
Agricultural research stations		3
Total		36

access inputs, financial services, information and new farming technologies.

Milk produced by smallholder dairy farms was sold through formal and informal dairy value chains. In some instances, farmers sold their milk directly to consumers. Farmers delivered their milk to bulking points, usually by the roadside, on foot, on horseback, or donkey-back. In a few instances, farmers formed cooperatives and farmer groups mainly to aggregate and sell their milk in bulk to processors or large traders who then sold it to other traders and/or consumers in urban areas.

> *"We don't buy raw milk directly from farmers* [..] *we buy from wholesalers who buy from farmers and aggregate it into large quantities. We refrigerate it in our facilities in those areas or they collect and bring it to us every morning, then we will process it and sell to our customers* [..] *hotels, supermarkets and agents"* (Milk processor)

Traders organized milk bulking by collecting and aggregating small quantities from individual farmers. Bulked milk was transported by traders with trucks who delivered it to the urban markets. Milk quality was tested using an alcohol test mainly and occasionally using a density test during bulking.

> *"When I receive it* [milk] *from wholesalers, I do test it with alcohol and lactoscan. That is why I do have many customers even though there are other sellers in our area"* (Milk trader 3)
>
> *"There are gaps in milk handling at different points along the chain. There are times when we have to reject milk at the collecting stations due to failing to fulfill requirements."* (Milk processor)

Farm-gate milk prices varied by season and location with low prices in rainy seasons and rural areas. Formal value chains had high retail milk prices selling pasteurized packaged milk. Informal value chain milk retail prices were lower; this was attributed to non-payment of tax by informal value chain actors.

> *"We pay farmers 20 birr per liter of raw milk* [..] *There's an unhealthy competition for raw milk in our area and due to low production. We are forced to look for suppliers in the neighboring federal region* [..] *That makes us spend a lot of money"* (Milk processor)

Processors faced more stringent milk quality requirements. For example, they were required to have refrigerated vehicles for milk transport. Formal value chain actors such as processors pasteurized their milk and sold it to supermarkets, hospitals, schools and other consumers.

> *"Milk quality control and regulation in our federal region are poor* [..] *There is a proclamation that was ratified by Oromia regional state even though it is not being fully implemented* [not enforced yet]. *It starts from handling, transportation, vehicle requirements, milk standards, and prohibition of adulteration* [..] *distributors must have an insulated refrigerated vehicle"* (Milk processor)

During the orthodox fasting season, milk traders were forced to add value to milk by making products that have a longer shelf-life. For example, traders were forced to make butter and cheeses during the fasting season, when milk sales are non-existent

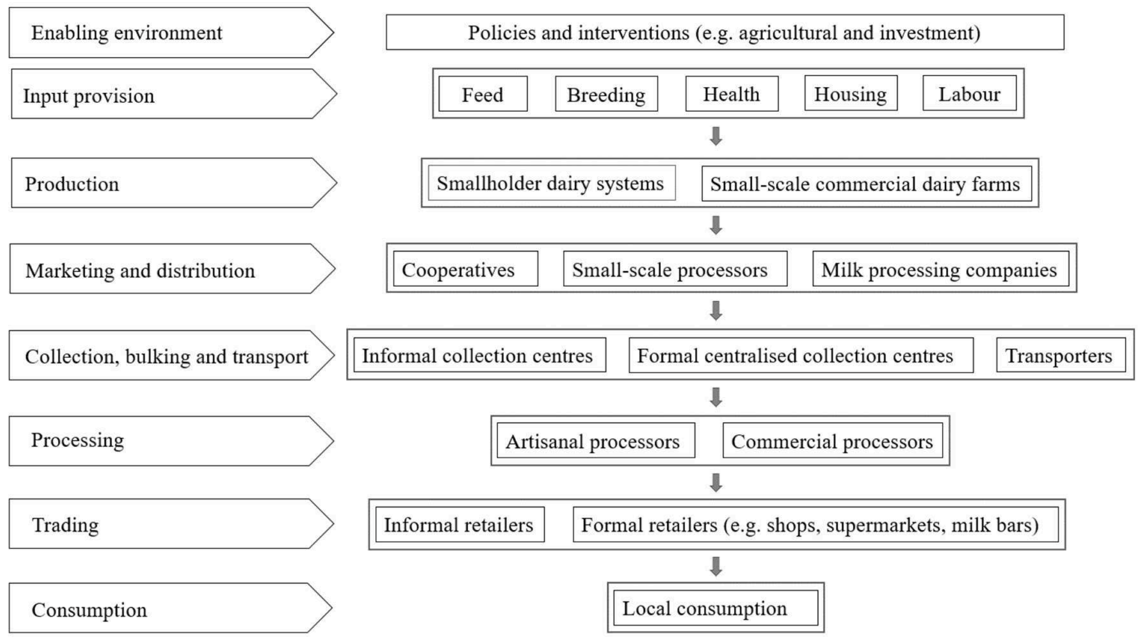

FIGURE 2
Milk value chain in Ethiopia.

to maintain their relationships with farmers and provide a milk market for producers. Processors produced ultraheat-treated milk, yogurt, cream, butter and cheeses which can be stored for longer.

> "*During fasting season, we process milk to cheese and butter since we cannot discontinue our contractual agreements or even reduce the amount of milk we buy. We receive* [milk] *from the union* [cooperative]. *Our agreement is renewed every 6 months* [...] *people do complain about our selling* [retail] *price by comparing it with farmers' selling price without taking cleanliness and quality into consideration. Price is their only measuring parameter*" (Milk trader 1)
>
> "*We have pasteurized milk, plain and flavored yogurt, cream, butter and cheeses*"
>
> (Milk processor)

3.2. Dairy value chain governance

This study explored value chain governance with an emphasis on horizontal and vertical coordination, regulation and quality control between the various value chain nodes where value addition takes place. There was limited vertical and horizontal integration of dairy value chain activities. Horizontal integration was characterized by limited organization of value chain activities and mostly occurred at the level of farmer groups and cooperatives (e.g., bulking of milk sold to processors and directly to consumers). Equally, there was limited vertical integration; nevertheless, there was some degree of interaction between processors, cooperatives and farmers. An analysis of the integration of value chain activities, personal relationships and the complexity of transactions indicates that several overlapping governance structures existed (Figure 3). In informal dairy value chains, spot market and relational governance structures dominated and were mainly based on product prices and personal relationships between value chain actors. Relational and modular governance structures were present in the formal dairy value chain and were based on short-term contractual relationships and aimed at enforcing milk quality standards stipulated by the Ethiopian government.

3.3. Dairy value chain food safety risks and quality management in selected regions of Ethiopia

3.3.1. Food safety risks and quality management at the farm level

Farmers identified several milk production and quality challenges, such as low access to veterinary and extension services which affected animal health and poor access to feed resources which affected milk production. Seasonal feed availability affected milk production while seasonal milk price variations affected farmers' profit margins and their willingness to invest in milk production and quality improvement. Milk handling hygiene at the farm level was observed as being unsatisfactory (i.e., not in line with standards set out by the Ethiopian Bureau of Standards). Observations revealed food safety compliance gaps during milking and milk storage and transport activities. The majority of farmers used non-food-grade plastic containers for milking and milk storage. Animals were milked in unhygienic conditions such as

TABLE 2 Key milk and meat value chains actors and their functions.

	Actors	Functions and activities
Input supply	Agrovets	• Supply inputs and extensions • Provision of animal health services
	Local feed traders	• Provision of various types of feed for dairy cattle and fattening animals
	AI providers	• Offer breeding services
	Extension agencies	• Provision of training and information to farmers • Funded by the county government
	Animal health/veterinary services	• Provision of animal health services
	Financial institutions	• Provision of credit services to farmers to purchase livestock, feed, medicines and other necessities for dairy and fattening activities
Production	Small medium and big farmers	• Produce livestock for meat and milk supply of animals for fattening
Supporting actors	Non-government organizations (NGOs) and community-based organizations (CBOs)	• Provide inputs, information and services to farmers • Research and present evidence to government agencies
	Research and academia	• Provision of innovations, technologies and research needed for production at the farm level. • Findings of research also inform policymaking
	Media	• Provide information to farmers and consumers
	Public health department	• Inspect premises to ensure they meet required standards • Issue health certificates to people handling food
	Ethiopia Bureau of Standards	• Set and enforces food safety standards
	National and federal governments	• Provide country-level policy and dairy plan, security, control of federal dairy planning and provide funds extension and livestock departments and national institutions
Processing, assembly and distribution	Milk processors	• Process raw milk into milk and dairy products (value addition)
	Farmer associations	• Process raw milk into milk and dairy products (value addition)
	Middlemen and traders	• Sell milk to consumers (mainly in small quantities) • Some pasteurize milk and some make yogurts
	Transporters	• Bulk and transport milk to processors and markets
	Feedlots	• Fatten beef cattle, goats and sheep
	Abattoirs	• Officially licensed to slaughter livestock to meat
Wholesaling	Farmer groups (can also be cooperatives or farmer groups)	• Help with milk marketing, provision of inputs and services to farmers • Bulk and market milk on behalf of farmers
	Wholesale traders	• Sell milk to consumers mainly trading in large quantities and sometimes serving as bulking agents. Some pasteurize milk and some make yogurts
Retailing	Retail traders	• Sell milk to consumers (mainly in small quantities)
	Supermarkets	• Sell packaged and pasteurized milk and dairy products (such as cheese, yogurts, etc.) to consumers
	Milk bars	• Sell milk to consumers mainly in small quantities and, sometimes, sell pasteurized milk or yogurts

cattle shed with dirty floors. Farmers reported a high incidence of mastitis and animal diseases, such as bTB, and cattle abortions. Poor udder hygiene was also observed, including the use of non-treated water, and use of the same water and towel to clean all the milking cows rather than using a new towel and clean water for each cow. Few farmers sieved their milk after milking to remove hairs and other foreign material or debris, however, this was not a common practice. Farmers sold milk immediately to consumers without boiling or pasteurization. Farmers used untreated water, stored in tanks or derived from a well, to clean milking equipment. Only those farmers who were located in urban areas had access to treated piped water.

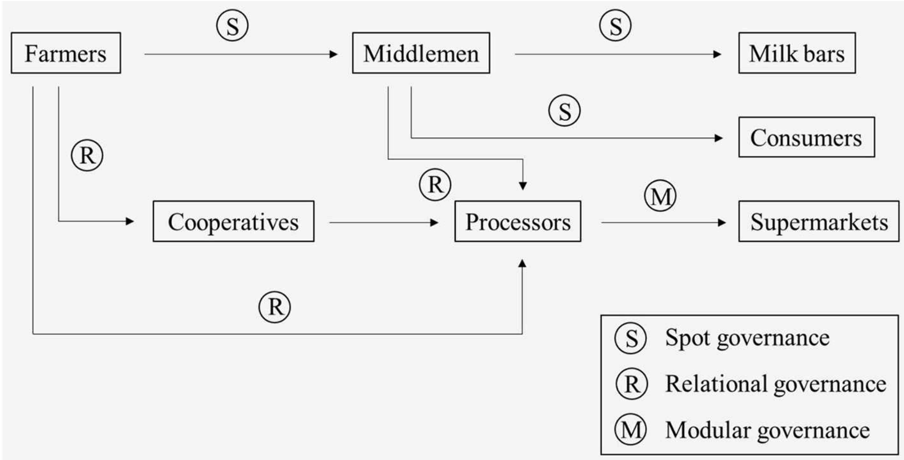

FIGURE 3
Governance structures in the milk value chain in Ethiopia.

3.3. Food safety governance dairy value chain in selected regions of Ethiopia

Regulatory authorities were tasked with ensuring that value chain actors adhere to public and private food safety standards and regulations meant to ensure that milk and dairy products are safe for human consumption.

> "*We do get regulated regularly by EFDA* (Ethiopian food and drug authority). *We do make sure that our workers are checked every 6 months. It is mandatory by law. We do have files. EFDA also makes regular check-ups. We cannot renew our license every year for each product without it.*" (Milk processor)
>
> "*You need a trading license if you are a working professional or not* [...] *if you are transporting and storing milk. They won't give you one if you have plastic containers. Their standards and precaution are good. We also have a permit for milk processing*" (Milk trader 1)

Formal dairy value chain actors, including milk bars and processors, were required to have and renew a public health certificate every 6 months. This public health certificate, issued after a medical examination was conducted, was designed to ensure that only healthy people were handling food.

> "We *undertake medical check-ups every 6 months* [and] *get a stamped certificate.* [the required working clothing is] *a gown, flat shoes, face mask, and a head covering which all are white* [...] *hands should be clean and it's better not to have long fingernails*" (Milk trader 2)

Formal traders and cooperatives were required to register their businesses and pay taxes. They were expected to renew this business certificate annually.

> "*Annually, people from the trade and industry bureau come to inspect my premises and see if it is fit for service or not* [..] *you submit your application stating that you want to start a business and then you process to get TIN* [tax] *number. Once you get that quality control personnel would come to assess whether your premise's standard is fit for the business*" (Milk trader 2)

Formal traders and cooperatives had milk cooling facilities which ensured that they were in a position to maintain milk quality and composition.

> "*We transport the milk by refrigerated truck and we have our refrigerator once it reaches here. We also take a permit for milk processing*" (Milk trader 3)

However, traders reported constant electricity outages which affected their business operations. Although the formal value chains had strict milk quality requirements and required tests to be met, farmers were not offered price premiums by other value chain actors for ensuring high food safety standards.

> "*It would be good to educate other stakeholders on how to be benefit by keeping the quality of the milk in the chain as they increase the price as well. I think when quality is kept, benefits will come as a result" (*Milk trader 1)

In contrast, informal actors operated outside the Ethiopian tax system and did not comply with food safety regulations which led to poor quality milk being sold in the informal value chain. Informal traders collected and bulked milk from farmers and moved from door to door, selling it to consumers. Operating outside the government agencies' working hours, they were not registered, and did not pay taxes and, thus, could afford to sell milk at a lower price than registered traders.

Other actors in the informal value chain understood the laws and legal requirements for handling food products. They attributed milk adulteration in the informal value chain to those actors who chose not to obtain the required public health certificates and comply with inspections.

> *"These individuals* [informal traders] *are taking our market share and compromising milk quality. People prefer to get milk delivered to their homes"* (Milk trader 1)
>
> *There was a woman who used to buy milk from me and resell it after diluting it with water. When I realized it, I reduced the amount an individual can purchase to two liters"*
>
> (Milk trader 1)
>
> *"Some* [traders are] *prioritizing making money and adulterate milk with different substances while others work in a morally acceptable way. The government has to do its part to follow up"*
>
> (Milk trader 2)

Government agencies' overlapping mandates complicated law enforcement. Laws were perceived as being difficult to enforce, particularly, in the informal value chain where actors avoided inspection of their milk and certificates.

> "[informal traders] *are one of the headaches for us, who work legally, renting a house, paying taxes and maintaining its quality spending a lot of expenses. We don't know where they get the milk from and they sell block to block in residential areas"*

Value chain actors operated based on verbal contracts rather than written contracts. Short-term relationships undermined long-term collaboration in implementing value chain activities while also disincentivising adherence to food safety standards. Short-term relationships also impacted actors' decisions to invest in the value chain upgrading required to realize improved milk quality.

Observations revealed that milk was bulked on the roadsides in non-food grade plastic containers by transporters and traders with minimal adherence to food safety standards. In all areas where farms were located and milk was bulked, there was infrastructure for cooling and maintaining hygiene such as hand-washing stations, latrines, etc. However, milk was bulked in trucks and transported uncooled. Milk from different sources was mixed after testing (i.e., density and alcohol test). Workers who were responsible for bulking did not wear recommended clothing (i.e., white overcoats, hair-nets).

3.4. Meat and livestock value chain structure and governance in selected regions of Ethiopia

Table 2, Figure 4 present the key actors, their functions and activities and their participation in the formal and informal meat value chains in Addis Ababa city and the Oromia region. The core activities in a meat value chain are input supply, production, trade (marketing), processing and consumption. The major actors in the meat value chain in the study areas were input suppliers, veterinarians, producers (farmers), brokers (middlemen), traders, abattoirs, government agencies (such as extension agencies) public health officers, butchers, supermarkets, hotels and individual consumers.

There was limited vertical and horizontal integration of value chain activities in the informal domestic meat value chain. Traders, butchers and transporters had short-term relationships based on trust and reciprocity. Actors reported that the limited integration of activities constrained the efficiency of the value chain.

> *"There is no uniformity and there are many actors in the market* [meat value chain] [..] *if someone buys* [livestock] *with weighing scale and the others decide not to do it, you are forced to do what they are doing"* (Export value chain transporter 2)

In contrast, in the formal export meat and livestock value chain, actors and activities were integrated. This ensured that standards (e.g., food safety, animal welfare, animal health etc.) were observed. Traders, transporters and export abattoirs coordinated their activities to ensure sufficient livestock numbers were available for daily slaughtering and export. A key driver of integration in the export value chain was that actors were motivated to secure an agreed number of cattle as outlined by a contract; traders supplied feedlots with animals, and feedlots supplied abattoirs with animals. Another driver was that actors wanted to ensure that stipulated quality standards were adhered to avoid legal hurdles associated with export markets.

Several overlapping governance structures existed in the meat value chain which reflected the integration of value chain activities, personal relationships and the complexity of transactions. These governance structures included spot market, relational and modular governance structures, as summarized in Figure 5. In the informal meat value chains spot market and relational governance structures dominated and were mainly based on product prices and personal relationships between meat value chain actors working with the domestic abattoirs and livestock markets. Relational and modular governance structures were present in the formal value chain and were based on short-term contractual relationships and aimed at enforcing food safety and quality standards stipulated by the Ethiopian government and middle-east export markets.

3.5. Meat value chain food safety risks and quality management in selected regions of Ethiopia

3.5.1. Livestock production, slaughtering and marketing

The majority of livestock destined for the meat value chain were kept in pastoral areas of rural Ethiopia, with only a small percentage kept in the urban and peri-urban areas. Input suppliers included feed suppliers, artificial insemination (AI), extension-, and veterinary service providers. Farmers who kept livestock for meat production procured inputs from the private market and extension- and animal health services from government agencies.

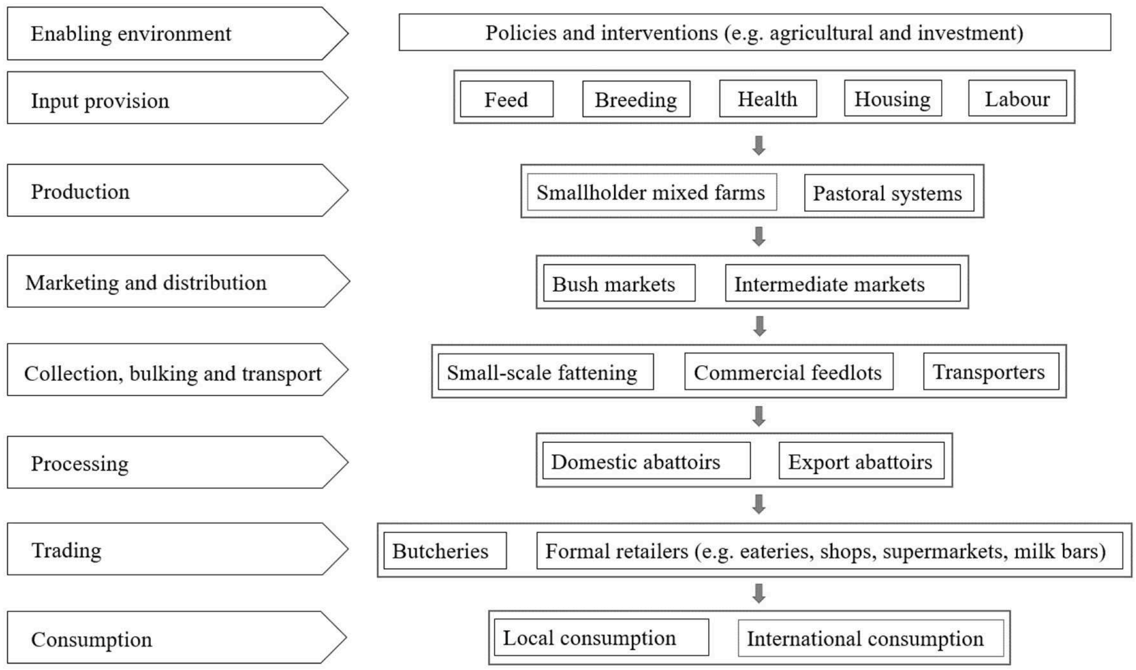

FIGURE 4
Meat value chain in Addis Ababa and Oromia Ethiopia.

The majority of animals sold and slaughtered in domestic and export abattoirs were sourced from smallholder farms located in Addis Ababa and the surrounding Oromia region, although a significant proportion came from other regions of Ethiopia. Livestock were aggregated in markets or farmers' homesteads and transported for slaughtering to urban markets and feedlots located in Modjo, Adama, and Addis Ababa urban centers. The cost of transport varied depending on location and road conditions. Livestock were often injured during transport due to poor loading practices and use of vehicles that were not designed for livestock transport.

> "*Animals travel long distances without transportation, even in the areas with road access [..] you pay 50-60 birr to transport animals from Adama to Mojjo, so we prefer to take goats on foot [...] from Harar road, access is limited and they [animals] are forced to travel long distances*" (Export value chain transporter 1)
>
> "*Sometimes animals come to the slaughterhouse with broken legs* [..] *animal welfare should be improved*" (Slaughterhouse worker 2)

3.6. Food safety governance in the meat value chain in selected regions of Ethiopia

In the meat value chain, there was an overlap of government agencies' mandates which resulted in a duplication of activities that frustrated value chain actors.

> "*We have two regulatory bodies in the agriculture bureau; meat inspectors and meat regulators' [mandates] are often conflicting*" (Public health officer 1)

In the domestic abattoir workers were required to have public health certificates to be allowed to work in an abattoir. These certificates were valid for 6 months only. Although they were required to wear white overcoats, gumboots and hair nets, there was lax enforcement of clothing regulations for domestic abattoir workers.

> "*We get our working gear (wear) from the agricultural bureau. The abattoir provides the workers with PPE (protective clothes and gear), although it is always not enough*" (Public health officer 1)

Domestic abattoirs lacked foot baths for disinfection at the door to prevent the risk of environmental contamination. Moreover, the abattoirs were not well-maintained, for example, the floors were cracked and there was no door screen to prevent contamination of carcasses and meat processed.

> "*There has been a hygiene problem in our abattoir and once in the past, the government health bureau closed it for 1 week due to safety issues. We had to take corrective action including adding a foot bath. We cannot fulfill everything to keep meat safe, but we are trying our best*"
>
> (Abattoir worker 1)

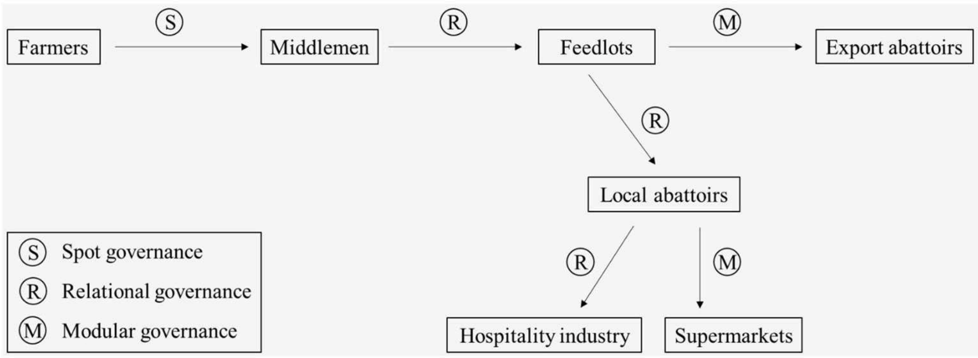

FIGURE 5
Governance structures in the meat value chain in Ethiopia.

There was a strict antemortem examination of livestock to be slaughtered which included overnight observation in the abattoir holding pen. Animal welfare, however, was poor.

> "*The lairage where animals stay until slaughter should be improved for animal welfare*"
> (Public health officer 1)

Abattoir actors and public health actors thought that camels, in particular, were inhumanely slaughtered due to poor stunning practices.

> "*The way we perform camel stun is not good and that does not give me comfort, because they stun by hitting the camel head*" (Abattoir public health inspector 1).
> "*Camel stunning methods, which we perform by hitting the head with a wood* [..] one *of the abattoir workers had an accident trying to hit a camel*" (Abattoir public health inspector 2).

To avoid conflict of interest, ante-mortem examinations were undertaken by government-employed public health workers and not by the abattoir workers.

> "*We perform examinations* [..] *in the antemortem examination, I look for the presence of a disease case and decide whether an animal is slaughtered or not*"
> (Abattoir public health inspector 2)

Livestock that passed the antemortem examinations were slaughtered in the morning hours. Meat value chain actors respected both orthodox and halal quality standards, with actors proceeded with prayers before slaughtering led by a priest or an imam, respectively, to satisfy domestic market demand. There was continuous cleaning of the carcass during the slaughtering process as it moved between the different workstations from stunning to flaying and quartering. Knives used for slaughtering were cleaned in hot water to minimize the risks of cross-contamination.

Post-mortem examination was undertaken on the carcass and offal, with carcasses stamped if deemed safe for human consumption. Zoonoses of public health importance, such as cysticercosis and bovine tuberculosis, had to be reported during post-mortem examinations.

> "*We observe for TB by looking at lymph nodes and if we suspect* [a case] *we will send for a laboratory diagnosis*" (Abattoir public health inspector 1).
> "*...in case of total condemnation due to TB, the report is given to the butcher association representative since they will compensate the owner*[..] *we will burn the affected carcass in an incinerator*" (Abattoir public health inspector 2).
> "*In* [the] *case* [of] *Cysticercus bovis, we decide* [carcass condemnation] *based on the number of the cyst if it is <20, we will recommend cooking the meat* [for fasciolosis] *which we find most of the time; it varies on season. If its prevalence reaches up to 30–40% in an abattoir, we condemn affected liver parts*" (Abattoir public health inspector 2).

There was a lack of training for meat value chain actors on quality and hygiene which constrained adherence to food safety standards.

> "*I did not get training* [on meat quality and hygiene], *most of the training given to us was on slaughtering techniques, how to keep meat safe, and hide removal*" (Abattoir worker 2)
> "*In our country, many things should be improved* [..] *hygiene in abattoirs should be improved, animal welfare training should be given to animal handlers, meat handling by butchers should be improved* [..] *butcher do meat cutting and cashiering* [handling money] *with the same hand*"
> (Public health officer 2)

Meat was transported to butcheries and local supermarkets in uncooled trucks. Loading and unloading activities were undertaken by transporters who wore special clothes; however, they did not regularly clean or disinfect between different jobs.

> "*The problem is workers who do meat loading and unloading do not have good quality wear, there are wearing overalls which get dirty with blood and they do not change it frequently. They are supplied every 3 months and they do not have overall for timely changing*" (Abattoir worker 1)

There was stricter food safety regulations compliance in export abattoirs, with workers adhering to strict clothing regulations and hygiene measures. Workers had to pass through a footbath to disinfect their footwear and had access to a hand-cleaning station. Moreover, door screens were installed to keep out contaminants (e.g., insects and dust). Livestock were slaughtered following halal requirements as the meat processed was destined for the Middle East market. Quality control occurred at every stage of slaughtering, with each step in the process monitored by a quality control manager. Livestock carcasses were continuously cleaned along the production line with piped, treated water. After the final quality inspection, the carcass was sprayed with ascorbic acid and immediately moved to a chiller room. The meat was transported for export in refrigerated trucks to its final destination.

Observations revealed that the common cultural practice in Ethiopia of consuming raw meat could expose consumers to the risk of foodborne zoonoses if hygiene was not properly maintained. Although some actors covered the meat with cling film to protect it from dust and flies, we observed that in most butcheries, meat was mostly hung on display in the open which exposed it to flies and dust contamination. We also observed butchery operators handling meat and money at the same time which was a contamination risk. Additionally, we observed that some butcheries and all the supermarkets had fridges and coolers to store meat so that it did not spoil. Meat was considered to be of higher quality at certain points of formal retail (e.g., higher in supermarkets compared to smaller butcheries).

Although it was illegal to slaughter at home for commercial purposes, we observed extensive home slaughtering, without inspection, during special festivals and occasions. Households bought live animals and slaughtered these animals at home, sharing the meat with their neighbors and relatives; this led them to bypass the formal procedures observed in abattoirs.

> *"During the holiday of 'Kerca' there is a lot of illegal slaughter and home slaughtering which affects the community health and affects our revenues"* (Abattoir worker 2)
>
> *"I do not know the specific rules, but I know if illegal slaughter is performed it has a penalty of 7000 birrs and above"* (Slaughterhouse worker 2)

4. Discussion

This study examined the composition and structure of milk and meat value chains and explored food safety risks and governance in Addis Ababa and Oromia regions in Ethiopia. Milk and meat value chains had diverse actors with limited integration and coordination of value chain activities. Observed food safety compliance gaps could lead to the contamination of meat and dairy products. Food safety is an integral part of food security (Kumar et al., 2020) and crucial to the achievement of Sustainable Development Goals (SDGs), particularly, SDG 2 which focuses on access to safe and quality food (Vipham et al., 2018). Studies suggest consumers in Ethiopia are willing to pay for quality foods and this has led to calls for food safety improvements in milk and meat value chains (Lemma et al., 2015; Amenu et al., 2019).

4.1. Milk and meat value chain structure and governance

The results of this study shows that milk and meat value chains in Ethiopia are complex in terms of their composition, and the relationships and governance structures which exist between actors, which is in agreement with previous studies conducted in Ethiopia (Lemma et al., 2015, 2018; Tigabu et al., 2015). The findings are also in line with studies that have found governance structures are a product of the complexity of transactions, power dynamics and the information asymmetries which exist in a given value chain (Gereffi et al., 2005; Trienekens, 2011; Abel et al., 2019).

The findings reveal low levels of vertical and horizontal integration between actors in milk and meat value chains constrains the coordination of value chain activities and food safety performance (Alemayehu, 2011; GebreMariam et al., 2013; Lemma et al., 2015; Tigabu et al., 2015). Informal value chain actors rely on spot market governance mechanisms, with value chain actors' behaviors and willingness to assess and prioritize food quality and safety moderated by trust (Hoang et al., 2021; Blackmore et al., 2022). In the formal value chain, governance structures are characterized by transactions, product prices and personal relationships (Lemma et al., 2015; Hoang et al., 2021). Modular and relational governance structures in formal value chains control the quantity of milk or livestock supplied and ensure actors meet stipulated quality parameters. Large processing companies depend on similar value chain linkages as informal value chain actors, although there is a greater level of integration and coordination of formal value chain activities and access to high-quality infrastructure (Alarcon et al., 2017). However, large companies with access to economic resources have the power to influence the behavior of the other actors in their value chain (Alarcon et al., 2017; Hoang et al., 2021). Understanding and leveraging governance structures including trust, power asymmetry and contractual relationships can lead to improved compliance with food safety regulations (Alarcon et al., 2017; Nyokabi et al., 2018b).

4.2. Food safety risks and management in value chains

The results of this study reveal food safety compliance gaps that could contaminate meat and milk products. Foodborne zoonoses negatively impact human health (Garedew et al., 2012; Cavalerie et al., 2021), with food serving as a medium for pathogen transmission if proper hygiene is not observed or implemented during the handling of ASF (Tigabu et al., 2015; Kumar et al., 2020). Previous studies have also reported that there are no differences between the formal and informal meat and milk value chains in the adoption levels of hygienic practices and practices (Minten et al., 2020; Seko et al., 2020). Low compliance and reluctance of meat and milk value chain actors to voluntarily and rigorously follow regulatory directives may be due to a lack of knowledge of the health and economic benefits of adopting these measures and a belief that adoption costs may exceed the derived benefits and poor enforcement of laws by officials

(Nyokabi et al., 2018a; Seko et al., 2020). The presence of a large informal value chain with a heterogenous set of actors in Ethiopia complicates government efforts to enforce food safety regulations as laws do not currently take into account the differing sizes and contexts of actors (Vipham et al., 2018; Blackmore et al., 2022).

4.2.1. Food safety risks and management at farm level

In Ethiopia, there is a growing demand for meat and milk value chain actors to adhere to food safety standards, in part, due to changing consumer preferences resulting from improved living standards (Deneke et al., 2022). An integrated "farm-to-table" approach to food safety is required to ensure food safety from farm to table and reduce the risk of food microbial, chemical and physical contamination associated with food production, handling and storage (Kumar et al., 2020). Among farmers, food companies and processors there is growing acceptance of the need for greater compliance with food safety regulations (Nyokabi et al., 2018b; Kumar et al., 2020); and the role that food handling plays in influencing food contamination (Roesel and Grace, 2014; Zavala Nacul and Revoredo-Giha, 2022).

The results reveal there is a risk of zoonoses, such as bTB, spreading between herds due to biosecurity measures not being observed (Sayers et al., 2013; Renault et al., 2018). This suggests a need to educate farmers on the risks of livestock disease transmission associated with through the introduction of new animals to the herd without observing a quarantine period and/or new animals being kept in shared pastures alongside existing herds (Alelign et al., 2019; Solomon et al., 2019). Zoonoses cause livestock diseases and deaths and impact the livelihood security of smallholder farmers and value chain actors in Ethiopia (Tigabu et al., 2015; Deneke et al., 2022). There is also a need to encourage farmers to engage public health inspectors when animals are slaughtered in the homestead to ensure that carcasses are safe for consumption (Nyokabi et al., 2018a).

4.2.2. Food safety risks and management in dairy value chains

The results of this study indicate milk handling hygiene at farm level was not in line with GAPs, with the majority of farmers using non-food-grade plastic containers for milking and storage, which could lead to microbial contamination (Tigabu et al., 2015; Deneke et al., 2022; Zavala Nacul and Revoredo-Giha, 2022). There is a low degree of organization in the informal dairy value chains in Ethiopia which dominates the dairy market (Alemayehu, 2011; Minten et al., 2020). Moreover, the results show that there is low investment in food safety infrastructure which could benefit milk value chain actors, including toilets, markets, bulking centers and milk cooling plants (Lemma et al., 2018; Deneke et al., 2022). Although the formal value chain, which includes modern processing companies selling branded pasteurized milk and supermarkets selling dairy and meat products, has higher food safety requirements, there are currently no economic incentives for farmers and other value chain traders to ensure that, milk and dairy products meet expected parameters (Jabbar and Admassu, 2009; Minten et al., 2020).

4.2.3. Food safety risks and management in value chains

Previous studies have documented gaps in food handling practices particularly in domestic abattoirs compared to export abattoirs (Alemayehu, 2011; Alarcon et al., 2017; Nyokabi et al., 2018a). There is an imperative for training-based interventions to improve abattoir workers' hygienic practices which could lead to improved meat safety (Seko et al., 2020). Additionally, there is a need for investment in hygiene infrastructure including changing rooms, latrines and toilets, foot baths and improved lairage to enable abattoir workers to comply with hygiene regulations (Nyokabi et al., 2018a; Seko et al., 2020). There is also a need to enforce the use of PPE given that close contact with livestock and contact with meat and blood has been shown to increase the risk of exposure to zoonoses (Deneke et al., 2022).

The results reveal that, in the meat value chain, animal welfare was poor which led to animal injuries and, occasionally, deaths. The slaughter of camels was below the required animal welfare standards and could affect the quality of meat and the welfare of the animals. Poor animal welfare goes against the Ethiopian Bureau of Standards' regulations and causes unnecessary suffering (Addisu et al., 2012; Legese et al., 2014). The results suggest an urgent to improve slaughterhouse practices by training workers on new humane stunning techniques (Legese et al., 2014) and ensure that local abattoirs meet the prescribed international standards and norms (Addisu et al., 2012; Legese et al., 2014).

4.3. Policy and institutional bottlenecks for food safety in value chains

The findings of this study reveal a lack of collaborative relationships between regulators and value chain actors, particularly in the informal value chain. Adversarial relationships between regulators and value chain actors create unnecessary transaction costs and lead to failure to capitalize on opportunities for enhancing livelihoods, food safety, and food security (Blackmore et al., 2022). The Ethiopian government's focus on taxation and formalization of value chains alienates actors rather than empowering them to improve food safety (Blackmore et al., 2022; Deneke et al., 2022). There is a need for collaborative efforts between the government, value chain actors and consumers to ensure coordination and integration of value chain activities which have the potential to improve food safety (Lemma et al., 2015; Blackmore et al., 2022; Zavala Nacul and Revoredo-Giha, 2022). There is also scope to improve hygiene in the meat value chain through better abattoir services, inducing behavioral change around meat sourcing, and educating the public on raw meat and raw milk consumption risks as a way to prevent and control the spread of zoonotic diseases (Deneke et al., 2022).

The national and federal governments are focused on the formalization of the informal value chain in Ethiopia mainly for

tax purposes and are not making concrete efforts to incentivise value chain actors to improve food safety. Government policy focused on formalization (i.e. licensing of informal value chain actors) fails to reward improved food safety practices and pushes actors to work on the periphery, particularly those who cannot afford to pay taxes and obtain licenses (Blackmore et al., 2022). Understaffing and underfunding of government agencies tasked with food safety regulations complicates and negates the oversight of food production and routine monitoring of foodborne hazards within value chains (Unnevehr and Hoffmann, 2015).

Low investment by the government in hygiene and food handling infrastructure in Ethiopia hampers food safety governance, has been reported by Vipham et al. (2018). There is a need for targeted public efforts to support the adoption of low-cost technologies such as access to infrastructure including clean water, electricity, sanitation, and refrigeration could mitigate food safety risks of public health importance (Unnevehr and Hoffmann, 2015; Blackmore et al., 2022). There are opportunities to leverage market incentives, such as halal branding, and increased demand for quality ASF to increase compliance with food safety standards (Unnevehr and Hoffmann, 2015). These market incentives hinge on consumer or buyer demand that rewards quality and supply chain coordination (Unnevehr and Hoffmann, 2015). There are opportunities for policy-makers to capitalize on existing approaches and efforts of actors in both formal and value chains to ensure food safety and quality by having open communication, engagement and constructive dialogue on inclusive and win-win pathways (Blackmore et al., 2022).

5. Conclusion

The results of this study show that the meat and milk value chains in Ethiopia are complex and comprise a diverse set of actors. This diversity underscores the extent to which milk, fattening animal and beef value chains play an important role in ensuring food security, providing employment and livelihood opportunities and contributing to the national economy. The results also reveal that there is a food safety compliance gap in both the formal and informal value chains. Food safety governance could be improved by encouraging value chain actors to move from spot market governance to more relational and hierarchical governance models that facilitate coordination and integration of value chain activities. These governance structures could incentivise actors' improved adherence to food safety standards, support the establishment of long-term contractual arrangements, and reward compliance with food safety standards. The results of this study suggest there is considerable scope for the Ethiopian livestock sector to provide price and other market incentives to milk and meat value chain actors and encourage these actors to invest in meeting standards, improving quality and expanding productivity. There is an imperative for the formal value chain to better reward improved food handling hygiene and food safety to increase the number of actors participating in this value chain. Crucially, the results underscore that the Ethiopian government should enact context-specific policies that enable small value-chain actors to comply with regulatory requirements given their low trade volumes and low-price margins.

Data availability statement

The datasets presented in this article are not readily available because the sample size was small and the participants may be indetifiable. Requests to access the datasets should be directed to n.nyokabi@ucl.c.uk.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Assessing milk products quality, safety, and influencing factors along the dairy value chain in eastern Democratic Republic of the Congo

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Dairying is one of the new promising economic sectors in eastern Democratic Republic of Congo (DRC), but still not explored enough to ensure consumers' safety. This study aimed to assess the health risks and nutritional profile of milk products along the value chain in South-Kivu and Tanganyika provinces. A total of 288 milk actors, including 160 producers, 35 collectors and 93 vendors, were concerned for interview and milk samples collection. A total of 302 milk samples (159 raw, 44 pasteurized, 76 fermented and 19 white cheese so-called "*Mashanza*") were collected for physicochemical [pH, fat, non-fat dry matter (NFDM), lactose, protein, freezing point, density] and microbiological (total *Aerobic Mesophilic Flora*, *Escherichia coli*, Total *Coliforms*, Fecal Coliforms, *Salmonella* and *Staphylococci*) analyses. Results revealed that the physicochemical characteristics of the milk mostly varied according to the type of milk and the regions. The pasteurized milk from Tanganyika presented the best physicochemical parameters [crude protein (CP) = 4.36%, Fat = 4.06%, NFDM = 12%, lactose = 5.4%, density = 1.02 and pH = 6.59] compared to other types of milk. For microbiology, no *E. coli* was recorded but *Salmonella* and *Staphylococci* were found in all the milk types with the values not exceeding 3×10^4 CFU ml^{-1} and 3×10^3 CFU ml^{-1}, respectively. This implies a long-term consumers' health issue if appropriate measures are not taken by milk actors along the value chain. The microbiological quality was influenced by the ecologies of production axis (representing the production zones) and by handling methods and infrastructures used by the actors involved along the value chain. Factors related to animal husbandry, milking method, milk processing and packaging had no significant effect on the physicochemical parameters under study. These results indicated that health risks for milk consumers are accrued by production practices and handling by milk actors due to shortage of required skills and appropriate

equipment along the milk value chain. Observance of hazard analysis critical control point (HACCP) measures is carefully required along the milk value chain nodes to improve the quality of milk produced and sold and thus reduce the risks among consumers in South-Kivu and Tanganyika provinces.

KEYWORDS

dairy products, health risk, microbiological aspects, milk value chain, nutritional profile, physicochemical characteristics

1. Introduction

Milk is defined by the United States Code of Federal Regulations as "the lacteal secretion, practically free from colostrum, obtained by the complete milking of cow and containing more than 8.25% of milk solids-not-fat and more than 3.25% of milk fat" (Goff and Hill, 1992). It is considered as one of the most important food sources and is a compensatory component of daily diets for people of all categories, mainly due to its high nutrients content (Abate and Addis, 2015). In Sub-Saharan Africa (SSA), milk production has social and nutritional considerations (Mutwedu et al., 2018; Ahikiriza et al., 2021).

In SSA, an increase in annual milk consumption was observed in the last two decades and a significant increase is expected by 2025 (Kabui et al., 2015). This indicates that good management of the dairy sector could serve as a powerful tool for poverty alleviation and wealth creation, especially in developing countries (FAO, 2021).

In the Democratic Republic of Congo (DRC), 9.2% of GDP comes from the livestock sector, which also plays a crucial role in the livelihoods of the rural farmers. Yet, cattle are the most important livestock contributing more than 80% of the total protein consumed and an estimated annual milk production of 1.18 billion liters (SNSA: Service National des Statistiques Agricoles, 2020). However, despite its economic, food and nutritional importance, the produced milk is reported to be of marginal qualities and marketed through traditional or informal networks. Up to 80–90% of the locally produced milk is handled in these informal markets while <10% is processed (Staal et al., 2001). Milk is produced mostly in rural areas by unorganized smallholder farmers and usually supplied to consumers in urban and rural areas by milk vendors or groceries (Ayagirwe and Mutwedu, 2021). Therefore, milking, handling, distribution to the consumers and conservation remains challenging.

This is a common situation in most African countries whereas informal milk value chain is predominant and represent more than 70% of milk traded (Nyokabi S. et al., 2021). The products traded in these informal value chains are traditionally processed and mainly concern *mashanza* and pasteurized or fermented milk. By traditional processing, we refer to small processing units that are typically carried out by isolated individuals or groups of individuals using local equipment such as jerricans, pans, calabashes, and local ferments (lactoserum, filtered local drink, lemon juice, etc.), without necessarily considering the classic norms to process food products. Informal value chains include licensed and unlicensed entities selling milk or dairy products directly to consumers through milk-bars, milk vending machines, corner-shops, street vendors and mobile vendors on bicycle or motorbike (Chepkoech, 2010; Odero-Waitituh, 2017; Alonso et al., 2018). The proportion of pasteurized milk traded in the informal value chains has been increasing due to growing demand for safe milk (Alonso et al., 2018; Bebe et al., 2018). However, milk is often re-contaminated after pasteurization due to unhygienic milk handling practices impairing its nutritional quality (Lindahl et al., 2018).

Taken together, the present state of milk handling and marketing may pose health risks to the public. These risks are linked to contamination of milk, growth and survival of harmful pathogens in the milk and increasing number of other micro-organisms caused by storage time and conditions such as temperature and humidity. Mutwedu et al. (2018) highlighted the implication of pasteurization process in physicochemical and microbiology characteristics of milk. Birali et al. (2019) observed a high variability of raw milk materials and the variation in processing procedures as source of milk product variability, composition, and shelf life. In fact, factors influencing milk composition (e.g., diet, breed and lactation stage) have been studied individually, while the interactions between several factors have been largely ignored. For example, the fatty acid profile of milk reacts quickly and is very sensitive to changes in diet (Schwendel et al., 2015). The milk handling practices and microbial health risks along the milk value chain in South-Kivu and Tanganyika provinces, Eastern of DRC are not known to ensure consumers' safety.

Therefore, the purpose of this study was to assess the health risks and nutritional profile of milk products as well as underlying factors along their value chain. Specifically, the current study sought to assess the physicochemical and microbiological composition of milk products as well as the risk factors associated with milk quality in South-Kivu and Tanganyika provinces.

2. Materials and methods

2.1. Study area

This study was carried out in the provinces of South-Kivu (territories of Kabare, Kalehe, Walungu, Uvira,

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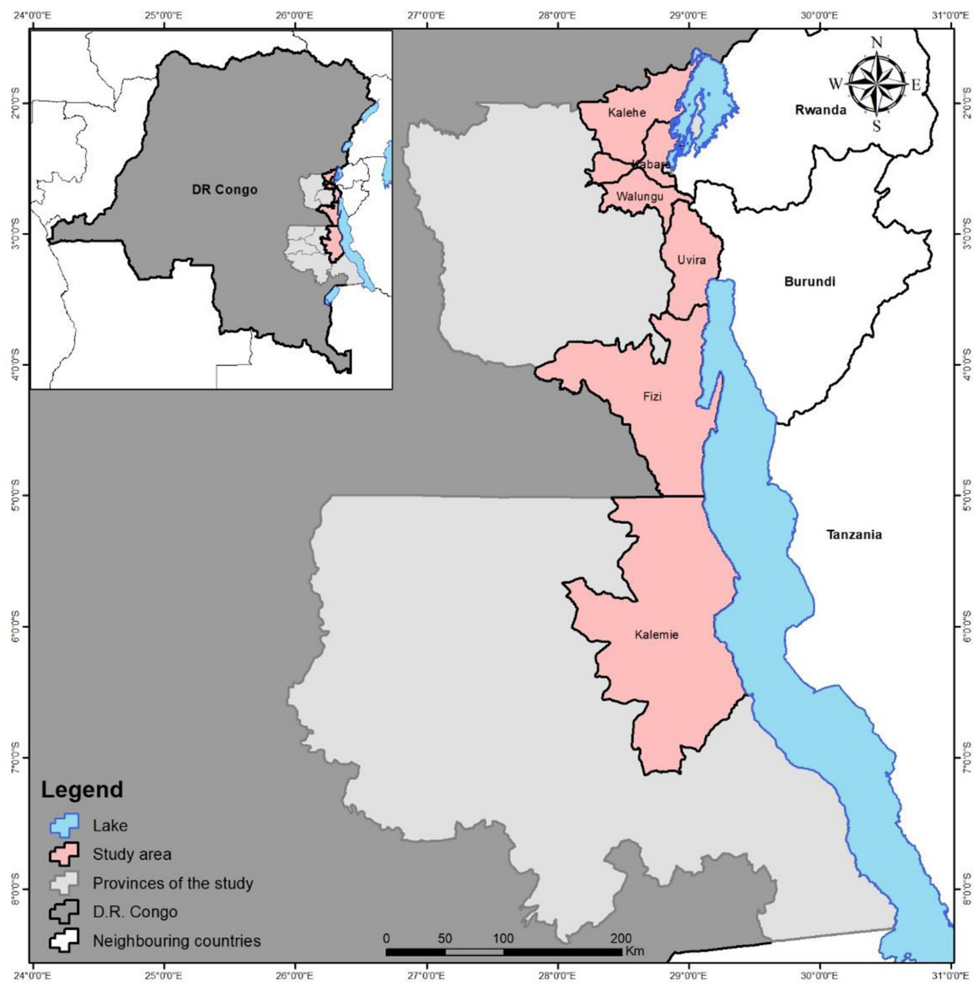

FIGURE 1
Map of the study area in the South-Kivu and Tanganyika provinces.

Fizi and the city of Bukavu) and Tanganyika (territory of Kalemie), located in Eastern of DRC (Figure 1). The selection of these sites was motivated by the high livestock population and therefore milk production (Defailly, 2000).

In South-Kivu, 3 axis were selected including Bukavu and its hinterland (Bukavu+) (villages of Miti, Mudaka, Kavumu, Katana, Kabamba, Bitale, Mulumemunene and Walungu center), the Ruzizi plain (Ruzizi) (Kamanyola, Luvungi, Bwegera, Luberizi, Sange, Kiliba) and the Fizi axis (Mboko, Baraka, Katanga, Fizi villages). In the context of the study area, ax means main production zone, mostly defined based on agroecological zones and livestock abundance.

In Tanganyika, 2 axis were concerned including the Kalemie-Malia axis (KLM-Ma) and the Kalemie-Kisondja axis (KLM-Ki). In KLM-Ma, 8 villages were selected including Kichanga, Kahengele, Tabac Congo, Kabutonga, PRP, Batumba and Malia; while in KLM-Ki, Kalemie town and the villages of Tundwa and Kisondja were concerned.

2.2. Data collection

2.2.1. Sampling strategy and survey

structured questionnaire was developed and formalized using the Kobo collect tool to gather information from three categories of milk actors, including milk producers, milk collectors and milk vendors. A cross-sectional study design was used where respondents were selected based on their experience in the specific category. All clients were asked to participate voluntarily, and confidentiality of information was ensured to respondents before giving their verbal consent to respond to questions. Direct and structured questions were used to obtain targeted qualitative information.

The questionnaire was designed to capture the information on demography, livestock and milk components. The questionnaire was developed after reviewing published papers in the same field (Dehinenet et al., 2013; Nyokabi S. et al., 2021; Nyokabi S. N. et al., 2021). Once the questionnaire was developed, it was submitted to a dairy expert from the Tanzania Livestock

TABLE 1 Distribution of milk samples collected.

Province	South-Kivu			Tanganyika		Total
Ax	Bukavu+	Ruzizi	Fizi	KLM-Ma	KLM-Ki	
Milk samples						
Raw Milk	65	33	13	34	14	159
Pasteurized milk	18	8	4	14	5	49
Fermented milk	27	15	3	10	21	76
Mashanza	8	6	-	-	4	18

Bukavu+, Bukavu and its hinterland; KLM-Ma, Kalemie-Malia axis; KLM-Ki, Kalemie-Kisonja axis.

Research Institute (TALIRI) for review and validation. A field trip was therefore organized to test the questionnaire to ensure that respondents understood the different questions and the answers given were consistent with the objectives of the study. A total of 288 persons were interviewed including 160 milk producers, 35 milk collectors and 93 milk vendors. Respondents were identified based on existing database of actors intervening in the livestock value chain, retrieved at the provincial divisions of livestock and fishery in South-Kivu and Tanganyika provinces. From the lists, respondents were selected randomly and personally identified on the ground though their cooperatives or associations. In Eastern DRC, almost all milk actors are organized into associations or cooperatives.

2.2.2. Collection of milk samples

A sample of 302 milk products were collected from the 288 actors and consisted of raw milk, pasteurized milk, fermented milk, and white cheese so-called "*Mashanza*" (Table 1).

The milk sampling considered 100 ml of each milk product, then homogenized using hermetically sealed white jars, labeled, and placed in cooling containers to maintain the temperature at +4°C and transferred to the lab within 4–6 h before being processed for lab analyses. After then, samples were stored in a freezer at −20°C while waiting for physicochemical analyses. All the analyses were carried out in the laboratory of food science and technology of the Université Evangélique en Afrique (UEA) in Bukavu, DRC. For liquid samples such as raw milk, they were directly analyzed. Fermented milk sometimes contains curds and therefore requires homogenization before analysis. However, a dilution was made for white cheese (so-called Mashanza), followed by a post-analysis homogenization.

2.2.3. Physico-chemical analyses

The physicochemical analyses evaluated the content in fat (%), protein (%), non-nitrogenous solid (%), freezing point (°C), density (kg/m^3) and lactose (%) by spectrophotometric method using a Lactostar as milk analyzer (Hoxha and Mara, 2012; Hossain and Dev, 2013). For this purpose, 5 ml of each sample was taken and sucked by the Lactostar. The pH value of each milk sample was determined by a pH meter brand 315i.

2.2.4. Microbiological analysis

The selective media were prepared in advance; dilutions and isolations were performed directly upon arrival of the samples in the lab. Reductase test was performed using the blue methylene to assess bacterial activity as described by Guiraud (2003). Decimal dilution was then elaborated according to Haas (1989) method.

The total mesophilic aerobic flora was quantified by counting colonies after growth on Plate Count Agar (PCA) inoculated and incubated for 72 h at 30°C. Colonies quantification was done on the boxes of 30–300 colonies and the number of microorganisms per ml was calculated using the formula developed by Houaria and Zohra (2018):

$N = \sum c/v\ (n1+0{,}1n2)\ d$

$\sum c$: total number of counted colonies.

n1: number of boxes scored in the first dilution.

n2: number of boxes scored in the second dilution.

V: volume of applied solution (1 ml).

d: the dilution factor from which the first counts will be observed.

Enumeration of total and fecal coliforms was performed. Coliforms are revealed in the presence of neutral red by the appearance of pink or red colonies on Mac Conkey Agar media. Separation between total coliforms (TC) and fecal coliforms (FC) was based on the incubation temperature of 37°C for 24 h for total coliform enumeration and 44°C for fecal coliforms (Yetis and Selek, 2015). The inoculation was performed in depth dilutions from 10^{-1} to 10^{-3}.

Staphylococcus aureus count was enumerated as bacteria that appear like black colonies resulting from the reduction of tellurite to tellurium, which are surrounded by a transparent halo indicating the presence of lipoproteinases. The medium used was Baird-Parker associated to egg yolk and potassium tellurite. A Gram stain test was used afterwards for confirmation.

For enumeration of *Salmonella*, Eosin Methylene Blue was used to determine the presence/absence of *Salmonella* and Hektoen agar was used as recommended by the Association of Official Analytical Chemists (1998) and the American Public Health Association (2001) for enumeration. It is a differential selective medium for enteropathogenic bacteria, particularly *Salmonella*. The composition of the medium allows the differentiation of colonies that ferment rapidly one of the three sugars (salicin, sucrose and lactose) by changing from blue to salmon-red and/or producing H_2S (black center). *Salmonella* which does not attack any of these carbohydrates, are able to produce H_2S from

TABLE 2 Physicochemical parameters according to production axis.

Province	South-Kivu				Tanganyika		
Axis	Bukavu+	Ruzizi	Fizi	Average	KLM-Ma	KLM-Ki	Average
Fermented milk							
pH	5.6 ± 0.4	5.8 ± 0.4	5.6 ± 0.4	5.6 ± 0.4	5.4 ± 0.4	5.9 ± 0.4	5.6 ± 0.4
Fat (%)	3.1 ± 0.6	3.3 ± 0.6	3.3 ± 0.7	3.3 ± 0.7	3.2 ± 0.6	3.3 ± 0.4	3.2 ± 0.5
NFDM (%)	12.5 ± 1.4	12.7 ± 1.4	11.8 ± 1.2	12.4 ± 1.5	12.0 ± 1.2	12.1 ± 1.4	12.1 ± 1.3
Lactose (%)	5.6 ± 0.7	6.1 ± 0.8	6.2 ± 0.7	6.0 ± 0.7	6.0 ± 0.6	5.6 ± 0.6	5.8 ± 0.6
Protein (%)	4.4 ± 0.5	4.7 ± 0.6	4.8 ± 0.5	4.6 ± 0.5	4.7 ± 0.4	4.1 ± 0.2	4.4 ± 0.3
FP (°C)	−0.4 ± 0.1b	−0.3 ± 0.1b	−0.3 ± 0.1b	−0.4 ± 0.1	−0.4 ± 0.1b	−0.6 ± 0.1a	−0.5 ± 0.1
Density	1.0 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Raw milk							
pH	6.6 ± 0.2a	6.1 ± 0.1b	6.0 ± 0.5b	6.3 ± 0.4B	6.7 ± 0.1a	6.6 ± 0.0a	6.7 ± 0.1A
Fat (%)	3.9 ± 1.4	3.0 ± 0.9	3.0 ± 1.1	3.7 ± 1.4	3.4 ± 0.4	3.5 ± 0.5	3.5 ± 0.4
NFDM (%)	12.4 ± 2.3a	11.7 ± 2.6a	7.3 ± 1.8b	11.7 ± 2.8	12.1 ± 1.9a	12.9 ± 1.7a	12.6 ± 1.8
Lactose (%)	6.2 ± 1.5a	4.5 ± 1.1b	2.8 ± 1.7c	5.5 ± 1.8	5.1 ± 0.5b	5.0 ± 0.8b	5.0 ± 0.7
Protein (%)	4.8 ± 1.7a	3.9 ± 1.4ab	2.3 ± 1.2b	4.3 ± 1.8	3.9 ± 0.7ab	3.8 ± 0.4ab	3.9 ± 0.5
FP (°C)	−0.5 ± 0.1b	−0.6 ± 0.1c	−0.3 ± 0.0a	−0.5 ± 0.1A	−0.5 ± 0.0bc	−0.5 ± 0.1bc	−0.5 ± 0.1B
Density	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0b	1.0 ± 0.0	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0
Pasteurized milk							
pH	6.0 ± 0.0a	6.1 ± 0.3a	6.1 ± 0.2b	6.1 ± 0.3B	6.6 ± 0.4a	6.1 ± 0.2a	6.4 ± 0.3A
Fat (%)	3.8 ± 0.0	3.0 ± 1.1	3.0 ± 1.2	3.1 ± 1.1	4.1 ± 0.2	4.0 ± 1.2	4.0 ± 0.2
NFDM (%)	13.3 ± 0.0a	12.5 ± 2.6a	7.2 ± 2.2b	10.2 ± 3.6	12.7 ± 1.6a	12.2 ± 2.2a	12.4 ± 1.9
Lactose (%)	3.8 ± 0.0ab	4.9 ± 0.7a	2.5 ± 1.9b	3.8 ± 1.7	5.4 ± 0.7a	5.2 ± 1.9a	5.3 ± 1.3
Protein (%)	4.3 ± 0.0ab	4.0 ± 1.1ab	1.8 ± 1.3b	3.1 ± 1.6	4.4 ± 0.4a	4.8 ± 1.3a	4.6 ± 0.9
FP (°C)	−0.5 ± 0.0bc	−0.6 ± 0.1c	−0.2 ± 0.0a	−0.4 ± 0.2	−0.5 ± 0.1a	−0.5 ± 0.0a	−0.5 ± 0.0
Density	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0b	1.0 ± 0.0	1.0 ± 0.0a	1.0 ± 0.0a	1.0 ± 0.0
Mashanza							
pH	5.6 ± 0.1a	5.3 ± 0.3b	–	5.5 ± 0.4	–	5.9 ± 0.0a	5.9 ± 0.0
Fat (%)	4.0 ± 0.6a	2.8 ± 0.7b	–	3.5 ± 0.8	–	3.3 ± 0.2ab	3.3 ± 0.2
NFDM (%)	11.4 ± 3.3	12.3 ± 2.5	–	11.8 ± 2.9	–	10.3 ± 0.1	10.3 ± 0.1
Lactose (%)	5.2 ± 0.7	4.6 ± 1.6	–	4.9 ± 1.1	–	3.6 ± 0.3	3.6 ± 0.3
Protein (%)	4.1 ± 1.2	3.3 ± 1.3	–	3.8 ± 1.2	–	3.2 ± 0.9	3.2 ± 0.9
FP (°C)	−0.4 ± 0.2	−0.5 ± 0.1	–	−0.4 ± 0.1	–	−0.5 ± 0.0	−0.5 ± 0.0
Density	1.0 ± 0.0	1.0 ± 0.0	–	1.0 ± 0.0	–	1.0 ± 0.0	1.0 ± 0.0

Values in this table are means ± standard deviations. For each parameter (each table row), statistical comparisons are made at two levels: at axis level in each province by using a,b and c (small letters) and at province level by using A and B (capital letters); Values with the same small letters (a,b,c) for the different axis in each province are statistically identical at the 0.05 significance level; Values with the same capital letters (A,B) for the two provinces are statistically identical at the 0.05 significance level; NFDM, Nonfat Dry Matter; FP, Freezing Point; Bukavu+, Bukavu and its hinterland; KLM-Ma, Kalemie-Malia axis; KLM-Ki, Kalemie-Kisonja axis.

thiosulfate in the medium. This result in blue-green colonies with a black center. Pre-enrichment was performed by suspending 25 ml of the sample in 225 ml of buffered peptone water (BPW). This broth was incubated at 37°C for 16–20 h, followed by enrichment on Selenite-Cysteine Broth (SCB) for 24 h at 37°C (Bachtarzi et al., 2015). The enumeration and isolation were performed on Hektoen medium by inoculating 1ml on the surface in the petri dish after incubation for 24 h at 37°C (Law et al., 2015).

For enumeration of *Escherichia coli,* Lysogeny media or Luria Broth (LB) was used. 1 ml of each sample from the selected decimal dilutions, was aseptically transferred to a sterile screw tube to which 15 ml of enrichment medium were added. These tubes were placed in the incubator at 37°C for 24 h. Tubes that color turned

black were then considered positive. These tubes were isolated on Urea indole for *Escherichia* that had been melted. The inoculated Petri dishes were incubated at 37°C for 48 h. Counting was done considering the result of the incubation of the tubes as well as the presence of colonies. For *E coli* enumeration, the most probable number procedure was applied. It is a statistical method based upon the probability theory. Samples were serially diluted to the point of extinction.

2.3. Statistical analysis

The data were summarized by means and standard deviations. In order to find the factors that induce statistically significant effects on the analyzed parameters, Wilcoxon-Mann-Whitney test or Student's T test were used to compare two samples' means while the analysis of variance or Kruskal-Wallis's test were used to compare more than two samples' means. In both cases, the choice of the statistical method was based on the results of the assessment of assumptions of normality and homoskedasticity. When these assumptions were met parametric methods (Student T test for two samples or analysis of variance for more than two samples) were used. Otherwise, non-parametric methods were used. All these analyses were performed using threshold $\alpha = 0.05$. In case of significant differences between more than two means, Dunn's test and Tukey HSD test were employed to determine homogeneous groups. The Redundancy analysis (RDA) technique was used to summarize the linear relationships between components of physicochemical and microbiological variables of milk products that are assumed to be redundant with their production, storage, and conservation conditions. All these statistical analyses were performed in R software version 4.0.5 (R Core Team, 2022).

3. Results

3.1. Nutritional profile of milk products in South-Kivu and Tanganyika provinces

3.1.1. Physicochemical parameters of collected milk samples according to the production axis

Table 2 shows that the evaluated physicochemical parameters for fermented milk did not vary according to the different axis, except the freezing point, which was higher ($p < 0.05$) in fermented milk from Kisondja than the other axis.

In raw milk, pH was lower ($p < 0.05$) in the milk collected in Fizi (6.0 ± 0.5) followed by that of Ruzizi plain (6.1 ± 0.1); the samples from Bukavu and its hinterland (6.6 ± 0.2), Kalemie-Kisondja (6.6 ± 0.0) and Kalemie-Malia (6.7 ± 0.1) had the highest pH. NFDM was lower ($p < 0.05$) in samples collected in Fizi (7.3 ± 1.8) compared to other sites. Lactose content was lowest ($p < 0.05$) in samples from Fizi (2.8 ± 1.7) followed by those from the Ruzizi plain (4.5 ± 1.1) and those from the Kalemie-Kisondja (5.0 ± 0.8) and Kalemie-Malia (5 ± 0.5) axis, while samples from the Bukavu and its hinterland had the highest lactose content (6.2 ± 1.5). Protein content was higher in samples from Bukavu and its hinterland (4.3 ± 0.0), followed by samples from the Kalemie-Kisondja (3.8 ± 0.4) and Kalemie-Malia (3.9 ± 0.7) axis and Ruzizi plain (3.9 ± 1.4), while samples from Fizi had the lowest protein content (2.3 ± 1.2). The freezing point was lower ($p < 0.05$) in Fizi samples (−0.3 ± 0.0) but higher in the Ruzizi Plain samples (−0.6 ± 0.1) while the density was lower ($p < 0.05$) in milk samples collected in Fizi (1.0 ± 0.0) compared to those from other sites.

Results obtained in pasteurized milk indicate that pH, NFDM and lactose were low in samples collected in Fizi (6.1 ± 0.2; 7.2 ± 2.2 and 2.5 ± 1.9 respectively) compared to samples collected in Bukavu and its hinterland, Ruzizi plain and Kalemie-Kisondja. Protein content was low ($p < 0.05$) in milk samples from Fizi (1.8 ± 1.3) followed by those from the Ruzizi plain (4 ± 1.1) and Bukavu and its hinterland (4.3 ± 0.0) while samples from the Kalemie-Malia axis presented the highest protein content (4.4 ± 0.4). The freezing point was lower in milk collected in Fizi (−0.2 ± 0.0) but higher in the samples from the Ruzizi plain (−0.6 ± 0.1) while the density was lower in the samples from Fizi (1.0 ± 0.0) than in those from other axis.

For *Mashanza*, beside the pH which was low in the Ruzizi plain samples (5.3 ± 0.3) compared to those from Bukavu and its hinterland (5.6 ± 0.1) and Kalemie-Kisondja axis (5.9 ± 0. 0), as well as the fat content which was higher in the samples collected in Bukavu and its hinterland (4.0 ± 0.6) and Kalemie-Kisondja axis (3.3 ± 0.2) than in the Plaine de la Ruzizi axis (2.8 ± 0.7), any other parameter varied following the axis.

3.1.2. Physicochemical parameters of milk products according to the value chain actors

Table 3 presents the findings on physicochemical parameters as influenced by actors along the milk value chain. For fermented milk, only the freezing point was statistically lower ($p < 0.05$) among milk producers in South-Kivu (−0.4 ± 0.1) compared to milk vendors in Tanganyika (−0.6 ± 0.1), where fermented milk is only produced. For raw milk, no significant difference was recorded ($p > 0.05$) among the different actors along the milk value chain in both South-Kivu and Tanganyika provinces.

Concerning the pasteurized milk, pH was high in the milk from producers in Tanganyika (6.6 ± 0.4) than those in South-Kivu (6.1 ± 0.3). The freezing point was high in samples from sellers of South-Kivu (−0.7 ± 0.1) but very low in South-Kivu farmers (−0.4 ± 0.2). For *Mashanza*, only the fat content was high in samples collected from South-Kivu farmers (4.1 ± 0.6) compared to those from sellers in Tanganyika (3.3 ± 0.2) and South-Kivu (3.0 ± 0.7).

3.2. Microbiological profile of milk products in South-Kivu and Tanganyika provinces

3.2.1. Microbiological parameters of milk products according to production axis

Table 4 indicates that, for fermented milk, all microbiological parameters did not vary significantly according to the different axis. For the raw milk, TAMF, total coliform and *Salmonella* counts were significantly lower ($p < 0.05$) in the milk samples collected

TABLE 3 Physicochemical parameters according to the value chain actors.

Province	South-Kivu				Tanganyika			
Actor	Producer	Collector	Vendor	Total	Producer	Collector	Vendor	Total
Fermented milk								
pH	5.4 ± 0.1	-	-	5.4 ± 0.1	-	-	5.9 ± 0.1	5.9 ± 0.1
Fat (%)	3.1 ± 0.6	-	-	3.1 ± 0.6	-	-	3.3 ± 0.4	3.3 ± 0.4
NFDM (%)	12.0 ± 1.4	-	-	12.0 ± 1.4	-	-	12.1 ± 1.4	12.1 ± 1.4
Lactose (%)	5.6 ± 0.7	-	-	5.6 ± 0.7	-	-	5.6 ± 0.6	5.6 ± 0.6
Protein (%)	4.4 ± 0.5	-	-	4.4 ± 0.5	-	-	4.1 ± 0.2	4.1 ± 0.2
FP (°C)	−0.4 ± 0.1	-	-	−0.4 ± 0.1A	-	-	−0.6 ± 0.1	−0.6 ± 0.1B
Density	1.0 ± 0.0a	-	-	1.02 ± 0.0A	-	-	1.0 ± 0.0a	1.0 ± 0.0A
Raw milk								
pH	6.3 ± 0.4	6.4 ± 0.4	6.4 ± 0.4	6.3 ± 0.4	6.7 ± 0.1	6.6 ± 0.0	6.6 ± 0.0	6.7 ± 0.1
Fat (%)	3.6 ± 1.4	2.8 ± 1.0	4.2 ± 1.4	3.7 ± 1.4	3.4 ± 0.4	3.5 ± 0.1	3.6 ± 0.5	3.5 ± 0.4
NFDM (%)	11.4 ± 2.9	11.7 ± 2.6	13.3 ± 2.1	11.7 ± 2.8	12.9 ± 2.0	12.2 ± 2.1	12.3 ± 1.6	12.6 ± 1.8
Lactose (%)	5.3 ± 1.9	6.1 ± 1.3	6.1 ± 1.3	5.5 ± 1.8	5.0 ± 0.6	5.3 ± 1.0	4.9 ± 0.9	5.0 ± 0.7
Protein (%)	4.2 ± 1.9	4.5 ± 1.1	4.9 ± 1.3	4.3 ± 1.8	4.0 ± 0.6	4.2 ± 0.1	3.5 ± 0.3	3.9 ± 0.5
FP (°C)	−0.5 ± 0.1	−0.4 ± 0.2	−0.4 ± 0.1	−0.5 ± 0.1	−0.5 ± 0.0	−0.5 ± 0.0	−0.5 ± 0.1	−0.5 ± 0.1
Density	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Pasteurized milk								
pH	6.2 ± 0.3ab	6.0 ± 0.0b	6.0 ± 0.1b	6.1 ± 0.3B	6.6 ± 0.4a	-	-	6.6 ± 0.4A
Fat (%)	3.1 ± 1.1	4.6 ± 0.0	2.6 ± 0.9	3.1 ± 1.1	4.1 ± 0.2	-	-	4.1 ± 0.2
NFDM (%)	9.4 ± 3.7	15.6 ± 0.0	11.1 ± 2.4	10.2 ± 3.6	12.7 ± 1.6	-	-	12.7 ± 1.6
Lactose (%)	3.5 ± 2.0	4.9 ± 0.0	4.3 ± 0.5	3.8 ± 1.7	5.4 ± 0.7	-	-	5.4 ± 0.7
Protein (%)	2.9 ± 1.9	3.8 ± 0.0	3.4 ± 0.6	3.1 ± 1.6	4.4 ± 0.4	-	-	4.4 ± 0.4
FP (°C)	−0.4 ± 0.2a	−0.5 ± 0.0ab	−0.7 ± 0.1b	−0.4 ± 0.2	−0.5 ± 0.1ab	-	-	−0.5 ± 0.1
Density	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	-	-	1.0 ± 0.0
Mashanza								
pH	5.3 ± 0.0	-	5.5 ± 0.3	5.5 ± 0.4	-	-	5.9 ± 0.0	5.9 ± 0.0
Fat (%)	4.1 ± 0.6a	-	3.0 ± 0.7b	3.5 ± 0.8	-	-	3.3 ± 0.2ab	3.3 ± 0.2
NFDM (%)	11.3 ± 4.1	-	12.1 ± 2.0	11.8 ± 2.9	-	-	10.3 ± 0.1	10.3 ± 0.1
Lactose (%)	5.2 ± 0.8	-	4.8 ± 1.3	4.9 ± 1.1	-	-	3.6 ± 0.3	3.6 ± 0.3
Protein (%)	3.9 ± 1.5	-	3.6 ± 1.1	3.8 ± 1.2	-	-	3.2 ± 0.9	3.2 ± 0.9
FP (°C)	−0.3 ± 0.2	-	−0.5 ± 0.1	−0.4 ± 0.1	-	-	−0.5 ± 0.0	−0.5 ± 0.0
Density	1.0 ± 0.0	-	1.0 ± 0.0	1.0 ± 0.0	-	-	1.0 ± 0.0	1.0 ± 0.0

Values in this table are means ± standard deviations. For each parameter (each table row), statistical comparisons are made at two levels: at axis level in each province by using a,b and c (small letters) and at province level by using A and B (capital letters); Values with the same small letters (a,b,c) for the different axis in each province are statistically identical at the 0.05 significance level; Values with the same capital letters (A,B) for the two provinces are statistically identical at the 0.05 significance level.
NFDM, Nonfat Dry Matter; FP, Freezing Point.

in Fizi (155 ± 44×10^3 CFU/ml; 121 ± 42 ×10^3 CFU/ml and 15 ± 3 ×10^3 CFU/ml, respectively) compared to other axis. The fecal coliforms and Staphylococci counts did not vary significantly following the different axis under study. For pasteurized milk, the TAMF (97 ± 17 ×10^3 CFU/ml) and total coliform (115 ± 31 ×10^3 CFU/ml) counts were lower ($p < 0.05$) in milk from Fizi than in the other axis, while there was no significant difference in fecal coliforms, *Salmonella* and *Staphylococcus* counts following axis. For *Mashanza*, apart from the total coliform load, which was lower in samples from the Ruzizi plain (294 ± 73 ×10^3 CFU/ml) and Kalemie-Kisondja (280 ± 85 ×10^3 CFU/ml) compared to Bukavu and its hinterland (486 ± 111 ×10^3 CFU/ml), no other parameter varied significantly across axis. *Escherichia coli* was not found in any of the milk samples.

TABLE 4 Microbiological parameters of milk products according to production axis (in $\times 10^4$).

Province	South-Kivu				Tanganyika		
Axis	Bukavu+	Ruzizi	Fizi	Total	KLM-Ma	KLM-Ki	Total
Fermented milk							
TAMF	40.5 ± 11.3a	46.9 ± 12.8a	41.0 ± 9.8a	42.9 ± 11.3A	40.1 ± 8.0a	38.7 ± 3.5a	38.7 ± 3.5A
E. Coli	-	-	-	-	-	-	-
Total Coliform	39.8 ± 12.5a	44.4 ± 14.0a	41.9 ± 12.5a	42.2 ± 12.7A	42.1 ± 10.6a	44.9 ± 6.2a	44.9 ± 6.2A
Fecal Coliform	26.6 ± 16.8a	33.2 ± 19.3a	31.6 ± 16.2a	29.5 ± 18.3A	31.8 ± 14.8a	23.9 ± 3.2a	23.9 ± 3.2A
Salmonella	2.4 ± 0.6a	3.8 ± 0.8a	3.7 ± 1.0a	3.3 ± 1.1A	3.3 ± 0.8a	2.2 ± 0.7a	2.2 ± 0.7A
Staphylococcus	0.3 ± 0.1a	0.4 ± 0.2a	0.4 ± 0.2a	0.4 ± 0.2A	0.4 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1A
Raw milk							
TAMF	42.9 ± 12.5a	34.1 ± 8.6a	15.5 ± 4.4b	34.1 ± 14.4A	41.6 ± 8.2a	36.6 ± 8.4a	38.5 ± 8.5A
E. Coli	-	-	-	-	-	-	-
Total Coliform	38.1 ± 11.8a	33.5 ± 8.5a	12.1 ± 4.2b	31.1 ± 13.8A	30.2 ± 12.8a	31.5 ± 14.0a	31.0 ± 13.2A
Fecal Coliform	32.8 ± 10.6a	30.2 ± 11.3a	29.5 ± 5.2a	31.2 ± 9.9A	32.6 ± 12.2a	31.2 ± 12.4a	31.8 ± 12.0A
Salmonella	2.0 ± 0.8ab	2.4 ± 0.8a	1.5 ± 0.3b	2.0 ± 0.8A	2.2 ± 0.7ab	1.9 ± 0.5ab	2.0 ± 0.6A
Staphylococcus	0.3 ± 0.1a	0.3 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1A	0.2 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1A
Pasteurized milk							
TAMF	14.7 ± 0.0ab	33.4 ± 5.3a	9.7 ± 1.7b	21.9 ± 12.5A	26.1 ± 11.5a	23.2 ± 8.6a	26.1 ± 11.5A
E. Coli	-	-	-	-	-	-	-
Total Coliform	27.8 ± 0.0ab	31.2 ± 10.8a	11.5 ± 3.1b	22.3 ± 12.5A	35.8 ± 13.2a	38.1 ± 10.2a	35.8 ± 13.2A
Fecal Coliform	16.6 ± 0.0a	29.3 ± 11.4a	28.2 ± 6.5a	28.0 ± 9.3A	29.4 ± 11.4a	27.9 ± 9.1a	29.4 ± 11.4A
Salmonella	2.6 ± 0.0a	2.6 ± 1.1a	1.9 ± 0.4a	2.3 ± 0.8A	1.7 ± 0.9a	1.7 ± 1.0a	1.7 ± 0.9A
Staphylococcus	0.2 ± 0.0a	0.3 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1A	0.2 ± 0.1a	0.2 ± 0.3a	0.2 ± 0.1A
Mashanza							
TAMF	57.9 ± 16.3a	34.9 ± 15.9a	-	43.5 ± 19.0A	-	25.6 ± 4.1a	25.6 ± 4.1A
E. Coli	-	-	-	-	-	-	-
Total Coliform	48.6 ± 11.1a	29.4 ± 7.3b	-	36.6 ± 12.8A	-	28.0 ± 8.5b	28.0 ± 8.5A
Fecal Coliform	29.6 ± 11.1a	23.4 ± 6.7a	-	25.7 ± 8.5A	-	18.7 ± 8.7a	18.7 ± 8.7A
Salmonella	2.1 ± 1.1a	3.2 ± 1.7a	-	2.7 ± 1.4A	-	2.0 ± 0.2a	2.0 ± 0.2A
Staphylococcus	0.3 ± 0.1a	0.2 ± 0.1a	-	0.2 ± 0.1A	-	0.2 ± 0.1a	0.2 ± 0.1A

Values in this table are means ± standard deviations of numbers of 10^4 colony-forming unit per milliliter (CFU ml^{-1}). For each pathogen (each table row), statistical comparisons are made at two levels: at axis level in each province by using a,b and c (small letters) and at province level by using A and B (capital letters); Values with the same small letters (a,b,c) for the different axis in each province are statistically identical at the 0.05 significance level; Values with the same capital letters (A,B) for the two provinces are statistically identical at the 0.05 significance level. TAMF, Total Aerobic Mesophilic Flora; Bukavu+, Bukavu and its hinterland; KLM-Ma, Kalemie-Malia axis; KLM-Ki, Kalemie-Kisonja axis.

3.2.2. Microbiological parameters of milk products according to the value chain actors

Table 5 indicates that, for fermented milk, only the *Staphylococcus* count was statistically higher at producers' level in South-Kivu ($3 \pm 1 \times 10^3$ CFU/ml) compared to milk samples collected from vendors in Tanganyika ($2 \pm 1 \times 10^3$ CFU/ml). For raw and pasteurized milk, the pathogens germs count did not significantly vary among the value chain actors ($p > 0.05$). In *Mashanza*, only TAMF count was statistically higher in samples collected from South-Kivu vendors ($441 \pm 105 \times 10^3$ CFU/ml) compared to vendors of Tanganyika ($256 \pm 41 \times 10^3$ CFU/ml). *Escherichia coli* was not found in any of these milk samples.

3.3. Factors influencing the quality of milk products in South-Kivu and Tanganyika provinces

3.3.1. Factors influencing the microbiological quality of milk products from producers

Results obtained with the CRA design indicate that pathogens tend to be more abundant in milk stored for a long time, even though during interviews farmers reported that most of them clean the milk storage equipment before use (92%) and wash their hands before milking (89%). In the Ruzizi plain, where plastic containers are the main packaging material (66.7%), *Salmonella*

TABLE 5 Microbiological parameters of milk products according to the value chain actors (in ×10^4).

Province	South-Kivu				Tanganyika			
Actor	Producer	Collector	Vendor	Total	Producer	Collector	Vendor	Total
Fermented milk								
TAMF	40.5 ± 11.3a	-	-	40.5 ± 11.3A	-	-	38.7 ± 3.5a	38.7 ± 3.5A
E. Coli	-	-	-	-	-	-	-	-
Total Coliform	39.8 ± 12.5a	-	-	39.8 ± 12.5A	-	-	44.9 ± 6.2a	44.9 ± 6.2A
Fecal Coliform	26.6 ± 16.8a	-	-	26.6 ± 16.8A	-	-	23.9 ± 3.2a	23.9 ± 3.2A
Salmonella	2.4 ± 0.6a	-	-	2.4 ± 0.6A	-	-	2.2 ± 0.7a	2.2 ± 0.7A
Staphylococcus	0.3 ± 0.1a	-	-	0.3 ± 0.1A	-	-	0.2 ± 0.1b	0.2 ± 0.1B
Raw milk								
TAMF	32.3 ± 14.0a	45.9 ± 8.1a	46.1 ± 15.3a	34.1 ± 14.4A	40.6 ± 8.9a	31.0 ± 11.7a	37.5 ± 6.9a	38.5 ± 8.5A
E. Coli	-	-	-	-	-	-	-	-
Total Coliform	29.6 ± 13.7a	37.2 ± 0.3a	43.1 ± 14.4a	31.1 ± 13.8A	27.4 ± 11.2a	27.0 ± 6.5a	37.0 ± 15.7a	31.0 ± 13.2A
Fecal Coliform	30.2 ± 9.6a	45.5 ± 2.8a	32.6 ± 10.0a	31.2 ± 9.9A	36.3 ± 9.9a	26.8 ± 5.8a	26.8 ± 14.2a	31.8 ± 12.0A
Salmonella	1.9 ± 0.8a	-	2.6 ± 0.7a	2.0 ± 0.8A	2.2 ± 0.5a	2.0 ± 0.2a	1.8 ± 0.6a	2.0 ± 0.6A
Staphylococcus	0.3 ± 0.1a	0.4 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1A	0.2 ± 0.1a	0.1 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1A
Pasteurized milk								
TAMF	18.7 ± 12.7a	-	29.2 ± 11.3a	21.9 ± 12.5A	26.1 ± 11.5a	-	-	26.1 ± 11.5A
E. Coli	-	-	-	-	-	-	-	-
Total Coliform	20.3 ± 14.5a	-	27.7 ± 5.0a	22.3 ± 12.5A	35.8 ± 13.2a	-	-	35.8 ± 13.2A
Fecal Coliform	28.7 ± 9.4a	-	25.7 ± 11.5a	28.0 ± 9.3A	29.4 ± 11.4a	-	-	29.4 ± 11.4A
Salmonella	2.1 ± 0.8a	-	2.8 ± 0.7a	2.3 ± 0.8A	1.7 ± 0.9a	-	-	1.7 ± 0.9A
Staphylococcus	0.2 ± 0.1a	-	0.3 ± 0.0a	0.2 ± 0.1A	0.2 ± 0.1a	-	-	0.2 ± 0.1A
Mashanza								
TAMF	-	-	44.1 ± 10.5a	44.1 ± 10.5A	-	-	25.6 ± 4.1b	25.6 ± 4.1B
E. Coli	-	-	-	-	-	-	-	-
Total Coliform	-	-	36.7 ± 13.8a	36.7 ± 13.8A	-	-	28.0 ± 8.5a	28.0 ± 8.5A
Fecal Coliform	-	-	26.7 ± 8.6a	26.7 ± 8.6A	-	-	18.7 ± 8.7a	18.7 ± 8.7A
Salmonella	-	-	2.7 ± 1.6a	2.7 ± 1.6A	-	-	2.0 ± 0.2a	2.0 ± 0.2A
Staphylococcus	-	-	0.3 ± 0.1a	0.3 ± 0.1A	-	-	0.2 ± 0.1a	0.2 ± 0.1A

Values in this table are means ± standard deviations of numbers of 10^4 colony-forming unit per milliliter (CFU ml^{-1}). For each pathogen (each table row), statistical comparisons are made at two levels: at axis level in each province by using a,b and c (small letters) and at province level by using A and B (capital letters); Values with the same small letters (a,b,c) for the different axis in each province are statistically identical at the 0.05 significance level; Values with the same capital letters (A,B) for the two provinces are statistically identical at the 0.05 significance level.
TAMF, Total Aerobic Mesophilic Flora.

germs are much more prevalent in milk samples. It was found a lower abundance of pathogens in milk collected from producers in Fizi axis compared to the other axis because all the farmers usually pasteurize the milk before delivery (100%). Moreover, in Tanganyika province, where raw milk is the main dairy product (83.7%), fecal coliforms are more abundant in milk.

3.3.2. Factors influencing the nutritionnal quality of milk products from producers

Figure 2 shows that milk products in the Kalemie-Malia and Kalemie-Kisondja axis have similar physico-chemical characteristics. These are milk products with high concentration in fat, non-fat dry matter, protein, and lactose contents. However, milk products from Fizi are mainly characterized by high freezing point and low contents of other physicochemical parameters. It was also noticed that when cattle are fed with concentrate as a supplement to pasture, the milk produced is of high density. This is particularly the case for milk from stall-feeding farms with crossbreds or improved dairy breeds compared to local breeds where a very low milk density was reported. It was also noticed that milk nutritional quality is influenced by the calving rank. In fact, milk collected from cows in their first lactation stage had less concentrated

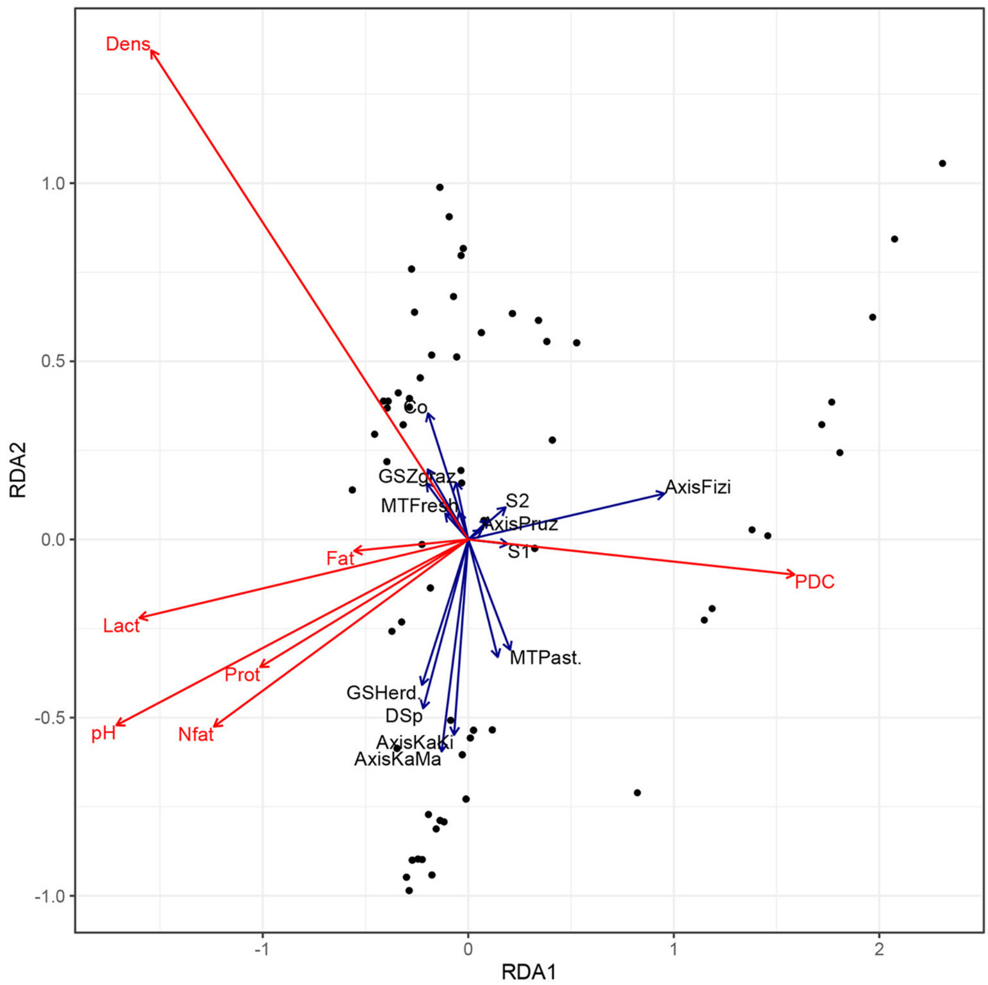

FIGURE 2
Biplot of Redundancy Analysis (RDA) of the nutritional quality of milk collected from farmers. MT, Milk type; GS, Grazing system; Co, feeding with concentrate; DSp, Dietary supplements provision; S1, First lactation rank; S2, Second lactation rank; S3, Third lactation rank; PDC, Freezing point; Lact, lactose; Prot, protein; Nfat, Nonfat dry matter.

milk compared to cows in the second lactation stage (see Figure 3).

3.3.3. Factors influencing the microbiological quality of milk products from collectors

Figure 4 indicates that transportation of milk by foot (71.4%), using plastic containers (82.9%) as well as the long time taken before milk stock disposal (around 4 days) are the main factors promoting the proliferation of fecal coliforms and staphylococci in milk samples. TAMF and total coliforms are common in Fizi axis, where collectors are supplied in milk directly at farm level (100%). However, in the Kalemie-Kisondja axis, where all collectors are used to clean their equipment for milk transportation and packaging (100%) and the cold chain management equipment is mostly available (72.73%), the milk collected had less pathogen counts. Globally, the cold chain and cleaning the equipment are the main factors that seem to reduce the abundance of pathogens in milk products at the collector's level.

3.3.4. Factors influencing the nutritionnal quality of milk products from collectors

Figure 5 shows that at the collectors' level, pasteurized milk is richer in fat and non-fat dry matter but with a low density and tend to be much acidic. Moreover, raw milk possesses a high quantity of lactose, with a high density and less acidity. Other parameters related to milk transportation, packaging and delivery did not impact the milk nutritional quality at collector's level.

3.3.5. Factors influencing the microbiological quality of milk products from vendors

Figure 6 indicates that the milk collected from vendors contains a low quantity of pathogens count when pasteurized and this was much found in milk products in the Fizi axis. The *Mashanza* samples collected from vendors are more characterized by a high accumulation of pathogen count mostly due to contamination during processing of raw milk into the *Mashanza* product.

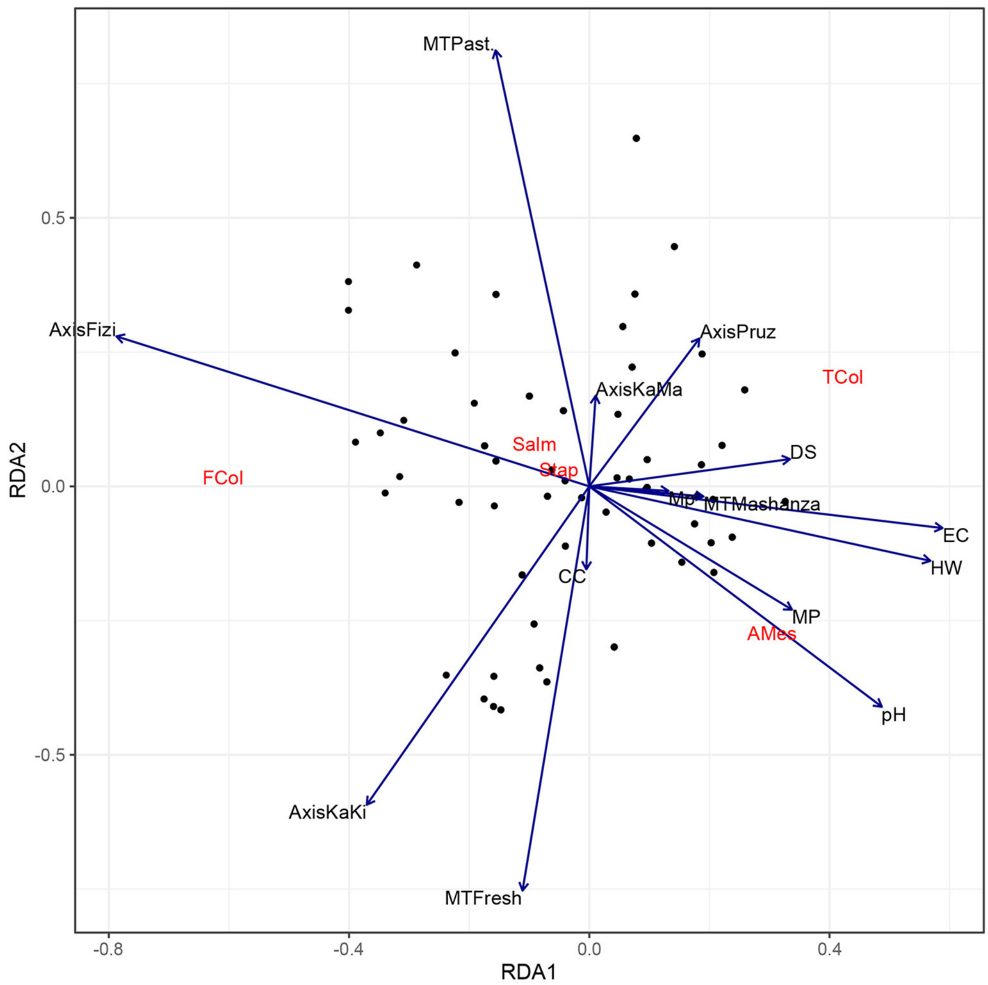

FIGURE 3
Biplot of Redundancy Analysis (RDA) of the encountered pathogens in milk collected from farmers. MT, Milk type; HW, Hand washing; EC, Equipment cleaning; CC, Cold chain equipment; MP, Milk processing; DS, Duration of stock depletion; AMes, Aerobic Mesophilic; TCol, Total Coliforms; FCol, Fecal Coliforms; Salm, *Salmonella*; Stap, *Staphylococcus*.

However, parameters related to milk transportation, packaging and delivery did not impact the milk nutritional quality at vendor's level mostly due to the short time taken before milk stock disposal (<1 days) and measures taken to avoid milk contamination during the sale, including the control by hygiene service, covering plastics containers with sachet, and placing it in water.

3.3.6. Factors influencing the nutritionnal quality of milk products from vendors

Figure 7 indicates that the milk products collected from vendors in Fizi and in Kalemie-Kisondja axis have very different physico-chemical characteristics. In fact, milk from Fizi have a high freezing point and low fat, non-fat dry matter, protein, and lactose contents and are more acidic. Other parameters related to milk transportation, packaging and delivery did not impact the milk nutritional quality at collector's level.

4. Discussion

In the two provinces, pH was lower in fermented milk and *Mashanza* compared to the raw and pasteurized milk. This result is in accordance with results found by Mutwedu et al. (2018) in *Mashanza* produced in South-Kivu province of DRC and Mukisa et al. (2020) in *Bongo*, a popular beverage in western and central Uganda produced traditionally by fermenting unpasteurized cows' milk. The diminution of the pH is desirable for fermented dairy products since it facilitates flavor development, coagulum formation and prevents growth of pathogenic and spoilage microbes (Downes and Ito, 2001; Mukisa et al., 2020). The fat content was high in *Mashanza* produced in South-Kivu compared to Tanganyika province. This result could be explained by the fact that the fat content in processed milk is mostly due to several factors including raw milk used, ferment used, condition of use (fermentation time, equipment), draining process (Fidler et al., 2001; Birali et al., 2019). The situation can also be explained by the fact that there more cows fed on concentrates and improved forages in South-Kivu than in Tanganyika. Fat is the main caloric-energetic

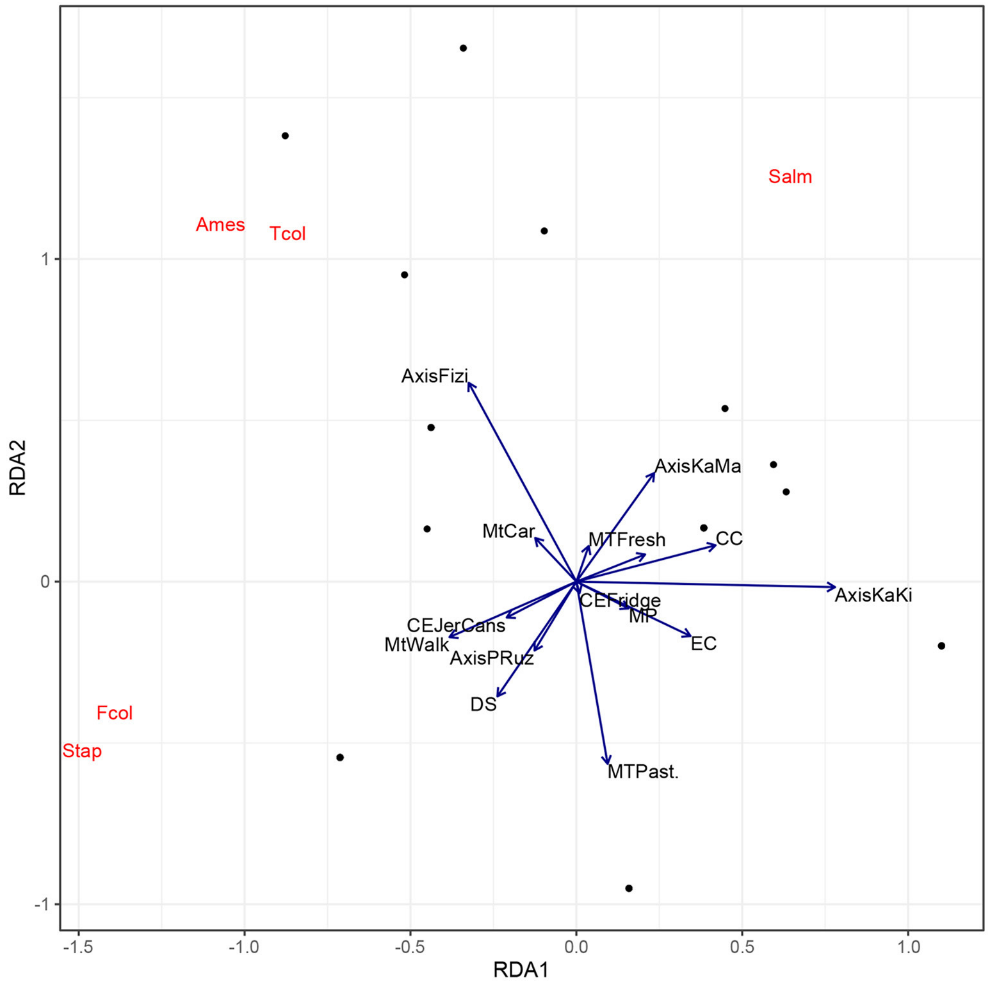

FIGURE 4
Biplot of Redundancy Analysis (RDA) of the encountered pathogens in collectors' milk. MT, Milk type; Mt, Means of transportation; CE, Conditioning equipment; EC, Equipment cleaning; CC, Cold chain equipment; MP, Milk processing; DS, Duration of stock depletion; AMes, Aerobic Mesophilic; TCol, Total Coliforms; FCol, Fecal Coliforms; Salm, *Salmonella*; Stap, *Staphylococcus*.

source of milk but is considered the most variable changeable constituent of milk (Shanler, 1985). Besides that, the repeated processing and ferment used can alter the fat globule structure in milk. This fact, associated with the use of plastic facilities to deliver milk to consumers, especially when this delivery process takes a long time, might be an explanation for the intense decline of fat content in the samples that were delivered by intensive biochemical and enzymatic processes (Metha et al., 1998; Thomaz et al., 1999). A decrease in lactose was observed in pasteurized milk compared to fermented, raw milk and *Mashanza*. The observed lactose values are in range with findings of Elbagermi et al. (2021) in cows' milk. Lactose is the major carbohydrate in milk and its reasonable concentration helps to maintain osmotic pressure of the milk by regulating its water content (Martin et al., 2016). In addition, lactose helps the absorption of calcium and other minerals contained in milk (Kalyanasundaram et al., 2021). In each milk type, protein content was not significantly influenced ($P<0.05$) by the production region. However, protein content was higher in raw and fermented milk compared to pasteurized milk and *Mashanza*. The decrease in protein content in pasteurized milk could be due to long time taken for the heat treatment and storage temperature and time, as reported in the present study. Lowe et al. (2004) reported that the degree of proteolysis depends on the intensity of heat treatment of milk. The decrease in protein content in *Mashanza* could be associated with the partial hydrolysis of proteins during its fabrication process. This hydrolysis could be the result of dripping during the processing period of *Mashanza*, native milk enzyme activity (plasmin) and/or proteases originating from incidental milk microflora (Ismail and Nielsen, 2010; Yasser et al., 2010).

Escherichia coli was not found in any of the milk samples. This should encourage efforts to preserve safe milk since *Escherichia coli* indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard and are therefore known as pathogenic bacteria causing severe intestinal and extra intestinal diseases for men (Kaper et al., 2004). Total Bacterial Counts (TBC) or Total Aerobic Mesophyll Flora (TAMF) reported in this study are in accordance with results

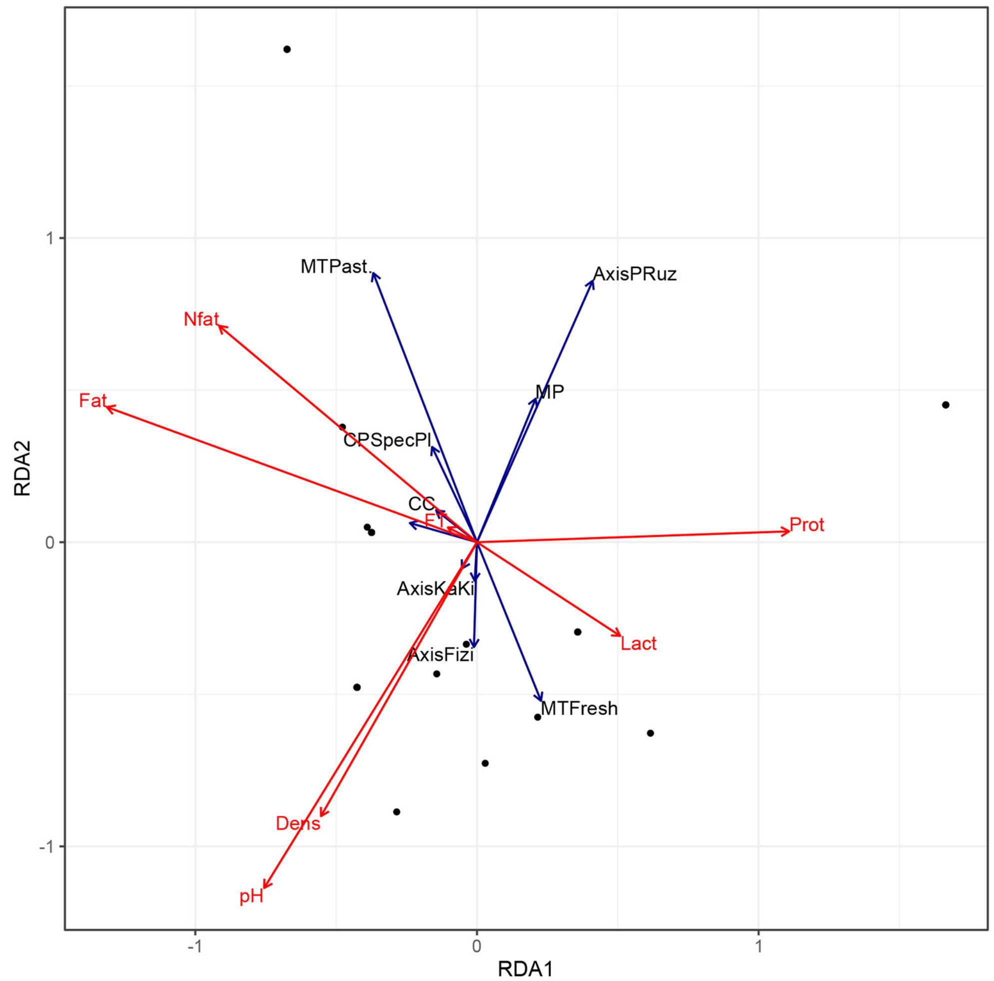

FIGURE 5
Biplot of Redundancy Analysis (RDA) of the nutritional quality of milk at collectors' level. MT, Milk type; CP, Collection place; CC, Cold chain equipment; MP, Milk processing; FT, Freezing point; Lact, lactose; Prot, protein; Nfat, Nonfat dry matter; PRuz, Ruzizi plain.

of Mpatswenumugabo et al. (2019) who assessed milk bacterial loads and micro-organisms associated with milk handling practices from dairy farmers, milk hawkers, milk collection centers and milk kiosks in the North-western region of Rwanda. Indeed, the observed TAMF varied from 14.7×10^4 CFU/ml (for pasteurized milk) to 57.9×10^4 CFU/ml (for Mashanza). These results concur with the EAC standards (10^6 CFU/ml), the AFNOR (France standard) (5×10^5 CFU/ml) and the American standard (3×10^3CFU/ml) (Mutwedu et al., 2018; Hoffmann et al., 2022). This contamination in the milk samples collected in the two provinces at farmers, collectors, vendors' level is influenced by different factors, including the hygienic condition of the milker and the mammary gland while milking (Mutwedu et al., 2018), storage and transport in unclean milk containers, prolonged time for milk storage and uncontrolled temperature during milk transportation (Mpatswenumugabo et al., 2019). In the study area, some farmers milked their cows in the morning hours and stored milk for about five hours at ambient temperature while waiting for milk collectors to take it for distribution to different customers like individual consumers, milk kiosks/restaurants. These collectors also had tendency of selling milk in the afternoon hours on public roads or milk "markets". Indeed, the milk market in eastern DRC is often designated as "informal" because most of the milk coming from smallholder farmers does not enter the regulatory food chain (Ayagirwe and Nfuamba, 2021), thus increasing the risks of delivering contaminated milk products to consumers.

In this study, coliforms were found abundant in all collected milk samples. All pasteurized milk samples were assessed to be out of the EAC standard (more than 10 CFU/ml). The same observation is made for non-pasteurized milk whose values are above the EAC accepted standard of 5×10^4 CFU/ml in almost all evaluated samples (Hoffmann et al., 2022). In fact, milk from emerging economies has been reported to contain very high coliform. For insistence, in Tanzania, mean coliform counts of 3-14×10^6 CFU/ml has been reported in milk that was examined along the informal value chain (Swai and Schoonman, 2011). In Zimbabwe, total coliform counts of 1.56–6.22 $\log_{10}$ CFU/ml

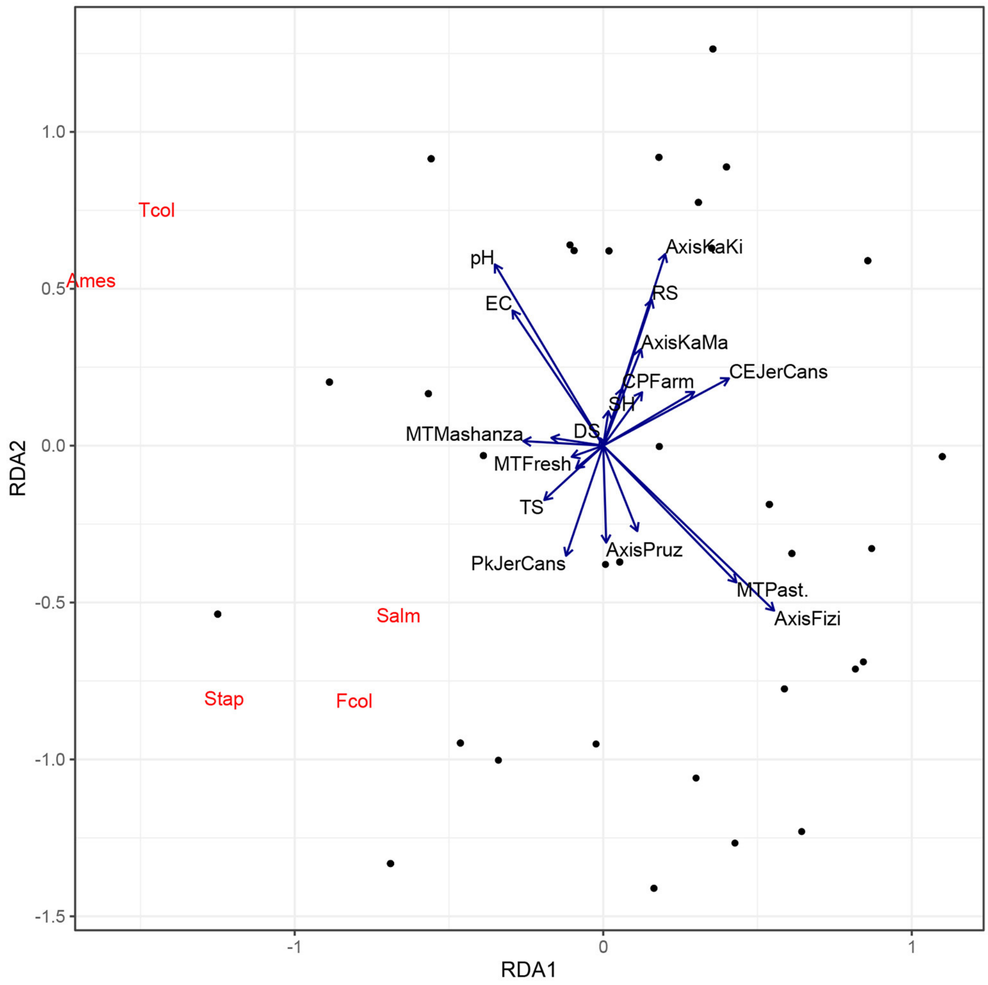

FIGURE 6
Biplot of Redundancy Analysis (RDA) of the encountered pathogens in sellers' milk. MT, Milk type; CP, Collection place; Pk, Pack; SH, Sale at home; RS, Roadside sale; TS, Time spent on the way; EC, Equipment cleaning; DS, Duration of stock depletion; Ames, Aerobic Mesophilic; Tcol, Total Coliforms; Fcol, Fecal Coliforms; Salm, *Salmonella*; Stap, *Staphylococcus*.

was reported in milk samples (Chimuti et al., 2016). Coliforms are a subset of bacteria that can grow at higher temperatures of 44.5-45.5°C, indicating that hot climate is one of the major ways of its proliferation (Wanjala et al., 2018). The principal sources of coliform contamination have been associated to sanitary infrastructures and establishments (Lues et al., 2003), non-hygienic milking equipment and udder (Miseikiene et al., 2015), containers (Wafula et al., 2016) and animal mastitis (Torkar and Teger, 2005).

Results of this study revealed the contamination in *Salmonella* in the tested milk products across axis. *Salmonella* is known to occur in raw milk, but pasteurized milk can be contaminated after heating and therefore transfer the pathogen to consumers (Holschbach and Peek, 2018), leading to salmonellosis, one of the most common causes of diarrhea globally (Sánchez-Vargas et al., 2011). The presence of *Salmonella* and *Staphylococcus* in food is typically prohibited (AFNOR, 1986; JORA, 1998). However, they are commonly found in contaminated food, especially when processing procedures are not adhered to. In this study, pasteurized milk samples were collected from local markets mainly characterized by non-observance of aseptic standards during milk processing and the storage and marketing conditions of milk products. This explains partly why pasteurized milk still contain *Salmonella* and *Staphylococcus*. Also, in the study area, milk buyers prefer to inspect the milk quality by opening the container or tasting the savor before they decide to purchase or not. This practice force vendors to open their products every time, exposing them to new contaminations. As the containers are not hermetically sealed and the products are not marketed under a cold chain, the ambient temperature accelerates the development of microorganisms and degrades the quality of the products.

The presence of *Staphylococcus* in tested milk samples is in accordance with findings of Kilango (2011) in milk samples collected in Dar es Salaam and of Addis et al. (2011) in milk samples collected in Ethiopia. Milk may carry a potential risk of poisoning with *Staphylococcus* along the value chain if the milk is subject to conditions and storage

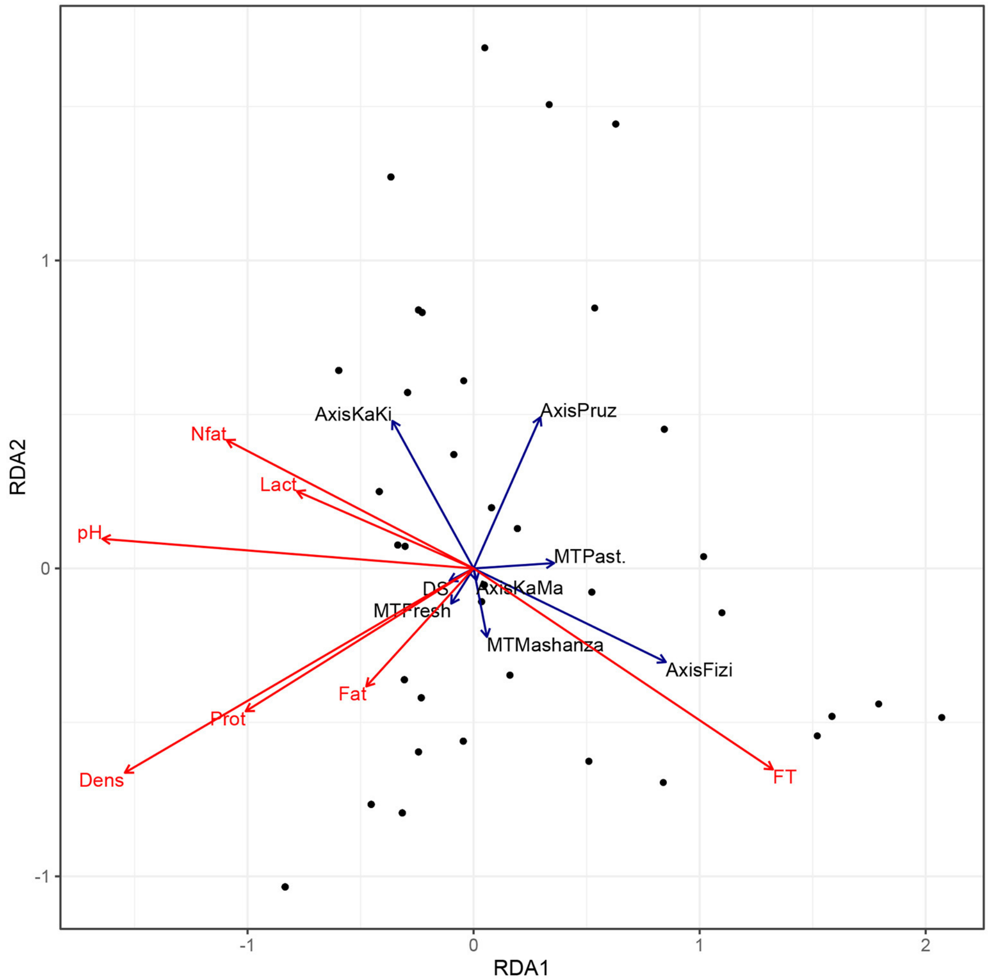

FIGURE 7
Biplot of Redundancy Analysis (RDA) of the nutritional quality of milk at sellers' level. MT, Milk type; EC, Equipment cleaning; CC, Cold chain equipment; DS, Duration of stock depletion; KaMa, Kalemie-Malia; PRuz, Ruzizi plain; KaKi, Kalemie-Kisondja; Past, Pasteurized milk; FT, Freezing point; Lact, lactose; Prot, protein; Nfat, Nonfat dry matter.

temperatures conducive to the multiplication of the pathogen, with subsequent production of enterotoxins (Nádia et al., 2012). Inappropriate handling of milk could result in bacterial growth and substantially increase the potential risk to consumers of milk products. Thus, vigilance in maintaining hygienic conditions in milking and in infrastructure along the milk value chain is of crucial importance (Van Kessel et al., 2004).

Generally, it was found that animal housing and feeding, animal health and management, practices of milk harvesting, storage, transportation, and retailing predisposed the milk to microbial contamination. The general hygiene at milking is known to affect the numbers of microorganisms in the milk (Kivaria et al., 2006). It is recommended that before milking, the animal house should be cleaned, the udder washed and dried before milking. After milking, teat dipping in suitable disinfectant is necessary to control entry of microorganisms through the teat canal (Shija, 2013).

From this study, occurrence of microorganisms at farm level could be associated with the fact that some farmers did not clean their hands, wash cow teats and clean animal houses before milking. Hand-milking using unwashed hands practiced by famers may transmit microorganisms to the milk (Shija, 2013). In addition, it was observed that milking was done either in the cowsheds or in a kraal with very dirty floor under traditional rearing system. This could be another risk practice that contributed to high microbial contamination of milk at producers' level. Worse enough, the longtime of milk storage at farmers, collectors and vendors' level was associated to the increase of microorganisms loads in collected milk in both South-Kivu and Tanganyika provinces. Indeed, storage and handling of milk under room temperature favors bacteria multiplication. The contamination of the milk stored in the freezer, especially at vendors' level, could be related to the irregularity of the electric power in the two provinces which can be unavailable for several hours per day, thus causing the bacterial multiplication when the cold chain is interrupted. Previous study by Swai and Schoonman (2011) in Tanga reported similar observations. Furthermore, other studies in Zimbabwe, Tanzania and Ghana reported that unhygienic practices and cold chain issues along the milk value chain predisposed

milk to high bacterial load (Gran et al., 2001; Omore et al., 2009).

The general microbial contamination in milk from vendors could be associated with the source of milk, bulking, cleanliness of the selling points and storage conditions. Dirty selling environment, lack of cold chain facilities and bulking were all together regarded among main risk factors that contributed to the high bacterial contamination of the milk products from vendors. These findings are in line with the study done in Dar es Salaam city by Kivaria et al. (2006).

It was noticed that the containers used during milking, transportation, storage and distribution were the wide and narrow necked plastic containers which sometimes are difficult to wash. The inner corners of narrow necked plastic containers are not easy to wash, this led to sticking of milk residues. In such a situation, microorganisms can rapidly build up in milk residues and may contaminate the milk products on subsequent uses. Similar observations were made by Bukuku (2013) who reported that plastic containers increased microbial count in milk. The plastic containers can thus be a source of several types of bacteria in milk. It is therefore not surprising that the milk storage containers played a significant role in the contamination of milk.

In the present study, milk collected from regions where farmers practice pasteurization had fewer numbers of pathogens compared to regions where most farmers deliver raw milk to collectors. In the latter, pathogens counts were higher such as fecal coliforms. In fact, pasteurization was reported to be positive effect on microbial contents in milk, which reduces the total bacteria count, Coliform bacteria count and pathogens (El Zubeir et al., 2007). Pasteurization can extend shelf life of milk while reducing its microbial load with no effect on chemical composition (AbdElrahman et al., 2013).

5. Conclusion

From the findings of this study, it can be concluded that physicochemical parameters in pasteurized, raw, fermented milk as well as in *Mashanza* depend on the feeding system, cattle breed kept by farmers and the calving rank. These parameters were not affected when milk was transferred from one stakeholder to another (producer, collector and, vendor). Nevertheless, microbiological parameters were highly affected by the types of milk products, production zone and stakeholders in the value chain. These microbiological parameters were mostly influenced by storage time, cleaning of equipment and hands, type of material for storage, the presence of the cold chain, contamination during milk transformation. However, in view of these results, the milk products sold along the milk value chain in South-Kivu and Tanganyika provinces are of poor quality and susceptible to negatively influence consumer's safety. It is therefore important to provide training on the health risk related to poor hygienic condition of marketed milk among actors along the milk value chain. Appropriate milk handling, conservation facilities and quality control services should be encouraged for consumers' safety.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization: SB and RA. Methodology: SB, RA, VM, and ZN. Software, data curation, and analysis: YM. Validation: MF and JM. Data collection: SB, RA, and VM. Laboratory analysis: RA, VM, and JZM. Writing—original draft preparation: SB and VM. Writing—review and editing: RA, ZN, PU, and JM. Supervision and funding acquisition: MF and JM. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Using a value chain framework for food safety assessment of edible offals in Nairobi, Kenya

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Edible offals comprise those parts of the animal considered fit for human consumption apart from the skeletal muscle, fat and attached skin. Edible offals form an important source of affordable animal protein and a delicacy for the larger Kenyan market. The offals are mainly traded in the informal markets which supplies at least 80% of the domestic market and where hygiene levels are low. The Meat Control Act Chapter 356, Laws of Kenya (Revised 2012) requires that offals be obtained from an officially declared food animal that has been slaughtered in a licensed slaughterhouse and declared fit for human consumption. According to the Meat Control Act, slaughterhouses in Kenya are classified as either export or local. The latter is subdivided into categories A, B, and C depending on their daily capacity, land size and the area they are authorized to supply meat and meat products. Each export slaughterhouse, category A and B slaughterhouses require a minimum of three meat inspectors to carry out inspection of carcasses and offals. The Directorate of Veterinary Services is the main regulator in Kenya on matters concerning safety of meat at the slaughterhouses. The Director of Veterinary Services appoints meat inspectors and veterinary officers who are responsible for ascertaining the safety of meat and meat products from the slaughterhouses. The meat inspectors also verify that slaughter facilities, staff, processes and equipment conform to food safety requirements. Offals, like carcass meat, should be handled under utmost hygiene conditions to minimize contamination or excessive growth of microorganisms. The current practices in slaughterhouses is that each set of edible offals is packaged separately in either polythene bags, plastic containers, meat transport containers or carriers and dispatched to the market. In some instances, stomachs and intestines are packaged for dispatch together with the lungs and hearts for transportation to distances over 50 km in either refrigerated or non-refrigerated containers or carriers. Some of these practices could compromise the safety of the offals. In this paper, we review the challenges faced in maintaining safety of edible offals especially at the slaughterhouses, distributors and retailers level from regulators' point of view.

KEYWORDS

offals, food safety, regulations, slaughterhouses, hygiene

1. Introduction

Offals are often defined as the edible, non-muscular parts of the carcasses of livestock including heart, kidney, liver, tongue, brain and intestines. Tails, feet, heads, and skin may also be considered offal (Codex, 2021). Many types of offals are nutrient dense, containing more iron and vitamin B12 than lean muscle meat, and historically were widely consumed

worldwide. However, offals became less popular in high-income countries (HICs), possibly because it was more difficult to prepare, associated with lower status, induced feelings of disgust by some consumers, and because of changes in value chains and concerns about health hazards such as heavy metals or diseases such as bovine spongiform encephalopathy (Hicks et al., 2018). In low- and middle-income countries (LMIC), offals remain widely consumed, and there are increasing efforts to promote their consumption in HICs as their consumption reduces food waste. In 2021, Kenyan exports of meat and edible offal was worth US$ 75.09 Million (Trading Economics, 2022).

The Kenya Standards-Offal Specification (DKS 2716:2017) categorizes offals into three namely: green offals (digestive tract organs i.e., rumen, reticulum, omasum, abomasum, small intestines, large intestines and colon), red offals (lungs, trachea, liver, hearts, kidneys, spleen, tongue, and pancreas) and white offals (brain, spine, bone marrow, testicles, pizzles, and udder). They are also loosely referred to as the "fifth quarter". Most of the green and red offals constitute edible offals in Kenya. Of the edible offals, the liver, kidney, gizzards and "matumbo" (rumen, reticulum, omasum, and abomasum) are most traded in the formal markets targeting high- and middle-income consumers. In the informal markets, traders sell intestines and tongue in addition to what is sold to the high- and middle-income consumers targeting low-income earners (Kenya Markets Trust, 2019).

The objective of this paper is to describe the red meat offal value chain in Kenya and challenges in maintaining food safety at different levels of the value chain from regulators' point of view following the structure below:

i The meat sector in Kenya: production, slaughter and retail line.
ii The yield of red meat offal in Kenya both from domestic and export sectors plus an estimate of the amount of red meat offal from the domestic sector.
iii Regulatory framework for meat safety in Kenya.
iv Offal handling practices.
v Offal condemnation.

2. The meat sector in Kenya: Production, slaughter and retail line

Kenya is administratively divided into forty seven counties and has a huge livestock resource base comprising 13.6 million heads of traditional/beef cattle, 2.2 million heads of dairy cattle, 19.3 million sheep, 28 million goats, 38.8 million poultry, 4.6 million camels, 1.2 million donkeys, and 0.4 million pigs (KNBS, 2019). About 60% of the livestock population is found in arid and semi-arid areas where it employs over 90% of the population (MOA, 2020). Beef, mutton, goat and camel meat comprise over 80% of meat consumed locally at a per capita meat consumption of 15 kg (Kenya Markets Trust, 2019). In 2018, meat production was as follows: 652,000 MT (beef), 25,271 MT (sheep and goat meat), 25,000 MT (pork) and 72,780 MT (camel meat) (FAOSTAT, 2018). The main market for the meat is domestic while a small proportion is sold internationally, mostly to Middle East countries, which are a major export destination for chilled sheep and goat carcasses. The main challenge in accessing other markets has been fulfilling the importing countries' sanitary standards particularly conditions on transboundary animal diseases and food safety.

In Kenya, slaughterhouses are classified as either export or local. There are several export slaughterhouses namely: Farmers Choice for pigs slaughter and Kenchic for chicken slaughter, whereas Choice Meats, Neema, Quality Meat Packers, KenMeat, Juja, and Kenya Meat Commission are mainly for slaughter of cattle, sheep and goats. Local slaughterhouses are over 2000 in number and are divided into: (1) Category A which is a large slaughterhouse with a daily capacity to process over 40 heads of cattle and is authorized to supply meat to any part of the country; (2) category B which is a medium slaughterhouse with a daily processing capacity of 6- 39 heads of cattle and is authorized to supply meat to towns or urban centers within 50 kilometers radius from its locality; (3) category C also called slaughter slabs, which processes less than 5 heads of cattle daily and is allowed to supply meat within its locality [Meat Control (local slaughterhouse) regulations, 2010 (Revised 2012)]. The slaughterhouses are further required to provide adequate land for each specific category to cater for slaughter stock holding area, location of auxiliary slaughterhouse facilities such as incinerator/condemnation pits, manure shed, waste water pre-treatment and changing rooms/ ablution. The primary aim of the slaughterhouses is to produce wholesome, healthy and clean meat and meat products (Ahmed et al., 2019).

3. The yield of red meat offal in Kenya both from domestic and export sectors plus an estimate of the amount of red meat offal from the domestic sector

The meat sector in Kenya is an important contributor to its economy. In 2020, the country exported 1,076 MT of beef valued at US$ 4,695,000; 12,508 MT of goat meat and mutton valued at US$ 55,790,000. In that year, 674,975 cattle, 2,634,766 goats, and 761,042 sheep were slaughtered. A small proportion of these was destined for the export market that mainly demands for whole carcass meat without offals. This left quite a significant quantity of offals to be handled and sold in the domestic market with a small proportion of this going for export. In 2020, Kenya exported edible offals worth US$ 1.45 million to Vietnam and Hong Kong making it the 64th largest exporter of edible offal in the world (OEC, 2023).

A high demand for ruminant offals is evident locally and is steadily growing owing to increased urban population coupled by expected doubling of red meat consumption by 2030. In Nairobi, Kenya's capital city, where the majority of export and large local slaughterhouses are located, the marketing channels for sale of offals are well established and known. The channels are distributed in formal (supermarkets and butcheries) and informal markets (street vendors, kiosks, and a popular chain of urban meat eateries). Kenya's informal sector is large and is increasingly expanding creating 8 out of 10 all new jobs outside the agricultural sector (KLMIS, 2021). The sector comprises enterprises that are not registered by the Registrar of Companies and are not covered by the government social security scheme and other employment

related government regulations (KLMIS, 2021). Informal markets are the main channels for distribution of offals. In areas where these are located, some county governments have tried to establish prerequisites for food safety such as running water, public toilets, waste disposal and electricity. However, hygiene controls in these markets are still inadequate and there is a need to build capacity of food business operators (FBOs) on hygiene responsibility and perform regular check-ups to enforce hygiene regulations.

4. Regulatory framework for meat safety in Kenya

In Kenya, various laws including Public Health Act Chapter 242, Food, Drugs, and Chemical Substances Act Chapter 254, and Meat Control Act Chapter 356 as well as the Kenya standards adopted from international organizations such as Codex Alimentarius Commission and International Standards Organization regulate meat safety. These laws and standards aim at protecting meat consumers from various health hazards arising from consumption of unsafe meat. Slaughterhouses or abattoirs in Kenya are encouraged to implement food safety management systems and are licensed by the Director of Veterinary Services based on compliance to the regulations outlined in the Meat Control Act Chapter 356 of the Laws of Kenya (Law, 2012). Emanating from this Act are regulations governing local slaughterhouses, export slaughterhouses, poultry meat inspection, transport of meat and licensing of meat transport carriers and containers. The Meat Control (local slaughterhouse) regulations of 2010 outlines minimum requirements for hygienic handling of offals (Table 1).

Besides the Meat Control Act, the Animal Diseases Act Chapter 364 is applicable in the Meat regulatory framework as it guides in animal disease management and livestock movement. Livestock movement is key in livestock traceability and thus meat product traceability from the farm to the fork. The Prevention of Cruelty to Animals Act, Chapter 360, guides on animal welfare practices for slaughter stock at the farm, during transportation and at slaughter. Upholding animal welfare practices for slaughter stock has a positive correlation with the keeping quality for the meat. Further, it prevents injuries including bruises and fractures on the slaughter stock that leads to economic loss. Kenya as an exporting country to several Middle Eastern countries has conformed to the sanitary conditions imposed by these countries. These sanitary conditions, which are generally outlined in the Health Attestation of the Export Certificates, contribute toward the regulatory framework of the Meat sector in Kenya.

TABLE 1 Summary of requirements for hygienic handling of offals as outlined in the meat control (local slaughterhouse) regulations of 2010.

Slaughter area	Hygiene measure
General	Any offal shall be kept identifiable with the carcasses until the inspection is completed
Chillers	Separate chillers for storing offal shall be provided
Cleaning	Separate rooms for offal cleaning shall be provided
Dispatch	Washed green offals shall be removed continuously from the facility but within four hours after evisceration
Dispatch	A sufficient number of suitable receptacles, if necessary with close-fitting covers, shall be furnished for offal collection or removal from the slaughterhouse
Dispatch	Dispatch areas should have equipment for sorting, quartering and marking of carcasses and red offals, as well as a door for dispatching and loading in a hygienic manner

5. Offal handling practices

To obtain offals, after stunning and bleeding the slaughter animal, hooves are removed and placed in a receptacle. Flaying is then done to remove the hide. The head is then removed and is placed on the inspection rack for post-mortem inspection. The testicles and pizzles are left on the hide to be removed in the hides and skins room and packaged for dispatch. Evisceration is then undertaken whereby the gastrointestinal tract is removed, post-mortem inspection is conducted, and afterwards they are cleaned and packaged ready for dispatch. The remaining offals are then removed and moved to the inspection area for post-mortem inspection after which they are packaged for dispatch. The kidney remains on the carcass for removal after inspection of the carcass. It is then collated with the other offals and dispatched. For dispatch, packaging is done separately for each set of offals in polythene bags or metal containers.

5.1. Offal handling practices at the slaughterhouses

It is assumed that offals are dispatched from the slaughterhouses while safe after having been inspected. However, there are challenges that pose increased risk of contamination of the offals thereby a risk of exposure to pathogens by slaughterhouse workers and local consumers that include, *inter alia*: (i) Inadequate knowledge to support good slaughtering practices, (ii) Lack of, or inadequate proper equipment such as aprons, boilers, cold storage facility and detention rooms, (iii) Water shortages that can compromise adequate cleaning of the products especially the "matumbo," (iv) Use of untreated water in the slaughterhouses, (v) Improper elimination of condemned slaughterhouse materials, (vi) Lack of value addition for extending the shelf life of the offals, and (vii) Lack of adherence to procedures for maintenance and hygiene of the slaughterhouses including meat transport containers and trucks.

5.2. Offal handling practices by distributors

Distributors source edible offals directly from slaughterhouses to distribute to formal and informal markets. The Meat Control Act Chapter 356 requires that the offals be transported in a hygienic manner in licensed meat carriers or containers. The following are

the key challenges in maintaining edible offal safety at distributor's level: (i) Weak enforcement of offal transportation regulations as per the Meat Control Act Chapter 356. There is minimal use of licensed meat carriers or containers or poor maintenance of the licensed ones, (ii) Practice of mixing red and green offals poses a risk of cross contamination, (iii) Long distance transportation in non-refrigerated containers to the markets, (iv) Inadequate or lack of monitoring product temperatures during transport and (v) Inadequate system for tracking and tracing the offal distribution system.

5.3. Offal handling practices by retailers

The key challenges in maintaining edible offal safety at retailer's level include: (i) Hygiene of the retail areas is occasionally overlooked if sold to informal markets as compared to formal ones such as butcheries, restaurants or hotels, (ii) Lack of industry association creating a barrier for access to capital to improve their infrastructure for hygiene, (iii) Lack of product traceability systems and (iv) Inadequate or lack of cold chain facilities.

6. Offal condemnation

Meat inspection in slaughterhouses refers to the expert supervision of the whole process of producing meat products with the objective of providing wholesome meat for human consumption and preventing danger to public health (Herenda et al., 1994).

The main reasons for condemnation of offals at the slaughterhouses include but not limited to parasitic conditions such as fascioliasis, hydatidosis, pimply gut/oesophagostomiasis; inflammatory conditions such as peritonitis, gastritis, enteritis, and pulmonary blood aspiration. A variety of internal parasites affect animals such as worms or helminths predominantly roundworms (nematodes), which are primarily parasites of the gastrointestinal tract with lung included, and the flat worms (trematodes) including liver flukes and tapeworms. Parasites often lead to abnormal conditions such as fascioliasis, peritonitis, hydatidosis and emphysema in livestock (Mareko et al., 2018). In a slaughterhouse survey in Ethiopia, out of 384 animals' slaughtered, postmortem inspection led to condemnation of 18.8% liver, 6.5% lung, 4.4% heart, 4.7% kidneys, and 4.7% tongue. The main causes for condemnation were fascioliasis (33.3%), calcification (16.7%), abscess (16.7%), and hydatid cysts (12.5%) (Ahmed et al., 2019).

A study in Kisumu County, Kenya conducted by Kanyari et al., (2012) revealed that in 2007 and 2008, a total of 12,332 and 10,509 cattle were slaughtered, respectively. Of these, 664 and 738 livers were condemned in 2007 and 2008. Of these, 52.04% and 56.4% for year 2007 and 2008 respectively were condemned due to fascioliasis. In 2007, the total monetary loss from liver fluke infections was KES 926,600 (US$ 12,034) while in 2008, this figure was KES 1,032,800 (US$ 13,413). On a monthly basis, the losses ranged from US$ 436 in February 2008 to US$ 13,413 in December of the same year (Kanyari et al., 2012). Regarding heart condemnations, in 2007 and 2008, the losses were KES 26,800 [US$ 348] and 10,780 [US$140] respectively. In 2007, all months recorded losses from cases of bovine muscular cysticercosis while in 2008, some months had very low or no cases at all (as observed in October). Between January and August, losses from heart condemnation fluctuated between the two years without a clear difference but from August, losses were consistently higher in 2007 compared to 2008. This somehow coincided with the higher losses being recorded from liver condemnations (Kanyari et al., 2012).

In addition, a cross sectional study conducted by Achollah et al. (2019) in Siaya County, Kenya revealed that 75 out of 112 cattle slaughtered, each had one or more organs condemned. Of the condemned organs, 51.8% had fascioliasis, 25% showed pimply gut and 0.9% had hepatic hydatidosis. Because of these condemnations, a total of US$ 935 in losses were incurred. The study concluded that many organs were condemned at slaughter due to controllable parasitic conditions and defects associated with poor slaughtering techniques. This causes wastage of edible organs and heavy economic losses for the livestock industry. The occurrence of hepatic fascioliasis and hydatidosis suggested an increased risk of transmission of the zoonotic agents to humans. However, World Health Organization Foodborne Disease Burden Epidemiology Reference Group ranked helminths as low-risk pathogens in Africa having been predicted to produce 105 illnesses per 100,000 population, respectively (WHO, 2015).

7. Discussion

Kanyari et al. (2012) postulates that malnutrition is a major cause of human mortalities in Sub-Saharan Africa and every effort must be made to conserve the available sources of protein for human wellbeing. Utilization of animal by-products such as the edible offals for human consumption contributes to conservation of animal source proteins and provision of key nutrients thereby combatting malnutrition especially in LMIC. Availability of these meats may be hampered by diseases such as the parasitic ones that constitute a major impediment to livestock production in Sub-Saharan Africa owing to the direct and indirect losses they cause. For example, it has been estimated that fascioliasis in Kenya leads to losses estimated at £7 million annually, through a combination of poor productivity, death of stock and condemnation of infected livers (Kanyari et al., 2012).

Offal handling practices by slaughterhouses, distributors and retailers affects the safety of offals either positively or negatively. Adherence to Meat Control Act regulations and generally improved sanitary conditions by these value chain actors will go a long way in improving the safety of meat and offals. Hygienic handling and adequate cooking of these meats by consumers will decrease the likelihood of meat borne diseases, bacterial or parasitic, being conveyed to human beings. The Directorate of Veterinary Services (DVS) has put in place measures to improve safety of meat in Kenya that include improving meat inspection services by introducing risk-based controls for better prioritization of resources and improving uniformity of inspections countrywide through developing guiding principles and values for inspectors. The DVS aims to regularly capacity build county

veterinary services on matters veterinary public health including slaughterhouse inspections. Furthermore, the DVS has developed the veterinary public health bill which when enacted into Veterinary Public Health Act will repeal the current Meat Control Act. The envisaged Veterinary Public Health Act will bestow the responsibility for food safety to FBOs. The FBOs will be required to implement immediate and safe disposal of condemned offals to break the cycle of parasites, promote value addition of offals to extend their shelf life and to promote investment in cold chain and monitoring of temperature of products throughout the value chain.

We conclude that continuous education on production of quality and safe meat along the value chain, coupled with strict enforcement of the legal framework, is inevitable so as to build consumer confidence on safety of these products.

Author contributions

AS and AM conceptualized the review topic and outline. AS and EM drafted the first manuscript with inputs from all co-authors. AS helped revise the manuscript after reviews. All authors approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence of extended-spectrum β-lactamase producing Enterobacterales in Africa's water-plant-food interface: A meta-analysis (2010–2022)

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Background: Multidrug-resistant extended-spectrum β-lactamase (ESBL)-producing Enterobacterales is regarded as a critical health issue, yet, surveillance in the water-plant-food interface remains low, especially in Africa.

Objectives: The objective of the study was to elucidate the distribution and prevalence of antimicrobial resistance in clinically significant members of the Enterobacterales order isolated from the water-plant-food interface in Africa.

Methods: A literature search was conducted using six online databases according to the PRISMA guidelines. All available published studies involving phenotypic and genotypic characterization of ESBL-producing Enterobacterales from water, fresh produce or soil in Africa were considered eligible. Identification and characterization methods used as well as a network analysis according to the isolation source and publication year were summarized. Analysis of *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* included the calculation of the multiple antibiotic resistance (MAR) index according to isolation sources and statistical analysis was performed using RStudio.

Results: Overall, 51 studies were included for further investigation. Twelve African countries were represented, with environmental AMR surveillance studies predominantly conducted in South Africa. In 76.47% of the studies, occurrence of antimicrobial resistant bacteria was investigated in irrigation water samples, while 50.98% of the studies included fresh produce samples. Analysis of bacterial phenotypic antimicrobial resistance profiles were reported in 94.12% of the studies, with the disk diffusion method predominantly used. When investigating the MAR indexes of the characterized *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* spp., from different sources (water, fresh produce or soil), no significant differences were seen across the countries. The only genetic determinant identified using PCR detection in all the studies was the $bla_{\text{CTX-M}}$ resistance gene. Only four studies used whole genome sequence analysis for molecular isolate characterization.

Discussion: Globally, AMR surveillance programmes recognize ESBL- and carbapenemase-producing Enterobacterales as vectors of great importance in AMR gene dissemination. However, in low- and middle-income countries,

such as those in Africa, challenges to implementing effective and sustainable AMR surveillance programmes remain. This review emphasizes the need for improved surveillance, standardized methods and documentation of resistance gene dissemination across the farm-to-fork continuum in Africa.

KEYWORDS

multidrug resistance (MDR), ESBLs, environmental AMR surveillance, foodborne pathogens, low and middle-income countries (LMICs), meta-analysis, Enterobacterales

1. Introduction

Antimicrobial resistance (AMR) is regarded as one of the top ten threats to global health (WHO, 2020). This follows as the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms continue to threaten the effectiveness of clinically important antibiotics to treat common infections (Koutsoumanis et al., 2021; Rahman et al., 2022). Globally, major organizations including the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), World Organization of Animal Health (OIE) and the European Commission (EC) have recognized the need for further investigation and a multidisciplinary approach to combatting AMR (Koutsoumanis et al., 2021). However, in low and middle income countries (LMICs), such as those in Africa, challenges to implementing effective and sustainable AMR surveillance programmes remain (Elton et al., 2020). This follows as LMICs often lack the necessary infrastructural and institutional capacities and effective reporting systems to roll out sustainable surveillance programmes (Elton et al., 2020).

Many different sources and routes for human acquisition of antimicrobial resistant bacteria are recognized, including human-to-human transmission, direct contact with food-producing animals and pets, foodborne transmission as well as the environment (Koutsoumanis et al., 2021). In LMICs, the main driver of AMR is reported to be transmission and not antimicrobial use (Koutsoumanis et al., 2021). Non-human sources of pathogens such as extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and plasmid mediated AmpC (pAmpC)-producing *E. coli* has been reported, with the need for longitudinal studies and continuous monitoring (Mughini-gras et al., 2019). Furthermore, the Centers for Disease Control and Prevention (CDC) recently reported that urgent AMR threats in the United States (US) include carbapenem-resistant Enterobacterales, while ESBL-producing Enterobacterales, drug-resistant nontyphoidal *Salmonella*, and drug-resistant *Salmonella* serotype Typhi are regarded as serious threats, among others (CDC, 2019). The Enterobacterales form part of the normal epiphytic microflora of fruit and vegetables, and include members ubiquitous in terrestrial and aquatic environments, as well as human foodborne pathogens including pathogenic *E. coli* and *Salmonella* spp. (Rajwar et al., 2015). Moreover, Enterobacterales are adapted to sharing genetic material and often clinically significant resistance genes through carriage on mobile genetic elements (Partridge, 2015). Recently, a comprehensive assessment of the global burden of AMR stated that the six leading pathogens for deaths associated with resistance included *E. coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Murray et al., 2022).

In the last decade, an increased emphasis on the role of the environment in dissemination of AMR has been reported (WHO, 2015; Koutsoumanis et al., 2021). Furthermore, authors have reported on the importance of an integrated One Health approach for developing and implementing mitigation strategies in combatting AMR (White and Hughes, 2019; Ikhimiukor et al., 2022). The One Health concept in AMR mitigation strategies recognizes that humans, animals (including wildlife), environments, and ecosystems are key priorities (White and Hughes, 2019). To date, most AMR surveillance studies, especially in LMICs in Africa, have focussed on humans and animals. As an example, from 901 studies in LMIC-based studies in 2000-2018, the rapid increasing trends of AMR in the food-animal sector for common indicator pathogens such as *E. coli*, *Campylobacter* spp., *Salmonella* spp., and *S. aureus* have been reported (Ikhimiukor et al., 2022). The main objective of the current study was to elucidate the distribution and prevalence of AMR in clinically significant members of the Enterobacterales family isolated from the water-plant-food nexus in Africa.

2. Materials and methods

The review and meta-analysis of occurrence included published articles from January 2010 – December 2022 and was compiled according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Page et al., 2021).

2.1. Search strategy

Information on published articles from all countries on the African continent as defined by the African Union (African Union Commission, 2022) that reported on the occurrence of multidrug resistant Enterobacterales in the water-plant-food interface were included. Two authors independently performed comprehensive literature searches using five online databases: Google Scholar, PubMed Database, EBSCOhost Online Research Databases, Science Direct and Semantic Scholar. Boolean operators ("AND", "OR") were applied to search the articles and only English publications were included. The predefined search terms "(extended-spectrum

beta-lactamase or extended-spectrum or beta-lactamase or ESBL or ESBL-producing) AND (Enterobacteriaceae or Enterobacterales) AND Africa AND (water or irrigation water or vegetables or fruit or fresh produce or produce or soil)" were used to retrieve relevant articles published within the chosen timeframe. The WHO Global Action Plan to tackle AMR (GAP-AMR) as well as the Global Antimicrobial Resistance and Use Surveillance System (GLASS) was established in 2015 and national action plans developed in individual countries subsequently followed (WHO, 2020; Willemsen et al., 2022). For the current review, the authors chose a timeframe that included environmental AMR surveillance studies 5 years prior to the launch of GLASS up to the most recent articles available in 2022, to provide an overview of current analysis of environmental AMR profiles in African countries over at least a decade.

2.2. Study inclusion and exclusion criteria

Publications were independently reviewed by two authors (LR and ED) to determine eligibility and duplicate entries were identified by considering the title, authors and the year of publication. Inclusion criteria comprised of all available full text articles involving phenotypic and genotypic characterization of ESBL-producing Enterobacterales from water, fresh produce or soil in Africa. More specifically, publications that described the occurrence of antimicrobial resistant bacteria in fresh produce production, including soil, harvested produce and associated irrigation water, as well as fresh produce at retail were considered eligible. Given the diversity of plant-associated bacteria, only members within the Enterobacterales order were included in the current review. This follows as a dramatic escalation in AMR among bacteria, especially members of the Enterobacteriaceae have been noted globally, resulting in ESBL-producing Enerobacteriaceae forming part of the WHO list of critical priority pathogens that pose the greatest threat to human health (WHO, 2017a; Lynch and Clark, 2021). Studies that focused on wastewater, human- or animal health and studies that did not identify the bacterial organisms to at least genus level were excluded (Figure 1). Furthermore, studies that focused on transmission of AMR or multidrug resistant bacteria through non-plant food sources (e.g. dairy, aquaculture or meat products) were not considered eligible for the current review. All published articles available on the selected databases at the time of data extraction ($n = 922$) were individually reviewed and those not meeting the pre-defined inclusion criteria were excluded from the final articles for analysis.

2.3. Data synthesis, analysis and reporting

Overall, 51 studies were included for further investigation. The publication year, isolation source (water, soil or fresh produce), percentage bacterial occurrence, and identification and characterization methods used were summarized for all publications (Supplementary Table 1). Network analysis was carried out using UCINET® 6 for Windows (Borgatti et al., 2002), with matrices created of the articles based on the isolation source and publication year and country. NetDraw2.175 was used to draw the network.

For selected bacterial species (*E. coli*, *K. pneumoniae* and *Salmonella* spp.) isolated from the different sources, where the information was not already included in the published results and possible to calculate, the multiple antibiotic resistance (MAR) indexes were calculated for analysis of the potential health risk (data not shown). The MAR index for each bacterial species in the respective studies was calculated as $x/(y.z)$ where x represents the aggregate resistance of antibiotics to all isolates, while y represents the total number of antibiotics and z the number of isolates from the isolation source (Riaz et al., 2011). The β-lactamase genes detected in *E. coli*, *K. pneumoniae* and *Salmonella* spp. across the different countries were represented using DataWrapper (Lorenz et al., 2012) and Microsoft Excel and PowerPoint.

2.4. Statistical analysis

Data were analyzed using RStudio (RStudio Team, 2020). The Shapiro-Wilk test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965). ANOVA was used to test for significant differences between the MAR indexes of the characterized *E. coli*, *K. pneumoniae* and *Salmonella* spp. per country and publication year, respectively. Student's protected t-LSD (Least significant difference) were calculated at a 5% significance level to compare significant source effects of the MAR indexes for the characterized isolates (Snedecor and Cochran, 1980).

3. Results

3.1. General overview

Based on the eligibility criteria (Figure 1), a total of 51 articles were included in the systematic review. The included studies represented 12 African countries (Figure 2). The majority of the studies were conducted in South Africa ($n = 20$), followed by Nigeria ($n = 10$), Tunisia ($n = 6$), Algeria ($n = 4$), Benin ($n = 2$), Ghana ($n = 2$), Morocco ($n = 2$), Egypt (=1), Tanzania ($n = 1$), Sudan ($n = 1$), Kenya ($n = 1$), and Democratic Republic of the Congo ($n = 1$).

The occurrence of antimicrobial resistant bacteria in irrigation water samples were evaluated in the majority (76.47%) of the studies (Figure 3). Where indicated, the type of irrigation water included predominantly surface water (river) sources, followed by borehole, ponds, wells, streams and/or canals. In nine studies, irrigation water in conjunction with associated irrigated fresh produce were analyzed, while another eight studies focussed on bacterial isolation and characterization from water and soil in the agricultural environments (Figure 3). Six studies included analysis of irrigation water, soil and associated fresh produce, while eleven studies focussed on isolation and characterization of bacteria from fresh produce only and one focussed on soil analysis only (Figure 3). Overall, the studies that included water, soil and/or fresh produce samples were predominantly conducted in South

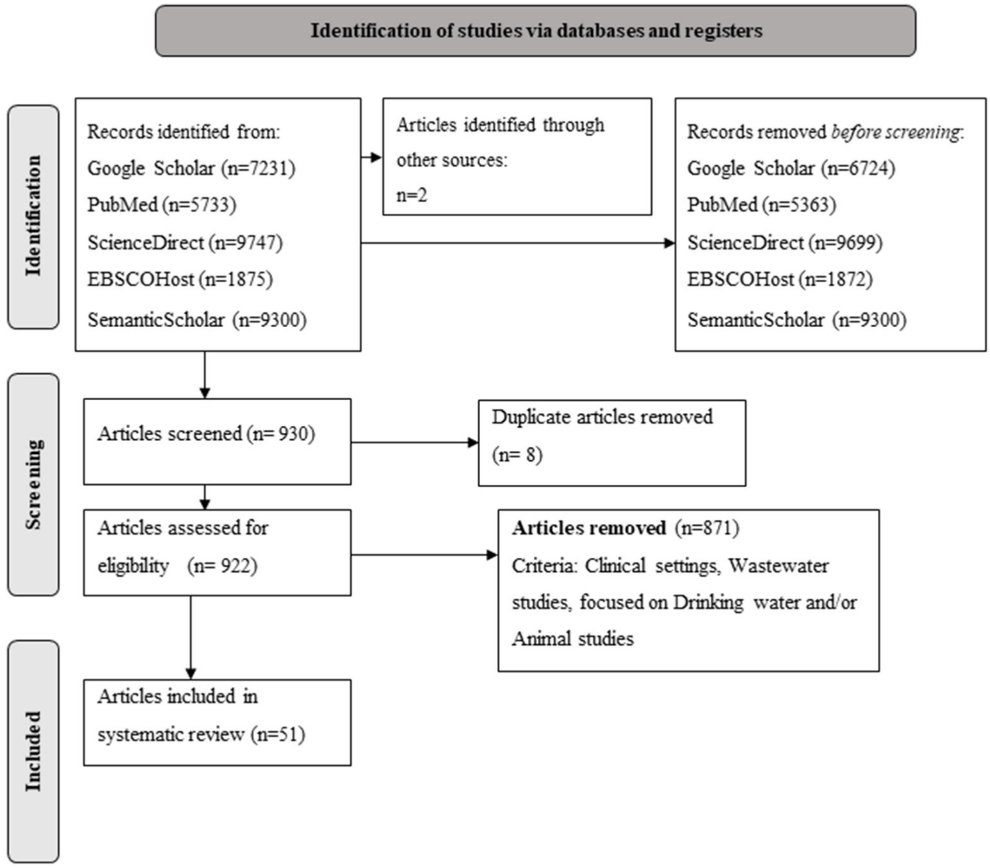

FIGURE 1
PRISMA flow diagram (Page et al., 2021) summarizing the process and selection of literature on occurrence and characterization of extended-spectrum beta-lactamase producing Enterobacterales in the water-plant-food nexus in Africa.

Africa (n = 13), while studies that focussed on fresh produce or water samples only, were conducted mostly in Nigeria (n = 8) (Figure 3). The majority of the studies were published in 2020 (n = 13), followed by 2015 and 2022 (n = 7, respectively) and 2021 (n = 5).

3.2. Identification and characterization of bacterial isolates

Enterobacterales from 15 different genera were isolated and characterized for antimicrobial resistance, either phenotypically, genotypically, or both, in 12 African countries. Bacterial isolate identification was performed using principally three methods, alone or in combination, that included biochemical tests, PCR and/or mass spectrometry (Supplementary Table 1). Matrix assisted laser desorption ionization time of flight (MALDI-ToF) mass spectrometry identification appeared to be the gold standard for isolate identification in South African studies, used in 60% (n = 12), followed by 16S rDNA PCR identification (n = 5), biochemical tests (n = 2) and one study that used the OmniLog system for identification. In the other African countries, biochemical tests [analytical profile index (API) or indole testing] were predominantly used for isolate identification (n = 18), followed by MALDI-ToF (n = 7) and 16S rDNA PCR identification (n = 4). One study in the Democratic Republic of the Congo used only selective media for isolate identification, while the Phoenix 100 phenotyping system and a combination of biochemical tests and PCR was used in two studies in Tunisia, respectively.

The 51 studies included in the current review reported on isolation and characterization of 15 different Enterobacterales genera from irrigation water sources, soil and fresh produce (Supplementary Table 1). In total, 20 (39.22%) articles focussed on *E. coli* only, three (5.88%) on *Klebsiella pneumoniae*, two (3.92%) on *Salmonella* spp., and one each on *Citrobacter* spp., and *Enterobacter* spp. In the remaining 24 articles that focused on the Enterobacterales family, the most frequently reported bacteria were *Klebsiella* spp. (43.14%), followed by *Citrobacter* spp. (35.29%), *Enterobacter* spp. and *E. coli* (33.33% each), *Serratia* spp. (15.69%) and *Salmonella* spp. and *Proteus* spp. (11.76% each).

Analysis of phenotypic antimicrobial resistance profiles of the isolates were reported in 94.12% of the studies, with the disk diffusion method predominantly used (Supplementary Table 1). Phenotypic results analysis mainly relied on the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI, including NCCLS, n = 31), followed by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, n = 3) and the

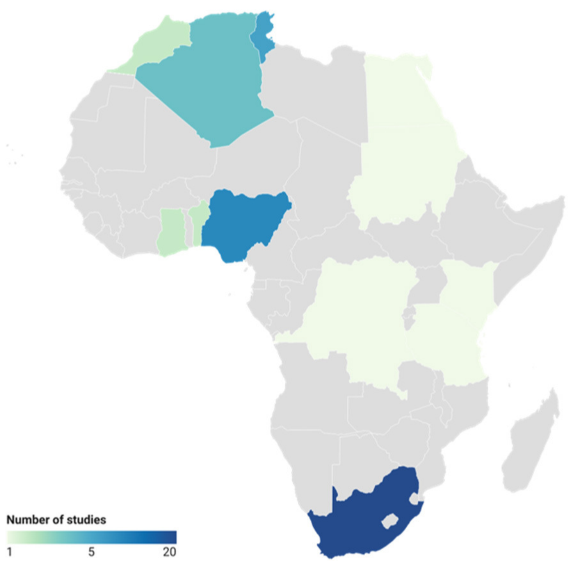

FIGURE 2
African countries ($n = 12$) represented by the data analyzed across studies ($n = 51$) related to antimicrobial resistance.

Antibiogram Committee of the French Society of Microbiology ($n = 4$). Furthermore, in seven studies, both the CLSI and EUCAST criteria were used for results interpretation. Additionally, 72.55% of the studies ($n = 37$) included PCR detection of the resistance genes, with DNA sequencing as a complementary test to the PCR included in 31.37% of the studies. Of the 31.37%, only 7.84% ($n = 4$) used the whole genome sequencing (WGS) technique for further characterization.

3.3. Shared resistance genes in potential human pathogens within the water-plant-food interface

The 37 studies that included PCR analysis of resistance genes were predominantly in South Africa ($n = 13$), followed by Tunisia and Nigeria ($n = 6$ each), Benin, Morocco and Algeria ($n = 2$ each) and one study each in Kenya, Sudan, Ghana, Egypt and the Democratic Republic of Congo. Overall, the greatest number of resistance genes were identified in isolates from water samples, however, this comes with a caveat that more studies focussed on water sample analysis ($n = 39$) alone or in combination with fresh produce and/or soil (Figure 3). In South Africa, Nigeria, Algeria and Tunisia, the bla_{CTX-M} ESBL resistance gene was identified in isolates from water, fresh produce and/or soil. Furthermore, the bla_{CTX-M} resistance gene was the only genetic determinant identified in most of the studies that included PCR analysis, with the exception of studies in Ghana, Morocco and Egypt where the bla_{TEM} gene was predominantly identified. Additionally, beta-lactamase genes including bla_{SHV}, bla_{TEM}, bla_{OXA}, as well as AmpC resistance genetic determinants (bla_{FOX}, bla_{MOX}, bla_{CIT}), sulfonamides and tetracyclines were identified in isolates from water, fresh produce and soil in the South African studies. Isolates from water samples in Nigeria predominantly harbored genes which contributed to resistance against tetracyclines, followed by aminoglycosides (aminoglycoside kinase, *aph*). In water sample isolates from both South Africa and Tunisia, the bla_{VIM} and bla_{IMP} carbapenem resistance genes were identified. Additionally, the bla_{KPC} and bla_{NDM} carbapenem resistance genes were identified in isolates from water samples, while the bla_{GES} carbapenem resistance gene was present in isolates from water and fresh produce, and the *mcr* gene was detected from isolates in soil samples, all in studies conducted in South Africa. The NDM carbapenem resistance gene was also identified in isolates from soil samples in Nigeria and water samples from Egypt.

3.4. Further analysis of selected potential human pathogens from the water-soil-fresh produce environment

3.4.1. *Escherichia coli*

In studies from all nine countries where *E. coli* was isolated, water samples predominantly included river water used for fresh produce irrigation in urban areas. River water was reported to be impacted by anthropogenic activities (agricultural, industrial and/or domestic) in all the included studies that investigated the presence of multidrug resistant Enterobacterales. The fresh produce samples were purchased at open air markets or formal retailers and farm fresh produce and soil samples included soil from the field where fresh produce was harvested. At least nine classes of antibiotics were included for phenotypic antimicrobial resistance screening in most of the studies that focussed on characterization of *E. coli*. The dominant resistance patterns of the *E. coli* isolates included resistance to antibiotics within the tetracycline, penicillin and sulfonamide antibiotic classes, followed by aminoglycosides and quinolones. For the studies where calculations were possible, isolated *E. coli* had multiple antimicrobial resistance (MAR) indexes of ≥ 0.2, except for two studies in South Africa in 2014 and 2016 (Supplementary Table 2). These studies were conducted in Tunisia, Nigeria, Algeria, Morocco, Sudan, Ghana or South Africa, with significant differences in the MAR indexes between certain countries ($p = 0.006$) (Figure 4). With a one-way ANOVA, sufficient evidence was given that the MAR indexes of characterized *E. coli* per publication year did not differ significantly ($p = 0.18$). Furthermore, no significant differences were seen in the overall MAR indexes of the characterized *E. coli* from different sources being either water ($p = 0.215$), fresh produce ($p = 0.435$) or soil ($p = 0.471$) samples throughout the study period. Overall, 17 studies that included PCR analysis (either presence/absence detection or further sequencing), screened for resistance genes in *E. coli* isolated from water, soil or fresh produce samples. The greatest diversity of β-lactamase genes was found in *E. coli* isolated from samples analyzed in South Africa (Figure 5). In isolates from Tunisia, South Africa, Nigeria, Kenya, Algeria, Sudan, Morocco and Benin, both the bla_{TEM} and bla_{CTX-M} genetic determinants were found (Figure 5). Where sequencing was done, the bla_{TEM} genetic determinants included TEM-1, TEM-2, TEM-3 and TEM-215 in *E. coli* isolates from South African studies and TEM-15 in isolates

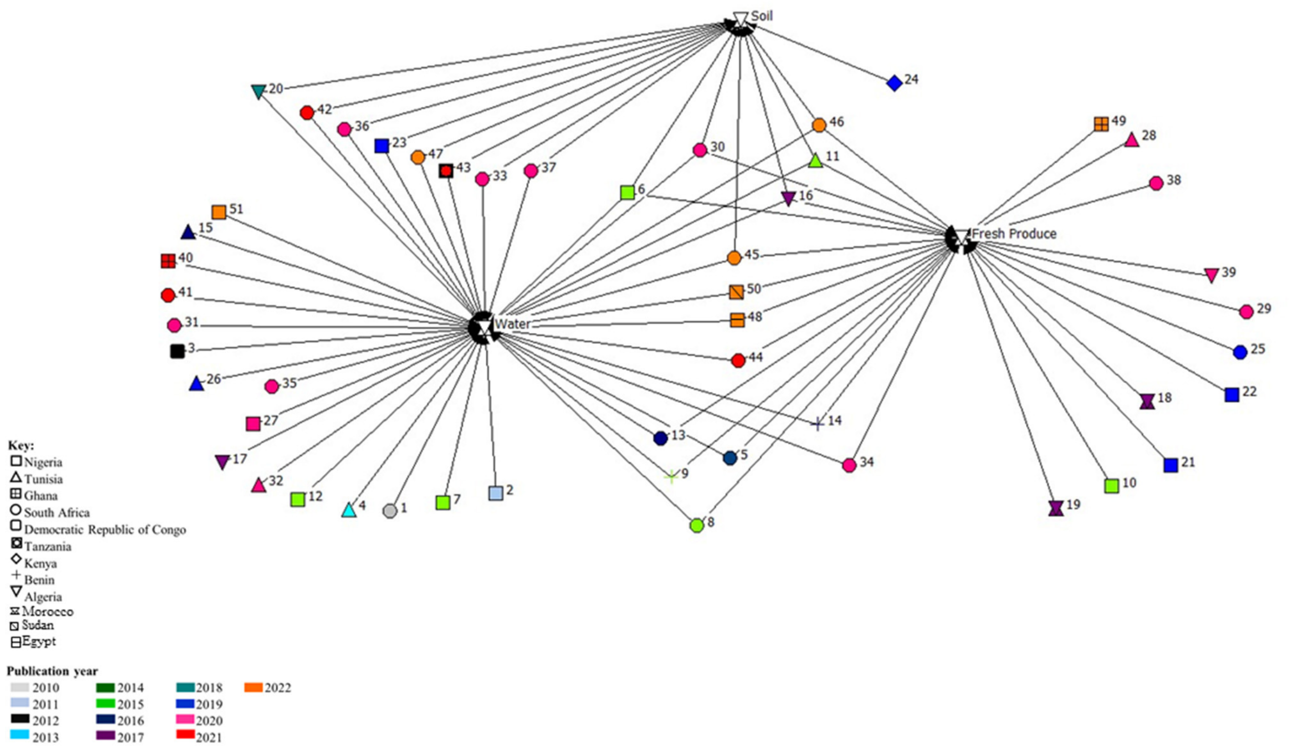

FIGURE 3
Network analysis of studies done in Africa on the occurrence and characterization of antimicrobial resistant Enterobacterales isolated from the water-soil-plant environment from 2010 to 2022. The numbers represent the articles (n = 51) included in the systematic review, while the colors represent the publication year and shapes the different countries.

from Tunisia. The $bla_{\text{CTX-M}}$ genetic determinants included CTX-M-15 (Tunisia, South Africa, Sudan, Algeria and Nigeria), CTX-M-55 (Tunisia and South Africa), CTX-M-14 (Morocco) and CTX-M-1, CTX-M-3, CTX-M-2, CTX-M-14, CTX-M-8/25, CTX-M-27, CTX-M-9 (South Africa).

3.4.2. *Klebsiella pneumoniae*

The samples where *K. pneumoniae* were isolated included mainly water, followed by fresh produce and soil. The soil sampled in the included studies were either close to food vending sites or where fresh produce were harvested in the field. The articles that focussed on characterization of *K. pneumoniae* only and included phenotypic characterization (Mouss et al., 2016; Zekar et al., 2020) tested seven classes of antibiotics for phenotypic resistance screening. This included penicillins, cephalosporins, carbapenems, aminoglycosides, quinolones, fluoroquinolones and sulfonamides. Similarly, where several members of the Enterobacterales family (including *K. pneumoniae*) were characterized, antibiotics which are usually used to treat infections by these pathogens, were included. Resistance to penicillin such as amoxycillin/clavulanic acid (Augmentin) were dominant, followed by resistance to aminoglycosides (including tobramycin) and cephalosporins such as ceftriaxone and cefotaxime. Overall, the MAR indexes ranged between 0.12 and 0.83 (Supplementary Table 2), which is an indicator that the *K. pneumoniae* isolates were from potentially high-risk environments where antibiotics are indiscriminately used (Krumperman, 1983; Fadare et al., 2020; Veloo et al., 2022). Significant differences were observed in the MAR indexes of the characterized *K. pneumoniae* per country (p = 0.04) as well as publication year (p = 0.07). However, the MAR indexes of the characterized *K. pneumoniae* from the different isolation sources i.e., water (p = 0.84), fresh produce (p = 0.63) and soil (p = 0.84) were not significantly different. In all nine countries where PCR analysis were included, *K. pneumoniae* strains harboring CTX-M genetic determinants were found (Figure 5). Where sequencing was done, the CTX-M variants included CTX-M-1 in isolates from the Democratic Republic of the Congo and CTX-M-15 in *K. pneumoniae* isolates from Tunisia, Nigeria and South Africa. Carbapenem resistance genes identified in *K. pneumoniae* included VIM and IMP variants in isolates from Tunisia, GES and OXA-48 genetic determinants in *K. pneumoniae* from South Africa and Algeria and the NDM-1 carbapenem resistance determinant in *K. pneumoniae* isolated from samples in Nigeria and Egypt.

3.4.3. *Salmonella* spp.

The studies where *Salmonella* spp. were detected were all conducted in Nigeria or South Africa. From the studies in Nigeria, samples that tested positive for *Salmonella* spp. predominantly included irrigation water, followed by fresh produce and soil. The *Salmonella* spp. positive samples from studies conducted in South Africa predominantly water samples, followed by soil samples from the fields in fresh produce production in selected studies. All samples came from urban areas where the river water used for irrigation. The three *Salmonella* spp. focussed articles

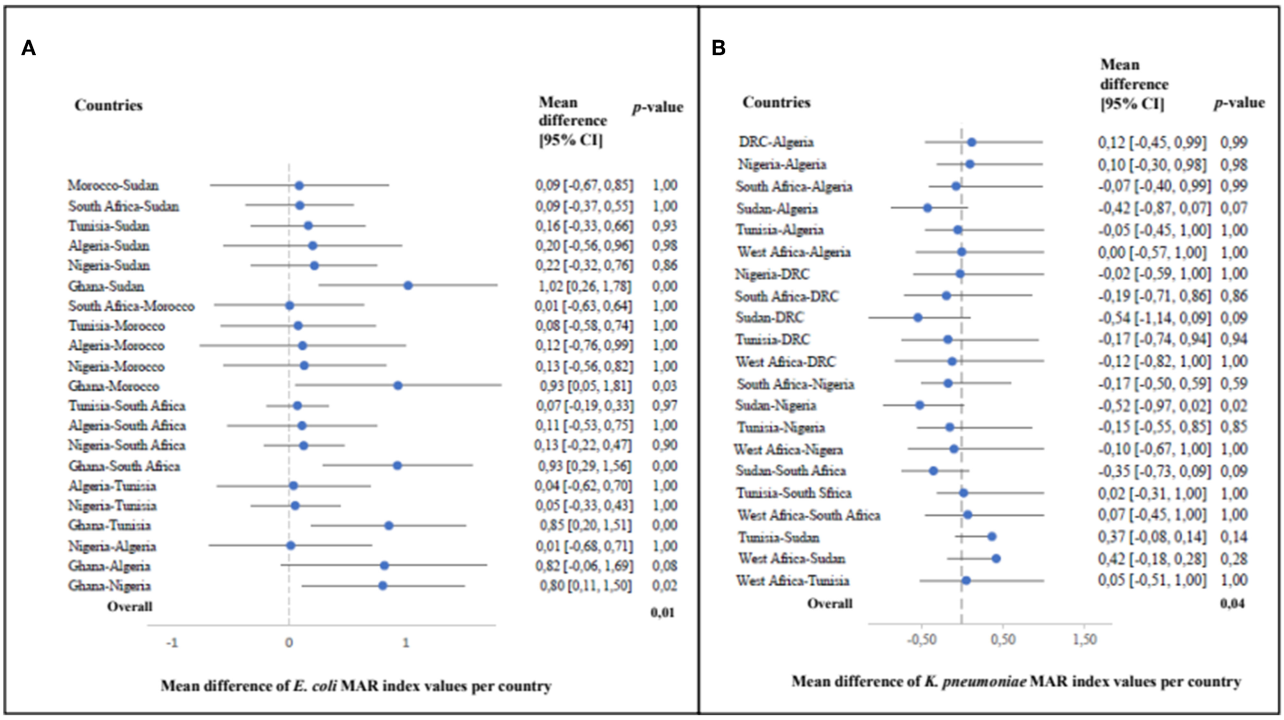

FIGURE 4
The mean differences in multiple antimicrobial resistance (MAR) index values of *Escherichia coli* **(A)** and *Klebsiella pneumoniae* **(B)** between different countries with corresponding 95% confidence intervals in the individual studies (*E. coli*: $p = 0.01$ and *K. pneumoniae*: $p = 0.04$) systematically reviewed across African countries (2010–2022).

were conducted in Nigeria and South Africa between 2011 and 2015 (Akinyemi et al., 2011; Abakpa et al., 2015; Raseala et al., 2020). Different antibiotics were tested in the respective studies, however, dominant resistance patterns in all three studies included resistance against antibiotics within the penicillin class (ampicillin or augmentin), sulfonomides, tetracyline and/or aminoglycosides. Similarly, multiple resistance to antibiotics from at least three different classes were seen in the isolates from the four studies that focussed on isolation of Enterobacterales and detected *Salmonella* spp. (Odigie et al., 2013; Richter et al., 2019; Iwu et al., 2020; Akinola et al., 2022). The MAR indexes were ≥ 0.2 in six of the studies (Supplementary Table 2), with no significant difference in MAR index values of characterized *Salmonella* spp. per country ($p = 0.51$) or publication year ($p = 0.92$). The MAR indexes of the characterized *Salmonella* spp. from water, fresh produce and soil were not significantly different ($p = 0.64$, $p = 0.62$, $p = 0.87$, respectively). Only three studies included PCR analysis of resistance genes in isolated *Salmonella* spp. (Figure 4). In these studies (Nigeria and South Africa), the β-lactamase genetic determinants included bla_{TEM} and bla_{CTX-M}, and bla_{OXA} while *Salmonella* spp. isolated from samples in Nigeria additionally harbored VIM, IMP and KPC genetic determinants.

4. Discussion

The role of the environment in dissemination of AMR is increasingly being reported (Koutsoumanis et al., 2021). This meta-analysis summarized the published data available on the occurrence of clinically significant Enterobacterales harboring antibiotic resistance genes, including genes expressing broad-spectrum β-lactamases, ESBLs and/or AmpC β-lactamases, isolated from the water-plant-food nexus in Africa. Environmental antibiotic resistant bacteria are typically classified as (i) carriers, that have a role in dissemination of resistance genes but cannot colonize the human body, and (ii) vectors, comprising of ARB that can colonize and invade the human body (Manaia, 2017). Globally, AMR surveillance programmes recognize ESBL- and carbapenemase-producing Enterobacterales as vectors of great importance in AMR gene dissemination (WHO, 2014, 2020). Based on eligibility criteria within the systematic approach used, 51 studies were identified for the current meta-analysis.

It is well known that the environment contains a natural antimicrobial resistance gene pool as well as resistance genes resulting from anthropogenic activities (Manaia, 2017). Previous research have further demonstrated that water is a unifying transmission pathway for dissemination of AMR throughout different environments (Liguori et al., 2022). Moreover, irrigation water is regarded as one of the main routes of transmission of human pathogenic bacteria onto fresh produce. At least 13 of the studies in the current analysis, from six different countries, all demonstrated that multidrug resistant potential pathogens such as *E. coli*, *K. pneumoniae*, *Salmonella* spp., *Enterobacter* spp., *Serratia fonticola* and *Citrobacter freundii* were present in fresh produce and associated irrigation water (Supplementary Table 1), which corresponds to global studies linking irrigation water to fresh produce contamination (Blaak et al., 2014; Ye et al., 2017; Vital et al., 2018; Banach and Van Der Fels-Klerx, 2020).

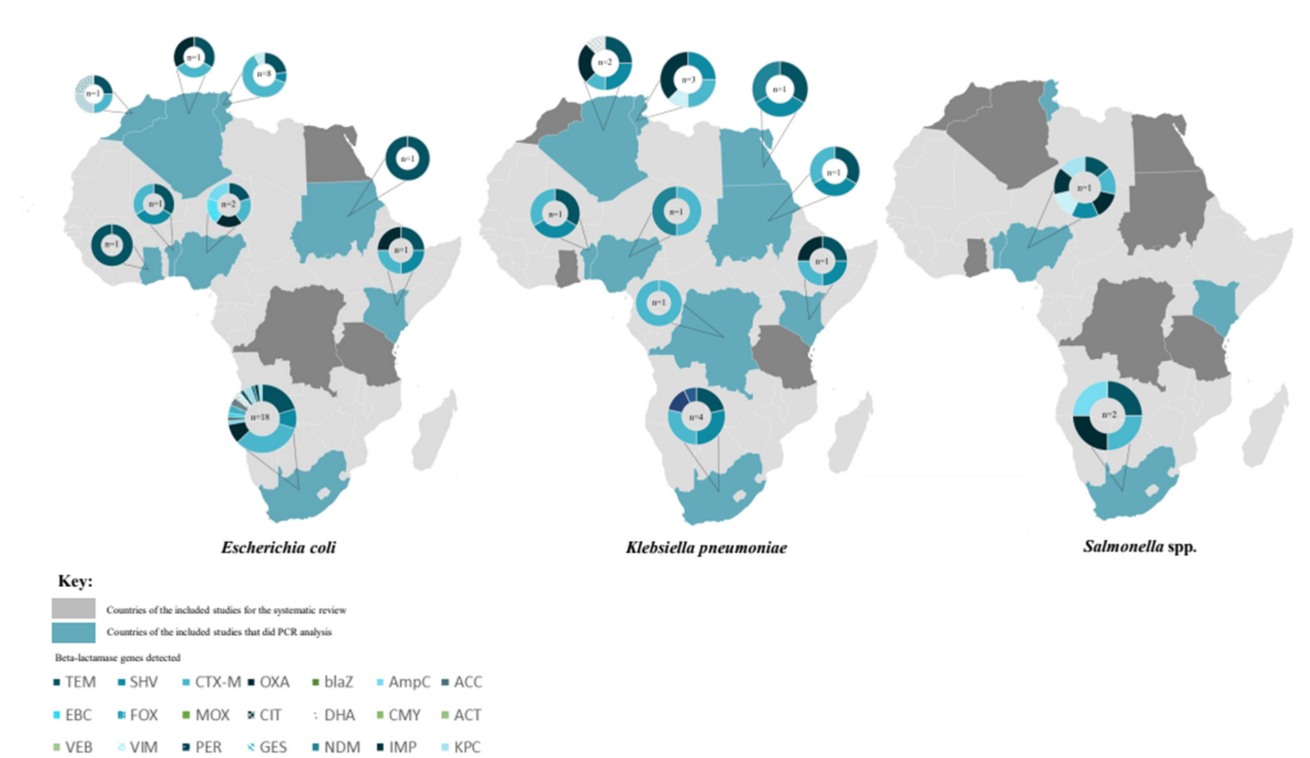

FIGURE 5
Beta-lactamase genes detected in *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* spp. isolated from water, soil and/or fresh produce samples in different African countries between 2010 and 2022. The numbers in the middle of each circle indicates the number of studies where the genes were detected. In total, 21 different β-lactamase genes were detected across the different countries, indicated with different colors or patterns on the same circle.

The current review also elucidated that water was mainly investigated in environmental AMR studies in Africa between 2010 and 2022. These studies had heterogeneous study locations and methods used, with *E. coli* predominantly isolated and characterized. The WHO recently published an integrated global surveillance protocol on ESBL-producing *E. coli* as an indicator (WHO, 2021). The procedures described were specifically designed to be conducted in a harmonized manner to provide the opportunity to increase capacities and to build national integrated surveillance systems for AMR within a One Health approach (WHO, 2021). The environmental aspect of this protocol proposes to detect and quantify ESBL-producing *E. coli* in contamination hotspot sources including surface water such as rivers that receive wastewater (WHO, 2021). However, the current metadata analysis showed that the methodology, including the frequency and number of samples analyzed, isolation, identification and characterization methods used (even within countries) differed, which contrasts the WHO proposed protocol to conduct research in a harmonized manner.

The results from the current review showed that for studies in South Africa and Algeria, MALDI-ToF was predominantly used for identification of the potential pathogens, while countries including Nigeria, Tunisia, Ghana, Egypt, Morocco and Kenya among others, predominantly used biochemical tests such as API strips for isolate identification. Recently, the reproducibility and accuracy of MALDI-ToF mass spectrometry was evaluated through comprehensive comparison studies in the clinical field (Hou et al., 2019). The authors concluded that the high-throughput, cost effective method with high accuracy resulted in MALDI-ToF mass spectrometry superseding previous conventional molecular or biochemical identification systems (Hou et al., 2019). Moreover, globally, MALDI-ToF is increasingly being used in food safety following approval by the U.S. Food and Drug Administration (Cheng et al., 2016). This follows as fast and consistent detection of foodborne pathogens in a cost-effective manner is vital in food safety analyses where time sensitivity represents an important factor (Cheng et al., 2016; Elbehiry et al., 2017). In many LMICs however, researchers still rely on biochemical tests for isolate identification due to infrastructural or funding constraints. This was evident in the current review where selected studies in Nigeria, South Africa and Tunisia as well as the studies from Benin, Ghana, Egypt, Morocco, Kenya and Tanzania all utilized biochemical tests, predominantly the API20E panels, which are reported to give efficient economical identification (Popov et al., 2022). From the current study, ESBL-producing Enterobacterales predominantly included *E. coli* and *K. pneumoniae* isolates. Of note was that 39.22% of the studies focussed on isolation and characterization of *E. coli* only, therefore, firm conclusions on the prevalence of ESBL-producers in the water-plant-food environment from these African countries cannot be drawn from this data alone. The WHO has reported that *E. coli* and *K. pneumoniae*, amongst other third-generation cephalosporin-resistant Enterobacterales, are categorized as a critical priority for global antimicrobial resistance research and development (WHO, 2017a).

Across all countries from the current meta-analysis, the Kirby-Bauer disk diffusion method was mainly used for phenotypic AMR analysis, with the CLSI criteria predominantly followed. However, the antibiotics included in analysis differed across studies and countries, therefore, no conclusion regarding phenotypic resistance patterns of potential pathogens isolated from the different matrices could be reported. Of note is that multidrug resistance (MDR), which is defined as resistance to more than one antibiotic class (Magiorakos et al., 2012), was reported in the majority of the studies. In 2014, the WHO released the first report on AMR surveillance and highlighted the need for an improved and coordinated global effort, while the FAO reported in 2018 on the need for increased environmental antimicrobial resistance surveillance (WHO, 2014; FAO, 2018). Phenotypic resistance to antimicrobials considered as critically important in clinical treatment were detected in the isolates from the water-plant-food nexus in the current meta-analysis. As an example, *E. coli* isolated from fresh produce and water across all countries showed resistance against β-lactams including penicillins like amoxicillin/clavulanic acid, cephalosporins, carbapenems and aminoglycosides including gentamycin, among others. Furthermore, both Abakpa et al. (2015) and Richter et al. (2020) reported on the occurrence of MDR *Salmonella* spp. isolated from irrigation water and fresh produce. Antibiotics from classes including β-lactams (amoxicillin/clavulanic acid, cefoxitin, imipenem), cephalosporins (cefepime), tetracylines, and aminoglycosides (gentamycin) amongst other, formed part of the antibiogram profiles in Nigeria and South Africa, respectively. Previous studies have reported that penicillins (specifically amoxicillin/clavulanic acid) are the most commonly prescribed antibiotics in hospital settings in Nigeria as well as South Africa (Okoro et al., 2019; Alabi and Essack, 2022). Tetracycline resistance was also evident in a high number of environmental bacterial isolates from the current review. The use of antibiotics in food animals and overuse in clinical settings have been linked to transmission and rise in environmental multidrug resistant bacteria (Jones-Dias et al., 2016). Along with spread of multidrug-resistance bacteria across all One Health domains, outbreaks of ESBL- and carbapenemase-producing bacteria present a serious challenge to clinicians, with the increasing occurrence in environments posing a public health concern.

The $bla_{\text{CTX-M}}$ ESBL resistance gene was identified in all the studies that included PCR analysis in the current review. Similarly, Muthupandian et al. (2018) reported that class A and class D ESBLs are common in bacteria from clinical settings in Africa, with the CTX-M-15 gene being the most prevalent. Screening of resistance to carbapenem antibiotics were included in 25 (59.5%) of the studies from seven different countries in the current review. Imipenem, conferring resistance to Ambler Class B carbapenem-resistant metallo-β-lactamases (Sawa et al., 2020) was the dominant antibiotic included in phenotypic screening, followed by meropenem, which confers resistance to Ambler Class A carbapenemases (Sawa et al., 2020). Carbapenem antimicrobials are considered the last-resort antibiotics for treatment of infections caused by third- and fourth generation cephalosporin resistant and multidrug-resistant bacteria (Sheu et al., 2019). Similar to the results from the current review, Brunn et al. (2022) reported on the presence of carbapenem resistance in environmental reservoirs globally and suggested that wastewater treatment plant effluents were a primary environmental contaminating source. In the subset of studies that included genotypic analysis in the current review, only selected studies ($n = 9$) screened for presence of carbapenem resistance genes. The KPC genetic determinant was identified in isolates from water samples, while the GES carbapenem genetic determinant was detected in isolates from water and fresh produce samples.

Class A carbapenemases, which include KPC and GES, are plasmid-encoded and frequently detected in clinically significant *Klebsiella* spp. and *Pseudomonas aeruginosa* (Sawa et al., 2020). In the current review, these genes were present in *E. coli* and *K. pneumoniae* isolates in selected studies conducted in South Africa. Perovic et al. (2016) reported that the introduction of carbapenemase-producing Enterobacterales in South Africa was molecularly confirmed at the end 2011. Furthermore, following environmental introduction, that rapid gene dissemination occurs (Perovic et al., 2016). Recently, Ragheb et al. (2022) reported on the genetic environments of carbapenmases from a One Health perspective in Africa. The authors concluded that the most commonly found carbapenemases were variations of NDM, OXA-48 and VIM being reported from clinical, animal and environmental samples.

Class B carbapenemases are typically encoded on a plasmid, transposon, integron, or chromosome and include the IMP, VIM and NDM genetic determinants (Sawa et al., 2020), supporting rapid gene dissemination across the different one health domains. Interestingly, studies in South Africa from the current review only reported on presence of the VIM and IMP genetic determinants in *E. coli* isolates, while the same genetic determinants were reported in *K. pneumoniae* only, in similar studies in Tunisia. Additionally, the NDM carbapenem resistance gene was detected in soil *K. pneumoniae* isolates from Nigeria, water *E. coli* isolates from South Africa and fresh produce *K. pneumoniae* isolates from Egypt. The results from the current review reiterates that clinically significant antibiotic resistant bacteria are no longer restricted to hospital settings, supporting the WHO (2017c) findings that global research and development strategies should include antibiotics active against more common community bacteria.

Similar to Ragheb et al. (2022), the results from the current review showed that detection of ESBL- and carbapenemase-producing Enterobacterales in Africa were relatively low as only nine countries from the entire continent have done environmental AMR surveillance in the current review. Moreover, only studies from six countries have included PCR analysis of β-lactamase genes. However, it must be reiterated that the low prevalence of these multidrug-resistant critical priority pathogens does not represent low environmental occurrence, but rather limited research on the role of the environment in dissemination of AMR within Africa. In selected studies from the current review, multidrug resistant potential pathogens were detected in different samples along the fresh produce farm-to-fork continuum. The mobility of antimicrobial resistance genes in association with mobile genetic elements and the subsequent potential of these genes to move across species, highlights the vital importance of standardized methods, achievable for LMICs as well as, to establish internationally comparable baseline environmental occurrence

data (Ikhimiukor et al., 2022; Liguori et al., 2022). From a food safety perspective, knowledge of AMR dissemination along the food supply chain, especially in food crops commonly consumed raw, is critically important for risk communication, intervention and public health (WHO, 2017b).

The inclusion of WGS has been reported as a promising tool for estimation of ARB in a one health context (Aslam et al., 2021). In the current review, only four studies included WGS analysis (Adelowo et al., 2020; Le Terrier et al., 2020; Zekar et al., 2020; Altayb et al., 2022), conducted in Nigeria, Algeria and Sudan, respectively. Additionally, a follow-up WGS analysis study for further characterization of isolates previously published was found in South Africa (Richter et al., 2021), however, this study was not included in the current meta-analysis, to avoid duplication of isolate occurrence information. Whole genome sequencing has successfully been incorporated in integrated food safety surveillance programs in high-income countries (Brown et al., 2019). Across borders, platforms such as GenomeTrakr and PulseNet International are used to share outbreak investigation data, however, there is still a long way to go in the ability to attribute sporadic illness to specific food categories (Brown et al., 2019). As sequencing costs reduce and methods for designing and interpreting metagenomic studies improve, the use of WGS in surveillance studies, especially in infrastructure and capacity-limited LMICs, are increasingly becoming a possibility (Ikhimiukor et al., 2022). The globalization of food supply chains necessitates an integrated surveillance system and platform to share molecular data for food safety purposes. The addition of WGS analysis to phenotypic AMR surveillance studies can aid in understanding the genetic basis of AMR mechanisms and distinguish isolates with phenotypically identical antibiograms, in addition to virulence genes and single-nucleotide polymorphism (SNP) analysis typically used in food safety surveillance and foodborne outbreak investigations (Brown et al., 2019; WHO, 2020). Subsequently, valuable information on the pathways of AMR dissemination across all sectors can be obtained.

5. Conclusion

The available data on occurrence of multidrug-resistant Enterobacterales in environmental settings in Africa emphasizes the need for improved surveillance and documentation of resistance gene dissemination across the farm-to-fork continuum globally. This follows as clinically significant bacterial isolates were found in various water sources and fresh produce. Furthermore, these human pathogenic microbes harbored resistance traits that corresponded to antibiotics often used in clinical settings as well as animal husbandry. The information obtained from the current review could however not be used to determine the extent of the human health risk in consumption of fresh produce where ESBL-producing potential pathogens were present. In addition to a need for harmonized methodology, the cost-effectiveness of One Health AMR surveillance systems, especially in LMICs, should also be considered and further investigated to inform the development and effective and efficient systems in Africa. The results further posed a challenge in comparative studies, as standardized methods were not utilized across the board. Although limited environmental AMR surveillance studies were found in comparison to published data on AMR surveillance in human and animal health, this review showed the vital importance of including information from the water-plant-food nexus in food safety surveillance programs related to AMR in a One Health context. It was further highlighted that comparable indicators to monitor AMR in food crop value chains is necessary. Establishing WGS as a surveillance tool in addition to phenotypic data in AMR surveillance studies will provide comprehensive information to inform comparable national and international actions plans against AMR.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LR, ED, SD, and LK contributed to the conceptualization of the study. LR and ED performed the data extraction. LR summarized the data, prepared all figures, and performed the statistical analysis. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1106082/full#supplementary-material

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Diarrhea illness in livestock keeping households in Cambodia: An analysis using a One Health framework

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Background: Most of human diarrheal pathogens are zoonotic, and transmission of the pathogens can occur by contaminated food, water, environment and direct contact with animals especially for livestock keepers. Yet little is known of the relative importance of different risk factors especially in under-studied countries. The objectives of this study were to identify risk factors for diarrhea in livestock keepers in Cambodia and detect diarrhea-causing pathogenic bacteria in both humans and livestock within a One Health approach. Of special interest were the links between diarrhea and food consumption and livestock-keeping.

Materials and methods: We used an existing dataset from a questionnaire survey conducted in 400 livestock farms in Prey Veng and Kampot Prefectures between February and March 2013 as well as laboratory results on bacterial isolation from fecal and swab samples from livestock and poultry, and human stool samples. Laboratory results were available for up to three animals of each species kept by a household, and for up to three human samples from households reporting at least one case of human diarrhea in the previous 2 weeks. Presence of *Escherichia coli*, *Shigella* spp. and *Salmonella* spp. was investigated in both animal and human samples, in addition to *Aeromonas* spp., *Vibrio* spp. and *Plesiomonas* spp. in animal samples and *Campylobacter* spp. in human samples. Univariable and multivariable risk factor analyses were performed by generalized linear mixed model.

Results: Household-level diarrhea incidence rate was 9.0% (36/400). The most statistically significant factor associated with diarrhea in multivariable analysis was water treatment for drinking and cooking (OR = 0.33, 95%CI: 0.16–0.69, p = 0.003), followed by number of days consuming egg within 2 weeks (OR = 1.16, 95%CI: 1.04–1.29, p = 0.008), number of children under 5 years old (OR = 1.99, 95%CI: 1.14–3.49, p = 0.016) and keeping poultry (OR = 0.36, 95%CI: 0.14–0.92, p = 0.033). Animal samples for bacterial culture test were collected at 279 cattle, 165 pig and 327 poultry farms, and bacteria were detected from 6 farms with the isolation of *Escherichia coli* O157 (non H7) from 1 cattle and 1 pig sample, *Aeromonas caviae* from 1 pig sample and *Salmonella* spp. from 3 chicken samples. In human samples, 17 out of 67 individual samples were positive for the culture test, detecting *Escherichia coli* O157 (non H7) from 7 samples and *Shigella* spp. from 10 samples. None of the households where target bacteria

were detected from animal samples had human samples collected due to lack of diarrhea episodes in the household.

Conclusions: It has often been hypothesized that keeping livestock may increase the incidence of diarrhea through multiple pathways. Contrary to this, we found livestock-keeping was not associated with increased risk, but food-related behavior and children under 5 years of age were strongly associated with increased risk. We discuss mediating and confounding factors and make recommendations for reducing the burden of diarrheal disease in Cambodia and more widely in low- and middle-income countries.

KEYWORDS

diarrhea, animal husbandry, risk factor, One Health, Cambodia

1. Introduction

Diarrhea is one of the most common symptoms of foodborne illness (Kosek et al., 2003). It is responsible for about 3.6% of the total disability-adjusted life year (DALY) global burden of disease, and causes ∼1.45 million deaths annually worldwide (Lozano et al., 2012; Murray et al., 2012). Most deaths are among children under the age of 5 years, with diarrhea being the second largest cause of mortality in this age group globally. Children's death by diarrhea is often associated with underlying malnutrition, which makes them more vulnerable to diarrhea and many infectious diseases (Pelletier et al., 1993; Schroeder and Brown, 1994).

In 2005 in Cambodia, diarrhea was responsible for 17% of deaths among children under the age of 5 (Borapich and Warsh, 2010). Over the past decades, Cambodia has made significant improvements and has been able to reduce by 80% the diarrhea-related DALYs from 1990 to 2010. This decreasing trend still continues, according to data available until 2019 (Institute for Health Metrics Evaluation, 2022). Despite these improvements, diarrhea is still a significant problem responsible for 6% of all deaths in children under 5 years of age in 2015 in Cambodia (Cambodia Health Data, 2015), and its impact is considerable especially in rural areas due to the poor resources of medical facilities. Diarrhea is a preventable but widespread condition in the country, and understanding its drivers and causes remains essential for its control.

Diarrheal diseases are caused by ingestion of bacterial, viral or parasitic pathogens mainly through contaminated food or water. Environmental contamination from human feces is also considered important in the epidemiology of human diarrhea (Laborde et al., 1993). In addition, two-thirds of emerging and re-emerging diseases are considered to be zoonotic and contact between animals and humans increases the risk of the transmission of diseases (Jones et al., 2008; Christou, 2011; Coker et al., 2011). In this regard, animals can also be causes of human diarrheal cases through contact with humans and environmental contamination from animal feces. For example, enteric pathogens including *Escherichia coli* O157:H7, *Campylobacter* spp., *Giardia* spp., *Salmonella* spp. and *Cryptosporidium* spp. are found in animals and are known for their zoonotic transmission (Feachem et al., 1983; Crawford and Vermund, 1988).

Livestock and poultry raising in close distance to the human living environment is common in many parts of the world, especially in low- and middle-income countries (LMICs), where animal husbandry is closely linked with human lifestyle and is a primary source of income and nutritious foods such as milk and meat (Sansoucy et al., 1995). Household-livestock keeping increases the opportunity of direct contact of humans with animals and the risk of fecal contamination within the household living environment, indicating potentially high transmission risk of zoonoses. However, solid evidence on the links between livestock keeping and contact with animals and its role in diarrhea is still limited (Coker et al., 2011).

The objective of this study was to identify risk factors for diarrhea among livestock-keeping households in Cambodia and to investigate the presence of foodborne pathogens concurrently in animals and humans living in close contact. The results will shed light on the intrahousehold transmission of zoonotic pathogens and household and farming practices that may be associated with diarrhea in humans.

2. Materials and methods

2.1. Study site selection and sampling of households

We used an existing dataset from a cross-sectional survey conducted among 400 livestock farms in four Districts in Prey Veng and Kampot Provinces between February and March 2013, as part of the *Ecosystem approaches to the better management of zoonotic emerging infectious diseases in the Southeast Asia region* (EcoZD) project (https://www.ilri.org/ecozd). The study that generated the data worked in two provinces that had contrasting agro-ecologies: Prey Veng Province is located in lower Mekong flood plain, which is a main agricultural production area in Cambodia; Kampot Province is located in the coastal area where rising sea levels and increased salinization are having a great impact in farming and fishery industries and in the availability of safe drinking water (Figure 1). Within each province, 2 districts were selected for the study to capture areas with contrasting diarrhea incidence. To do that, the study used 2010 bloody diarrhea incidence estimates to identify the districts with the highest and lowest

bloody diarrhea incidence within each province; Preah Sdach (high: 3,492/100,000 people) and Prey Veng (low: 291/100,000 people) Districts were selected from Prey Veng Province, and Kampot (high: 1,386/100,000 people) and Angkor Chey (low: 974/100,000 people) Districts from Kampot Province (National Institute of Statistics, Directorate General for Health, and ICF Macro, 2011). Using the sampling frame of all the villages in each district, two villages were randomly selected from each district, and 50 livestock-keeping households were randomly selected from each village based on a sampling frame built with the help of the community. Thus, 100 households from each district were included in the study for a total of 400 households. Data were obtained *via* a structured questionnaire designed to collect information on diarrhea episodes in the household in the 2 weeks prior to the visit, as well as household and farming factors potentially associated with diarrhea, including household characteristics, animals kept, food consumption practices and water sources. An episode of diarrhea was defined as having soft stools at least three times within 24 h in the 2 weeks prior to the visit, and a household with at least one household member having experienced at least one episode of diarrhea was considered a diarrhea-positive household. The questionnaire was designed in English and translated into Khmer, the national language of Cambodia.

Data were also obtained from laboratory results on bacterial isolation from fecal and swab samples collected from livestock and poultry, as well as human stool samples from consenting households where at least one person had diarrhea in the previous 2 weeks. One to three individual animals of each species kept by the household were sampled; when more than one animal was sampled of a given species, the samples were pooled into a single tube. All animal species kept in the household were tried being sampled. In consenting diarrhea-positive households (i.e., where at least one person reported having experienced diarrhea in the 2 weeks prior to the visit), human samples were collected from one and up to three household members, with particular interest in those who prepared food, children and those having contact with animals. All samples were collected and placed in Cary-Blair transport medium and kept in a cool box until sent to the National Institute of Public Health (NIPH), in Phnom Penh, Cambodia. The samples were stored at −20°C at NIPH.

Samples were tested following published protocols for isolation and identification of the target pathogens (Ernest Jawetz et al., 1989; Henry and Todd, 1991; Centers for Disease Control and Prevention, 1999; Forbes et al., 2002; Lynne and Henry, 2007). Human samples were tested at NIPH for presence of *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. Briefly, to detect *Escherichia coli*, *Salmonella* and *Shigella*, the samples were directly inoculated in MacConkey Agar (MAC) and Salmonella Shigella Agar (SSA) and then incubated at 37°C for 18–24 h. Moreover, to increase the sensitivity of the culture test, enrichment of bacteria included in the samples was done using Selenite broth with incubation of 37°C for 18–24 h. After the incubation, the Selenite broth was subjected to SSA and then the SSA was incubated. Screening and identification were performed based on the morphology and biochemistry characteristics using Triple Sugar Iron (TSI) tube agar, Sulfide Indol Motility (SIM) tube agar, Simmons citrate agar, Analytical Profile Index 20E kit (API20E, bioMérieux) and oxidase test. *Escherichia coli* isolates were further tested for serotype O157:H7 by agglutination test. To detect *Campylobacter* spp., the sample was inoculated in *Campylobacter* agar with Blood and Blaser formula supplemented and incubated in microaerophilic condition at 42°C for 48 h. Colony identification was done using Gram stain, Oxidase and Catalase tests.

Animal samples were sent to Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand and tested for the presence of *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Plesiomonas* spp., *Vibrio* spp. and *Aeromonas* spp. The samples from animals were cultured using MAC, SSA and Thiosulfate Citrate Bile-Salts Sucrose agar (TCBS) with the incubation of 37°C for 18–24 h. In addition, for enrichment of bacteria, the samples were also placed into Alkaline Peptone Water (APW) tubes and the tubes were incubated at 37°C for 6–8 h. Then the APW were inoculated to TCBS and the TCBS were incubated as described above. Colony identification was performed using Kligler Iron Agar, Manitol Mobility agar, Urea Indole broth, API20E, Oxidase and Catalase tests.

2.2. Data analysis

Data from the questionnaires were obtained electronically in Microsoft Excel files and data cleaning was performed. Statistical analyses were performed using statistic software R version 3.5.0 (R Core Team, 2018). For comparisons of the occurrence of diarrhea in the households between provinces and between districts, Pearson's Chi-squared test with Yates' continuity correction was used. Univariable risk factor analysis was performed by generalized linear mixed model (GLMM) with binomial errors using lme4 package (Bates et al., 2011) to identify household-level diarrhea risk factors. Only variables for which a plausible biological link to diarrhea were included as explanatory variables in the analysis. Univariable models included the occurrence of diarrhea in households as response variable and prefectures and districts as random effects.

Multivariable analyses were performed using GLMM. The models included variables having p values < 0.1 in the univariable analyses as explanatory variables and diarrhea occurrence as response variable. The association between the selected explanatory variables was checked using GLMM with cut-off $p < 0.05$. Backward stepwise simplification was conducted using the likelihood ratio test.

3. Results

3.1. Descriptive statistics

Table 1 shows the characteristics of the households participating in the study. Households had an average of 4–5 members in all districts. Rice farming was a major activity in most of the households across the two provinces. Livestock farming (animal husbandry) was practiced by the majority of households in Kampot Province, but was rarely practiced by households in Prey Veng Province. Crop farming was also most common in Kampot Province than in Prey Veng Province. The majority of

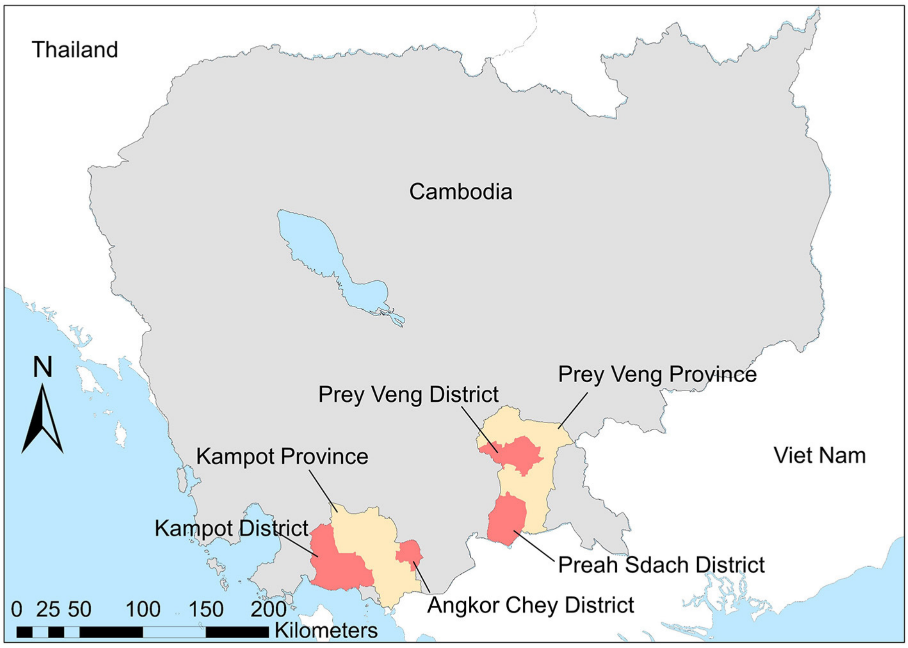

FIGURE 1
Map of the study area.

the households kept cattle and chicken, and pigs and ducks were kept by a moderate number of households. Livestock farms were smallholders, keeping on average 2–3 heads of large livestock, 2–6 pigs and from 15 to 30 chickens.

3.2. Diarrhea prevalence

The number of households reporting at least one member experiencing diarrhea over 2 weeks prior to the visit was 36, for a household-level diarrhea prevalence of 9.0% (95% CI: 6.5–12.3). The diarrhea prevalence in Prey Veng Province was 16.0% (95%CI: 9.7–25.0) and 6.0% (95%CI: 2.5–13.1) in Preah Sdach and Prey Veng Districts, respectively. In Kampot Province, the prevalence was 5.0% (95%CI: 1.9–11.8) and 9.0% (95%CI: 4.5–16.8) in Kampot and Angkor Chey Districts, respectively. In the comparison of the district prevalence within the provinces, a statistically significant difference was observed between Preah Sdach and Prey Veng Districts in Prey Veng Province ($p = 0.04$), while the difference between Kampot and Angkor Chey Districts in Kampot Province was not statistically significant ($p = 0.41$). In addition, the difference of the prevalence between Prey Veng and Kampot Provinces was not statistically significant ($p = 0.22$).

3.3. Bacteria culture of human and animal samples

A total of 67 human stool samples were collected from 25 diarrhea-positive households. Pathogenic bacteria were detected in 17 human samples (25.4%); *Escherichia coli* O157 (non O157:H7) was found in 7 samples and *Shigella* spp in 10 samples (Table 2).

Animal samples were collected from 95.9% (279/291), 91.7% (165/180) and 91.3% (327/358) of households keeping cattle, pigs and poultry, respectively. *Escherichia coli* O157 (non O157:H7) was isolated from 1 cattle and 1 pig sample, *Aeromonas caviae* from 1 pig sample and *Salmonella* spp. was isolated from 3 chicken samples. All the 6 culture-positive samples belonged to different households. None of the households where animal samples were found to be carrying the target bacteria had human samples collected due to lack of diarrhea episodes in the household in the 2 weeks prior to the visit. The same pathogen was not detected simultaneously in different animal species and human samples belonging to the same household. Among households reporting diarrhea in the previous 2 weeks, only one had an animal positive for any of the target bacteria; in particular one poultry sample carrying *Salmonella* spp.

3.4. Risk factors for diarrhea

Tables 3, 4 show the results of univariable and multivariable analysis for diarrhea. At univariable analysis, water treatment for drinking and cooking appeared as a potential preventive factor, and family size, number of children under 5 years old, not having a toilet facility, and number of days consuming eggs within the previous 2 weeks appeared as risk factors for diarrhea. In multivariable analysis, the final model included four factors (Table 4). In line with the results of univariable analysis, water treatment for drinking and cooking appeared as a protective factor for diarrhea in the household, while frequency of egg consumption and children under

TABLE 1 Household characteristics ($N = 100$ per district).

	Prey Veng Province		Kampot Province	
	Preah Sdach District	Prey Veng District	Kampot District	Angkor Chey District
	(High incidence)*	(Low incidence)*	(High incidence)*	(Low incidence)*
	n (%)	*n* (%)	*n* (%)	*n* (%)
Remunerative activities of household members				
Rice farming	96 (96%)	96 (96%)	68 (68%)	98 (98%)
Crop farming	2 (2%)	0 (0%)	10 (10%)	41 (41%)
Animal husbandry	2 (2%)	17 (17%)	97 (97%)	96 (96%)
Trading animals	0 (0%)	1 (1%)	4 (4%)	2 (2%)
Trading fresh animal meat	1 (1%)	0 (0%)	3 (3%)	0 (0%)
Slaughtering livestock	0 (0%)	0 (0%)	1 (1%)	2 (2%)
Livestock kept				
Cattle	69 (69%)	69 (69%)	59 (59%)	94 (94%)
Buffalo	5 (5%)	7 (7%)	0 (0%)	0 (0%)
Pig	71 (71%)	18 (18%)	58 (58%)	33 (33%)
Chicken	83 (83%)	79 (79%)	92 (92%)	95 (95%)
Duck	47 (47%)	27 (27%)	37 (37%)	33 (33%)
Highest level of education among household members				
No education	4 (4%)	3 (3%)	2 (2%)	1 (1%)
Primary	56 (56%)	23 (23%)	25 (25%)	26 (26%)
Secondary	32 (32%)	48 (48%)	29 (29%)	33 (33%)
High school	8 (8%)	25 (25%)	36 (36%)	34 (34%)
College/University	0 (0%)	1 (1%)	8 (8%)	6 (6%)
	Median (range)	Median (range)	Median (range)	Median (range)
Family size	4 (2–7)	4 (2–8)	5 (2–8)	4 (2–10)
Children under 5 years old	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–1)
Number of cattle**	3 (1–8)	3 (1–10)	2 (1–5)	3 (1–8)
Number of buffalo**	2 (2–6)	4 (2–9)	–***	–***
Number of pigs**	2 (1–15)	5 (1–18)	2 (1–80)	2 (1–9)
Number of poultry**	14 (1–750)	11 (1–95)	24 (3–512)	25 (2–104)

*High and low are based on bloody diarrhea incidence in 2010.
**The estimates are calculated only for households that keep the livestock species.
***None of the households raised buffalo.

5 years old appeared as risk factors. In addition, keeping poultry showed a negative association with diarrhea.

4. Discussion

Diarrhea is a symptom of infections caused by bacterial, viral and parasitic organisms. Common causative agents of diarrhea are zoonotic, and it has often been hypothesized that keeping livestock, which is closely associated with human lifestyle, especially in LMICs, may increase the incidence of diarrhea through multiple pathways such as direct contact with them and environmental and water contamination by the animal feces with infectious organisms. Our study did not find evidence supporting this hypothesis, at least for the pathogens that our studied targeted. We did not find higher diarrhea prevalence in the districts where households were most likely to keep livestock and, accordingly, livestock keeping did not appear as a risk factor in our analysis. Moreover, poultry keeping was revealed as a protective factor for diarrhea in our study. In a review paper exploring the association between human diarrhea and domestic animal husbandry, of the 23 studies included in the systematic review, 21 indicated having found a positive association

TABLE 2 Result of bacteria culture test of livestock fecal and swab samples and human rectal or stool swab samples.

	Number of households raising livestock	Number of households from which samples were collected*	Number of positive households (%)	Number of positive samples						
				Escherichia coli O157	*Shigella* spp.	*Salmonella* spp.	*Aeromonas caviae*	*Vibrio* spp.	*Plesiomonas* spp.	*Campylo bacter* spp.
Cattle	291	279	1 (0.4%)	1	0	0	0	0	0	NT
Pig	180	165	2 (0.6%)	1	0	0	1	0	0	NT
Chicken	358	327	3 (0.9%)	0	0	3	0	0	0	NT
Human	Number of households sampled	Number of individual samples	Number of positive samples (%)							
	25	67	17 (25.4%)	7	10	0	NT	NT	NT	0

*One to three individual animals of each species kept by the household were sampled, and when more than one animal were sampled of a given species, the samples were pooled into a single tube and subjected to pooled culture test.
NT, not tested.

between livestock keeping and human diarrhea (Zambrano et al., 2014). The other two studies included in the systematic review reported negative associations between animal husbandry and diarrhea, as is the case of our study (Huttly et al., 1987; Kimani et al., 2012; Zambrano et al., 2014). Moreover, we did not find that livestock and humans were sharing any of the target pathogens in our study, which further supports the idea that, in the context we studied, human diarrhea may more likely be driven by non-animal related factors. Zoonotic transmission is influenced by many factors, including the nature and closeness of interaction between animals and humans. It is likely that livestock keeping in our study areas, while practiced at home, does not involve co-habitation and instead involves separation of living areas for animals and humans. This certainly would reduce pathogen transmission, and explain the lack of pathogen sharing between animals and humans. These findings show the importance of contextualizing the investigation of livestock keeping and the meaning it has in veterinary public health. Considering diarrhea remains a challenge worldwide and the growing interest in livestock raising as an economic activity in emerging nations, more context-specific research is needed in this field. The One Health approach (Zinsstag, 2012; Cleaveland et al., 2017) should continue to be used to look at animal-human interactions in the context of diarrhea.

While no association was found between animal husbandry and human diarrhea, we found several significant factors associated with diarrhea occurrence. Water treatment was the most significant and protective factor identified in this study. Many previous studies have reported the protective association of safe water against diarrhea, suggesting access to safe drinking water is one of the key preventive measures (Quick et al., 2002; Arnold and Colford Jr, 2007; Daud et al., 2017). In addition, a randomized controlled intervention trial of drinking water filters was performed in a rural village in Cambodia, which is a similar setting to that of the current study, showing a preventive effect of diarrheal disease (Brown et al., 2008). Thus, great effort should be put into safe water security to mitigate human diarrheal illness. As food-related factors, consuming eggs and washing root vegetables showed an association with diarrhea, although the latter was not included in the final model in multivariable analysis. *Salmonella* spp. is a well-known diarrhea causative agent, and one of the most important pathogens associated with egg consumption (Mc and Eisele, 1951; Hennessy et al., 2004; Schroeder et al., 2005). Although the number of days consuming eggs within 2 weeks was a risk factor statistically significant in our analysis, the effect size showed an odds ratio −1.14, showing a relatively minor effect. In the current study, while washing other vegetables had no association, washing root vegetables showed a slight protective association ($p = 0.065$) with diarrhea in univariable analysis. Our result may suggest that root vegetables have high risk of contamination by enteropathogenic species in soil because they grow underground, and washing may more effectively remove the pathogens of diarrheal diseases from root vegetables. Nevertheless, several studies have reported the limitations of washing fruits and vegetables for preventing food-borne diseases, which is compatible with our general finding that washing vegetables practices did not appear to be associated with diarrhea in the household (Burnett and Beuchat, 2001; Sivapalasingam et al., 2004; Lynch et al., 2009).

TABLE 3 Univariable risk factor analysis for diarrhea using GLMM.

Variable	Categories	Diarrhea		Odds ratio (95% CI)	*p*-value
		Positive	Negative		
		n (%) or median (Range)	n (%) or median (Range)		
Socio-economic characteristics					
Highest education among household members	No education and Primary	9 (8.3%)	109 (91.7%)		
	Secondary	11 (7.8%)	131 (92.2%)	0.72 (0.31–1.64)	0.431
	High school or above	16 (12.9%)	124 (87.1%)	0.76 (0.30–1.95)	0.571
Family size		5 (2–9)	4 (2–8)	1.34 (1.08–1.67)	0.008
Number of children under 5 years old	0	21 (7.0%)	278 (93.0%)	2.00 (1.18–3.40)*	0.011
	1	11 (13.1%)	73 (86.9%)		
	2	4 (23.5%)	13 (76.5%)		
Livestock keeping					
Cattle and buffalo	Yes	27 (9.0%)	272 (91.0%)	1.00 (0.44–2.24)	0.994
	No	9 (8.9%)	92 (91.1%)		
Pigs	Yes	19 (10.6%)	161 (89.4%)	1.26 (0.60–2.64)	0.539
	No	17 (7.7%)	203 (92.3%)		
Poultry	Yes	29 (8.1%)	329 (91.9%)	0.44 (0.18–1.11)	0.082
	No	7 (16.7%)	35 (83.3%)		
Sanitation					
Toilet type					
Toilet	Yes	17 (7.5%)	209 (92.5%)	0.77 (0.36–1.68)	0.517
	No	18 (10.3%)	156 (89.7%)		
Latrine	Yes	7 (7.4%)	87 (92.6%)	0.66 (0.27–1.60)	0.357
	No	29 (9.5%)	277 (90.5%)		
No toilet facility	Yes	28 (12.0%)	206 (88.0%)	2.65 (1.11–6.31)	0.028
	No	8 (4.8%)	158 (95.2%)		
Water source for drinking and cooking					
Rain	Yes	4 (7.0%)	53 (93.0%)	0.71 (0.22–2.36)	0.580
	No	32 (9.3%)	311 (90.7%)		
Pond	Yes	10 (8.5%)	108 (91.5%)	0.97 (0.36–2.62)	0.945
	No	26 (9.2%)	256 (90.8%)		
Well	Yes	23 (9.7%)	213 (90.3%)	1.06 (0.39–2.84)	0.913
	No	13 (7.9%)	151 (92.1%)		
Tap	Yes	3 (6.3%)	45 (93.7%)	0.85 (0.20–3.62)	0.828
	No	33 (9.4%)	319 (90.6%)		
Water treatment	Yes	16 (5.8%)	261 (94.2%)	0.34 (0.16–0.71)	0.005
	No	19 (15.8%)	101 (84.2%)		

(Continued)

TABLE 3 (Continued)

Variable	Categories	Diarrhea		Odds ratio (95% CI)	*p*-value
		Positive	Negative		
		n (%) or median (Range)	n (%) or median (Range)		
Food consumption					
Raw vegetables					
Aerial vegetable	Yes	9 (9.8%)	83 (90.2%)	1.13 (0.51–2.54)	0.756
	No	27 (8.8%)	281 (91.2%)		
Aquatic vegetable	Yes	2 (13.3%)	13 (86.7%)	1.50 (0.32–7.03)	0.608
	No	34 (8.8%)	351 (91.2%)		
Fruit vegetable	Yes	14 (8.2%)	156 (91.8%)	0.91 (0.45–1.87)	0.800
	No	22 (9.6%)	208 (90.4%)		
Leaf vegetable	Yes	3 (13.6%)	19 (86.4%)	1.92 (0.52–7.03)	0.324
	No	33 (8.7%)	345 (91.3%)		
Root vegetable	Yes	0 (0.0%)	6 (100.0%)	–**	0.829
	No	36 (9.1%)	358 (90.9%)		
Number of days having food within 2 weeks					
Beef		0 (0-2)	0 (0-10)	0.79 (0.44–1.44)	0.445
Pork		4 (0-12)	5 (0-14)	0.95 (0.86–1.05)	0.308
Chicken		0 (0-7)	0 (0-12)	1.17 (0.96–1.43)	0.127
Eggs		2 (0-14)	0 (0-14)	1.12 (1.01–1.24)	0.029
Fish		10 (0-14)	10 (0-14)	1.01 (0.93–1.10)	0.796
Eat sick/dead animals					
Cattle	Yes	2 (25.0%)	6 (75.0%)	2.67 (0.48–14.91)	0.264
	No	34 (8.7%)	357 (91.3%)		
Pig	Yes	6 (16.7%)	30 (83.3%)	1.83 (0.60–5.63)	0.290
	No	30 (8.3%)	333 (91.7%)		
Chicken	Yes	11 (11.7%)	83 (88.3%)	1.30 (0.59–2.89)	0.513
	No	25 (8.2%)	280 (91.8%)		
Duck	Yes	3 (10.3%)	26 (89.7%)	0.92 (0.25–3.39)	0.899
	No	33 (8.9%)	337 (91.1%)		
Food hygiene practices					
Washing of vegetables before consuming					
Aerial vegetable	Yes	36 (9.4%)	345 (90.6%)	–**	0.995
	No	0 (0.0%)	19 (100.0%)		
Aquatic vegetable	Yes	6 (10.2%)	53 (89.8%)	1.09 (0.42–2.78)	0.864
	No	30 (8.8%)	311 (91.2%)		
Fruit vegetable	Yes	32 (8.6%)	342 (91.4%)	0.43 (0.14–1.39)	0.160
	No	4 (15.4%)	22 (84.6%)		
Leaf vegetable	Yes	13 (11.8%)	97 (88.2%)	1.63 (0.78–3.41)	0.192
	No	23 (7.9%)	267 (92.1%)		
Root vegetable	Yes	1 (1.7%)	57 (98.3%)	0.15 (0.02–1.12)	0.065
	No	35 (10.2%)	307 (89.8%)		

(Continued)

TABLE 3 (Continued)

Variable	Categories	Diarrhea		Odds ratio (95% CI)	p-value
		Positive	Negative		
		n (%) or median (Range)	n (%) or median (Range)		
Situation and frequency of handwashing using water					
Before cooking	Always or often	18 (11.5%)	157 (88.5%)	1.22 (0.60–2.46)	0.579
	Never or occasionally	18 (8.7%)	207 (91.3%)		
Before eating	Always or often	19 (11.3%)	168 (88.7%)	1.36 (0.67–2.76)	0.392
	Never or occasionally	16 (8.1%)	197 (91.9%)		
After toilet	Always or often	18 (13.0%)	138 (87.0%)	1.58 (0.79–3.16)	0.195
	Never or occasionally	18 (8.0%)	226 (92.0%)		
After animal handling	Always or often	11 (8.7%)	126 (91.3%)	0.83 (0.39–1.76)	0.634
	Never or occasionally	25 (10.5%)	238 (89.5%)		
Situation and frequency of handwashing using water with soap					
Before cooking	Always or often	9 (7.1%)	126 (92.9%)	0.70 (0.31–1.56)	0.390
	Never or occasionally	27 (11.3%)	238 (88.7%)		
Before eating	Always or often	6 (5.1%)	118 (94.9%)	0.45 (0.18–1.11)	0.082
	Never or occasionally	30 (12.2%)	246 (87.8%)		
After toilet	Always or often	13 (7.0%)	187 (93.0%)	0.58 (0.27–1.24)	0.159
	Never or occasionally	23 (13.0%)	177 (87.0%)		
After animal handling	Always or often	7 (5.4%)	130 (94.6%)	0.47 (0.19–1.11)	0.087
	Never or occasionally	29 (12.4%)	234 (87.6%)		

*Explanatory variable was analyzed as continuous variable.
**Not calculated due to 0 score in the cell.

Although not included in the final model in multivariable analysis, washing hands with soap before eating and after animal handling were moderate preventive factors ($p = 0.082$ and 0.087, respectively) and no access to toilet facility was a significant risk factor in univariable analyses. These factors were hypothesized by us to be important due to the previously reported evidence (Koopman, 1978; Black et al., 1981; Pickering et al., 1986; Luby et al., 2004; Asfaha et al., 2018; Ejemot-Nwadiaro et al., 2021). According to World Health Organization, along with access to safe drinking water and food hygiene, as mentioned above, use of improved sanitation including hand washing with soap and toilet facilities are the key preventive measures (World Health Organization, 2014).

Regarding the children under 5 years old as a risk factor for diarrhea, in line with our result, several studies have reported the incidence of diarrheal diseases are greatest among children in this age group (Fischer Walker et al., 2012; Farthing et al., 2013). Since diarrhea in children causes growth faltering, malnutrition, and impaired cognitive development as well as fatal cases in resource-limited countries, great attention should be paid to them (Pelletier et al., 1993).

In order to mitigate diarrheal disease, health and food safety education plays an important role as well as the factors mentioned above (World Health Organization, 2014). In addition, not only household-based interventions but also community-based interventions to reinforce the knowledge and practices toward diarrhea and other diseases have been recognized as effective disease mitigation strategies in developing countries (Sheth and Obrah, 2004; Haroun et al., 2010; Mashoto et al., 2014; Abdel-Aziz et al., 2015).

TABLE 4 Factors included in the final model of multivariable analysis for diarrhea using GLMM.

Variable	Odds ratio (95% CI)	p-value
Water treatment for drinking and cooking	0.33 (0.16–0.69)	0.003
Number of days having egg within 2 weeks	1.16 (1.04–1.29)	0.008
Number of children under 5 years old	1.99 (1.14–3.49)	0.016
Keeping poultry	0.36 (0.14–0.92)	0.033

In addition to risk factor analysis, this study also investigated the concurrent presence of bacterial organisms in both domestic

animal and human samples in a One Health framework. While most of other studies which focused on association between animal husbandry and human diarrhea set single pathogen as a target and only human samples were utilized for culture test, the strength of the current study is that it investigated several pathogenic bacteria simultaneously (O'Brien et al., 2001; Belongia et al., 2003; Alyousefi et al., 2011; Leung et al., 2013; Zambrano et al., 2014). Only six animal samples were positive to any of our target bacteria. Among the six positives, one chicken-positive household with *Salmonella* spp. was human diarrhea positive at household level in questionnaire survey, but no human samples were available for that case. As one of the limitations of this study, not all animals kept at each household were sampled, and the samples were pooled into a single tube for every animal species within each household for culture test. While this makes laboratory analysis more efficient, it does reduce the sensitivity of the test, and it may have led to false negative results. For bacteria culture of human samples targeting *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp., 67 individual samples were collected from diarrhea positive households and culture tests were positive for 17 (25.4%) of them, detecting *Escherichia coli* O157: non-H7 and *Shigella* spp. Although the individuals sampled in a household had not necessarily experienced diarrhea in the 2 weeks prior to the visit while at least one household member had done diarrhea in the period, other pathogens such as norovirus, rotavirus, *Cryptosporidium* and *Giardia*, which were not investigated in this study, may have caused diarrhea. Although there are limited data on diarrhea etiology from developing countries, a study conducted in Cambodia reported that among the stool samples collected from 600 children with acute diarrhea (cases) and 578 children without diarrhea (controls), the most frequently isolated pathogens in these cases were enteroaggregative *Escherichia coli* (20%) and rotavirus (26%) (Meng et al., 2011). In addition, from the perspective of the season, while bacterial diarrheal diseases often occur during rainy season, the sampling was performed in dry season in the current study, which may be behind the low overall diarrhea incidence observed (Bonkoungou et al., 2013; Kraay et al., 2020). Season could have also explained the low isolate rates of some of the target bacteria, which may be less prevalent in dry season (Picard and Goullet, 1987; Shigematsu et al., 2000; Barkocy-Gallagher et al., 2003; Perencevich et al., 2008; Akil et al., 2014; Bhattacharya et al., 2014; Lee et al., 2017). The available data does not allow to further investigate if this is indeed the case. Further research is desirable to understand the diarrhea situation more generally, in particular in the context of its zoonotic transmission.

General preventive measures against diarrhea, such as good food hygiene, water hygiene, handwashing with soap and good sanitary facilities, are effective methods to contain diarrhea in the studied livestock-keeping households in Cambodia. These are universally well known and effective methods to reduce gastro-intestinal disease. Livestock keeping, while it may be related to other zoonoses not targeted in our study, does not seem to be a factor that may be behind diarrhea in the households in our study areas. Livestock keeping remains an important source of livelihoods and food in many communities, and remains a potential risk activity in terms of a number of zoonotic infections. Further studies should expand, using the One Health approach, our understanding of what health risks for humans livestock keeping may pose to the communities in our study area.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by International Livestock Research Institute. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. The animal study was reviewed and approved by International Livestock Research Institute. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SAs performed data cleaning and analysis and wrote the majority of the manuscript. BK, SS, SP, CT, CP, and TS contributed to field surveys, data collection, and entry. SB contributed to bacterial culture test. JG and DG designed and coordinated the research. SAl designed and coordinated the research, advised analysis, and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Heavy metal contamination in retailed food in Bangladesh: a dietary public health risk assessment

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Introduction: Contamination with heavy and toxic metals along the food value chain is a public health concern in Bangladesh.

Methods: In this study, 608 fish and chicken samples from traditional and modern retail outlets in urban, peri-urban, and rural areas were collected and analyzed for chromium (Cr), cadmium (Cd), and lead (Pb) contamination, using atomic absorption spectrometry method. The daily intake, target hazard quotient and the target carcinogenic risk (for lead only) as a result of fish and chicken consumption was calculated based on mean results, and by Monte Carlo simulation in @Risk with 100,000 iterations (quantitative risk assessment).

Results: Cr and Cd were detected in 80–86% of both chicken meat and fish samples, while Pb positivity found in chicken meat and fish was 54.9 and 23.3%, respectively. The mean concentration (±SD) of Cr, Cd, and Pb in chicken meat were 0.66 ± 0.93, 0.02 ± 0.03, and 0.09 ± 0.10 mg/kg, respectively; and in fish were 0.49 ± 0.62, 0.02 ± 0.03, and 0.06 ± 0.09 mg/kg, respectively. The estimated daily intakes of Cr, Cd, and Pb from chicken and fish were lower than the maximum tolerable daily intake in all studied areas. In addition, the target carcinogenic risk for Pb in chicken was lower than the negligible range, which indicated the risk of cancer due to exposure to Pb through chicken meat and fish consumption was very low.

Discussion: The present study concludes that consumption of chicken meat and fish in Bangladesh, currently at very low levels, is unlikely to constitute a major health risk for humans in respect to these metals. However, continuous market surveillance for heavy metals in food stuff is recommended, especially since consumers may increase their meat intake.

KEYWORDS

food safety, chemical hazards, quantitative risk assessment, trace metal, dietary intake, lead, cadmium, chromium

1. Introduction

Heavy and toxic metals/metalloids are ubiquitous in the environment with natural and anthropogenic sources, including agriculture, industry, mining, land fill and transportation (Cui et al., 2005; Zheng et al., 2007; Islam et al., 2014). Heavy metals enter plant or animal-source foods through contaminated soil or water, and can accumulate along the food chain, in animals, and humans (Kachenko and Singh, 2006; Kumar Sharma et al., 2007). Environmental pollution with heavy metals is a serious threat because of their toxicity, bioaccumulation, and biomagnification in the food chain (Demirezen and Uruç, 2006). Although contamination of animal feed and water with toxic metals cannot be entirely avoided, there is a clear need for such contamination to be minimized, in order to reduce effects on animal and human health. Under the food value chain aspect, different production systems or raising locations may affect heavy metal contamination level due to different environment (water and air) and feed sources. Food at markets also may come from different areas, and therefore the final consumers may be exposed to higher levels depending on the origin of the products.

Heavy metal intoxication causes a range of adverse health effects. Some micronutrients [e.g., copper (Cu), chromium (Cr), and nickel (Ni)] are toxic at high concentration (McLaughlin et al., 1999; Rahman et al., 2014) although small quantities are essential for plant growth and human nutrition (Sankar et al., 2006; Kumar Sharma et al., 2007; Bundschuh et al., 2012; Ji et al., 2013; Rahman et al., 2014). Trace metals such as Cr, Ni, As, Cd, and Pb have been considered as the most toxic elements in the environment by the US Environmental Protection Agency (EPA) (Lei et al., 2009; Islam et al., 2014), and dietary intake is an important exposure pathway for these. For example, lead (Pb) hinders the cognitive development and intellectual performance of children, causes high blood pressure and cardiovascular disease in adults (Luckey and Venugopal, 1979; Flora et al., 2006), and is also associated with cancer (Steenland and Boffetta, 2000). Similarly, cadmium (Cd) intoxication leads to cancer, but also to impaired kidney function, poor reproductive capacity, hypertension, and hepatic dysfunction (Luckey and Venugopal, 1979; Wilbur et al., 2012). Cd, classed as a group 1 carcinogen, was estimated to cause over 2,000 deaths globally in 2015 (Gibb et al., 2019). Chromium (Cr) exposure may result in severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects, which may ultimately lead to death (Wilbur et al., 2012). It is found in two forms, trivalent and hexavalent. The latter has been classed as a group 1 carcinogen (carcinogenic to humans) (International Agency for Research on Cancer, 2012), and causes respiratory tract cancers, often due to occupational exposure.

Animal-source foods are important sources of protein, and major components of many diets (Alonso et al., 2019), particularly in low- and middle-income countries (LMICs). Consumption of poultry and fish is rising rapidly in LMICs as the result of increasing incomes, increasing population, and urbanization (Rae, 1998). Poultry can acquire heavy metals from different sources, including feed containing tannery waste, and heavy metal residues may accumulate in body tissue as well as in eggs (Nisianakis et al., 2009), while heavy metals easily sediment in the aquatic environment from and bioaccumulate in fish and other aquatic animals (Islam et al., 2015a; Ullah et al., 2017). The extensive tannery industry in Bangladesh is a risk factor for Cr toxicity. The human health burden of disease caused by Cd and Pb has been assessed globally by the World Health Organization, which estimated that the median disability adjusted life years (DALYs) lost in South-East Asian region D, where Bangladesh belongs, was 0.01 and 52 per 100,000 inhabitants, for Cd and Pb, respectively (Gibb et al., 2019). However, the evidence and available data related to human health risk due to consumption of different food items in Bangladesh is limited. The concentration of heavy metals in fish and chicken meat has been studied before in Bangladesh across urban and peri-urban areas, indicating varying levels of contamination, some in excess of the tolerable limit (Pintaeva et al., 2011; Hasan et al., 2013; Ahmed et al., 2015; Ullah et al., 2017), but not put in context of a risk assessment.

The present study was conducted as part of a broader project which aimed to identify priority food safety hazards in animal-source foods in Bangladesh. It aimed to determine concentrations of Pb, Cd, and Cr in fish and chicken meat sold in different markets in urban, peri-urban, and rural areas in order to produce a risk assessment for the Bangladeshi population.

2. Materials and methods

2.1. Study design and area

A cross-sectional study was conducted between November 2018 and June 2019. In total, 71 traditional markets and 41 modern markets were selected from urban (Dhaka), peri-urban (Savar) and rural (Netrokona) areas (Figure 1), purposively selected to represent areas of different degree of urbanization. In Bangladesh, traditional markets often sell different type food commodities (animal source food, fish, vegetable, dried food). Traditional markets have permanent locations close to the residential area, however, they often lack infrastructure to store food properly, and lack food quality control as well as food traceability. Modern markets are represented by convenience stores and supermarkets which are mainly available in the urban area, and have better infrastructure to keep and display food to sell. In each area, traditional markets were selected using predefined criteria, including markets that sold both chicken and fish. In addition, 41 modern markets were selected in urban area only.

2.2. Sample size and sampling

The selection of which food items to prioritize for the study was decided in collaboration with national stakeholders, including the Bangladesh Food Safety Authority (BFSA). In each selected traditional market, three to four chicken and two to three fish vendors were randomly selected, and chicken meat and fish were purchased. Each selected vendor was asked for one sample of either pangasius fish or chicken meat. Modern food retail outlets located in the same area as the traditional markets were also included, and in each modern food outlet, two chicken meat and fish (pangasius) samples were also collected. We hypothesized heavy metals would

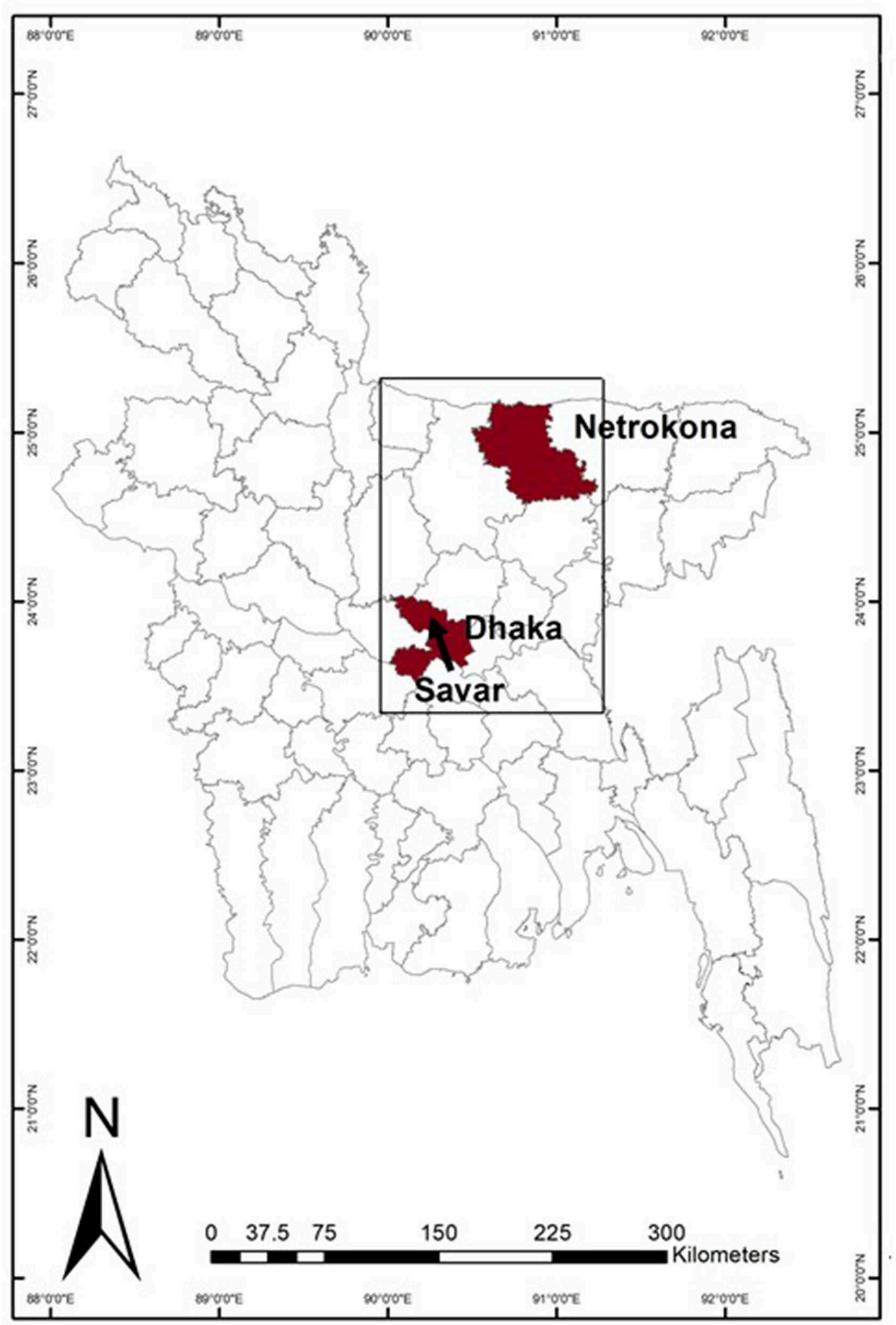

FIGURE 1
The study sites in Bangladesh, showing Dhaka and Netrokona district in dark, and an arrow indicating the location of Savar within the Dhaka district.

be lower in rural areas, as we considered industrial pollution to be the main source in Bangladesh and is lower in rural areas. The study aimed to collect 350 samples per site, and was designed to detect a difference of 10% between urban and rural contamination, 95% confidence and 0.8 power, with no clustering assumed. In total, 608 samples (359 chicken meat and 249 fish) were collected. All

collected samples were kept in cool box (0–4°C) after sampling and transported to the laboratory for analysis within 24 h.

For each shop where a sample was taken, one to two consumers were interviewed and asked how much of this product they would usually consume in the household, and how many members in the household consumed it. A total of 675 consumers in rural, peri-urban and urban areas were interviewed. An average meat amount (gram) consumed per person per day was calculated by dividing the total amount of fish or chicken/day/household by the number of household members.

Ethical approval was given by International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC) with approval number 2018-27.

2.3. Laboratory analysis

2.3.1. Sample processing and testing

The selection of the prioritized heavy metals Pb, Cr, and Cd was decided in collaboration with national stakeholders, including BFSA, based on the perceived priorities and concerns of the country. Approximately 5–10 g of meat was cut from four to six (depending on size of the specimen) different parts of the whole fish or chicken carcass sample (avoiding intestinal content or giblet), cut into small pieces and mixed properly. One gram of this homogenized sample was accurately weighted in a crucible, then an oven (Nüve, Turkey) drying step was done at 120°C for 1 h, until constant weight was obtained. The dried samples were placed in a muffle furnace (Witeg, Baden-Württemberg, Germany) at 450°C for 10 h to generate ash. The ash was transferred into the digestion vessel, a conical flask, by adding 5 ml of 5 M HCl (Merck, Darmstadt, Germany) and 10 ml of 0.01 M HNO_3 (Merck, Darmstadt, Germany) per sample (Idera et al., 2015). Then the vessel was heated to 120°C until the fume disappeared. After digestion step, a semi solid residue remains in the bottom of the tube that was diluted with deionized water to a final volume of 100 ml.

2.3.2. Instruments and reagents

Atomic absorption spectrometer (AAS-7000, Shimadzu, Japan) was used for the determination of Cr, Cd, and Pb. Hollow cathode lamps used for Pb, Cd, and Cr were 283.3 nm and slit 0.5 nm; 228.8 nm and slit 0.5 nm; and 357.9 nm and slit 0.2 nm, respectively. Analysis was carried out according to the conditions recommended by the manufacturer. Atomic signals were measured for Pb, Cd, and Cr in peak area. The AAS system was standardized and validated using the limit of detection (LOD), limit of quantification (LOQ), linearity test, precision, accuracy and recovery. Calibration standards for each heavy metal were prepared each day from the certified stock solution of 1,000 mg/kg (manufactured under ISO 9001 quality assurance system). At least five different concentrations of each metal were spiked into blank matrix before digestion. At least 10 individual replicates were prepared for each concentration. The concentration range was considered to give linear analytical response when the regression coefficient, $R^2 > 0.995$. All solutions were prepared in double-distilled water. All containers and glassware were cleaned by soaking in 0.1% HNO3 (Merck, Darmstadt, Germany) for at least 24 h and rinsed three times with deionized water before using.

2.4. Data analysis

Survey and laboratory data were recorded in Microsoft Excel 2007 (Microsoft Corporation, WA, USA) and imported into STATA 13 and 14.2 (StataCorp, College Station, TX 77845) for analysis. Means, standard deviations of heavy metal concentration, and percentage of samples contaminated with heavy metals calculated. Chi-square or Fisher exact tests (where appropriate) was used to compare proportions of heavy metals contamination by heavy metal types and study areas. One-way analysis of variance (ANOVA) with Bonferroni correction was applied to assess differences in values by heavy metal types and study areas. The correlation between the heavy metal in chicken and fish meat was assessed using Pearson correlation test. A *p*-value <0.05 was considered statistically significant. To account for variability in amount of meat consumed, heavy metal concentration and body weight, a stochastic risk assessment model was conducted using Monte Carlo simulation in @Risk 7.6 (Palisade, USA) with 100,000 iterations to estimate the distributions of the health risks.

2.5. Point estimation of daily intake and non-carcinogenic risk

The estimated daily intake (EDI) of metals due to consumption of contaminated food on fresh weight basis (FW) was calculated using the following formula:

$$EDI = \frac{\sum FIRxC}{BW}$$

Where, FIR (food ingestion rate, kg/day) was the average amount of meat (fish or chicken) (kg) eaten per day by fresh weight; C was the concentration of heavy metal in food (mg/kg FW); BW was average body weight, assumed to be 60 kg for an average adult. For the point estimate, average FIR used in the point estimate for adult consumers was 22.5 and 15.1 for meat and 67.9 and 60.6 g for fish on FW basis from urban/peri-urban and rural area respectively (HIES, 2019).

The oral reference dose (RfD) was set at 1.5 (EPA, 2000), 0.001 (EPA, 1989) and 0.0125 (FDA, 2019; Flannery et al., 2020) mg/kg/day for Cr, Cd, and Pb, respectively, following the United States Environmental Protection Agency (EPA). The EPA does not have an oral reference dose for lead so the United States Food and Drug Administration (FDA) interim reference level for lead for adults was used instead. The non-carcinogenic risk for each heavy metal through fish or meat consumption were assessed using the target hazard quotient (THQ) (EPA, 2022), the ratio of a single substance exposure level to the reference dose (RfD) for that substance. The equation used for estimating THQ is as followed:

$$\bullet THQ = \frac{EDI}{RfD}$$

Where, THQ is the target hazard quotient, EDI is the estimated daily intake (mg/kg/day), and RfD is an oral reference dose (mg/kg/day). Target carcinogenic risk factor (TR) was calculated by following formula:

$$\bullet \text{TR} = \frac{\text{FIRxCxCSFo}}{\text{BW}}$$

Where, CSFo is the oral carcinogenic slope factor, which is equal to 8.5×10^{-3} (mg/kg/day) for lead phosphate (OEHHA, 2009).

2.6. Stochastic risk assessment

A stochastic risk assessment model was built using @Risk add-in in MS Excel. The model used the same equations as in the point estimation of daily intake and non-carcinogenic risk (Section 2.5). Meat consumption, heavy metal concentration and body weight were parameterized to describe their variability by distributions. Different input parameters of relevant variables were generated from the data of consumer survey (meat consumption) and laboratory analysis (heavy metal concentration). To define body weight value of the adult, normal distribution was used, with mean of 60 kg and standard deviation of 10 kg. The RfD for Cr, Cd, and Pb, and oral carcinogenic slope factor for Pb were set as fixed values as the same as in Section 2.5. Variables, distributions, parameters and values used in the model were described in Table 1.

In brief, meat consumption data was fitted following Pert distribution using minimum, most likely, and maximum which were derived from the dataset of a consumer survey. Concentration of Pb, Cd, and Cr data were obtained in Section 2.3 above. Samples which had concentration below detectable level (BDL) were assumed to be equal to 0.0001 mg/kg. Sample groups were categorized by areas, heavy metal and meat types, and fitted with Exponential distribution using fitting distribution function in @RISK. "RiskExpon(λ,RiskShift(0.000001))" function was used to compute concentration of heavy metal as positive values. For each sample group, lambda (λ) values were obtained from fitting distribution in @RISK accordingly. The file containing data and simulations are provided as Supplementary material 1.

3. Results

3.1. Prevalence of heavy metal contamination in chicken meat and fish

Out of 359 chicken meat samples, 80.2%, 85.5%, and 55% were contaminated with Cr, Cd, and Pb, respectively; while out of 249 fish samples, 86%, 85%, and 23% were contaminated with Cr, Cd, and Pb, respectively. Least detected metal was Pb with 55% in chicken and 23% in fish, while the most detected metal was Cd in chicken, 86%, and Cr in fish, 86% (Table 2). Prevalence of heavy metal contamination in both chicken and fish samples was significantly lower in rural area compared to that in urban area ($p < 0.01$, Table 2).

3.2. Concentration of heavy metals

Average concentrations of heavy metals (mean ± SD) in chicken meat and fish are presented in Table 3. Highest mean concentration was found in chicken meat sold in different areas: 0.78 ± 0.077 mg/kg, 0.02 ± 0.04 mg/kg (urban) and 0.05 ± 0.11 mg/kg (rural) for Cr, Cd, and Pb, respectively, whereas the lowest mean concentration was found in the peri-urban markets. Regarding fish samples, the highest mean concentration of Cr, Cd, and Pb was 0.68 ± 0.67 mg/kg, 0.02 ± 0.03 mg/kg (urban) and 0.02 ± 0.08 mg/kg (rural), respectively. There were significant differences ($p < 0.05$) in heavy metals concentration of chicken and fish samples between study sites.

Correlation among the tested heavy metals in the meats tested with Pearson's correlation depicted in Table 4. Only Cr and Cd showed a clear pattern of positive correlation found in both chicken meat and fish ($p = 0.003$ for chicken and $p < 0.0001$ for fish).

3.3. Estimated daily intake

The consumption rates and estimated daily intakes (EDIs) of heavy metals in adult inhabitants from eating chicken and fish were listed in Table 5. Total daily intakes of Cr, Cd, and Pb by eating chicken meat were 0.00029, 0.000008, and 0.000011 mg/kg/day BW in urban area, while in rural area intakes were 0.00016, 0.000003, and 0.000013 mg/kg/day BW, respectively. The total daily intakes of Cr, Cd, and Pb by eating fish were 0.00077, 0.000023, and 0.000023 mg/kg/day BW in urban area, and 0.000283, 0.00001, and 0.00002 mg/kg/day BW in rural area, respectively. A higher contribution of dietary intake of metals came from fish, due to these being the most highly consumed protein-based food in urban and rural area (67.9 and 60.6 g/person/day, respectively) (HIES, 2019). Metal specific point estimates for the EDIs revealed that EDI of Cr, Cd and Pb from consumption of all examined foodstuffs were lower than the maximum tolerable daily intake (MTDI, Table 5). The stochastic model which allowed for slightly higher consumption had higher estimates, but still well below the MTDI.

3.4. Non-carcinogenic risk and target carcinogenic risk

THQs of individual heavy metal through chicken meat and fish consumption by average Bangladeshi adults are presented in Table 6. Average heavy metal concentration in chicken and fish (pangasius) was used to calculate THQ for the people of Bangladesh. The THQ value for the targeted heavy metals followed the order Cd > Pb > Cr in chicken and fish. Table 6 indicated that the THQ value for all three metal Cd, Cr and Pb were <1 in all study areas. The maximum THQ was the highest for Cd in chicken meat and fish across urban, peri-urban and rural areas. The average target carcinogenic risk of Pb due to exposure from the consumption of targeted chicken meat and fish samples were 9.6×10^{-8}, 1.9×10^{-7} (urban area) and 1.0×10^{-7}, 1.7×10^{-7} (rural area), respectively. Regarding consumption of chicken meat, the stochastic model showed 3.7 to 10 times higher average target

TABLE 1 Variables, distributions, parameters and source of data used in the stochastic risk assessment model.

Variables/input	Abbreviation	Unit	Distribution/parameter/value	Data source
Meat/Fish ingestion rate-FIR	FIR	kg/day	RiskPert(min, most likely, max)	This study
Heavy metal concentration				
Concentration of chromium	CCr	mg/kg FW	RiskExpon[λ,RiskShift(0.000001)]	This study
Concentration of cadmium	CCd	mg/kg FW	RiskExpon[λ,RiskShift(0.000001)]	This study
Concentration of lead	CPb	mg/kg FW	RiskExpon[λ,RiskShift(0.000001)]	This study
Body weight of adult people	BW	kg	RiskNormal(60,10)	Author's assumption
Daily intake of heavy metal				
Estimated daily intake (EDI)-Cr	EDI.Cr	mg/kg BW/day	(output): FIRxCCr/BW	
Estimated daily intake (EDI)-Cd	EDI.Cd	mg/kg BW/day	(output): FIRxCCd/BW	
Estimated daily intake (EDI)-Pb	EDI.Pb	mg/kg BW/day	(output): FIRxCPb/BW	
Oral reference dose				
Oral reference dose-Cr	RfD.Cr	mg/kg/day	Fix value: 1.5	EPA, 2000
Oral reference dose-Cd	RfD.Cd	mg/kg/day	Fix value: 0.001	EPA, 1989
Oral reference dose-Pb	RfD.Pb	mg/kg/day	Fix value: 0.0125	FDA, 2019; Flannery et al., 2020
Target hazard quotient				
Target hazard quotient-Cr	THQ.Cr	No unit	(output): EDI.Cr/RfD.Cr	
Target hazard quotient-Cd	THQ.Cd	No unit	(output): EDI.Cd/RfD.Cd	
Target hazard quotient-Pb	THQ.Pb	No unit	(output): EDI.Pb/RfD.Pb	
Oral carcinogenic slope factor-Pb	CSFo	mg/kg/day	Fix value: 8.5×10^{-3}	Flannery et al., 2020
Target carcinogenic risk factor-Pb	TR.Pb	No unit	(output): EDI.PbxCSFo	

TABLE 2 Number of chicken and fish samples collected and tested positive for heavy metals contamination in different areas of Bangladesh.

Areas	Chicken (%)				Fish (%)			
	n	Cr	Cd	Pb	*n*	Cr	Cd	Pb
Urban	**170**	160 (94.1)[a]	158(92.9)[a]	124 (72.9)[b]	**123**	115 (93.5)[a]	112 (91.1)[a]	19 (15.4)[e]
Peri-urban	**94**	68 (72.3)[b]	86 (91.5)[a]	44 (46.8)[c]	**65**	51 (78.5)[b]	62 (95.4)[a]	26 (40.0)[c]
Rural	**95**	60 (63.2)[b]	63 (66.3)[b]	29 (30.5)[d]	**61**	47 (77.0)[b]	37 (60.7)[b]	13 (21.3)[d,e]
Total	**359**	288 (80.2)	307 (85.5)	197 (54.9)	**249**	213 (85.5)	211(84.7)	58 (23.3)

Different superscripts indicate statistically significant differences.

carcinogenic risk of Pb. Whereas average target carcinogenic risk of Pb due to consumption of fish calculated between point estimate and the stochastic model was almost similar (Table 6).

4. Discussion

Determination of heavy metal concentration in different food types is important from a public health aspect. This present study highlighted the heavy metal contamination in fish and chicken meat in different areas of Bangladesh, and provided an estimation of the risk for consumers. We found that the contamination levels varied both between and within the different sampling sites. These observed variations in heavy metal concentrations in foodstuffs could be due to various absorption and accumulation capabilities (Pandey and Pandey, 2008), growth period and stages during food production (Saha and Zaman, 2013) as well as climatic differences of the study areas (Santos et al., 2004).

The presence of chromium (mean ± SD) in chicken meat were found as 0.64 ± 1.24, 0.04 ± 0.05 and 0.78 ± 0.077 mg/kg in rural, peri-urban and urban, while in fish samples, mean value were 0.28 ± 0.50, 0.06 ± 0.09 and 0.68 ± 0.67 mg/kg in rural, peri-urban and urban, respectively. This could indicate a great concern for the public, but the levels observed here were generally lower than what was found in other studies. An earlier study conducted in Bangladesh stated that mean concentration of Cr in chicken was 1.4 ± 0.31 mg/kg (Islam et al., 2015a), which is almost double the levels detected in our study. In fish species caught from different rivers,

TABLE 3 Metal concentration [mg/kg fresh weight (FW)] in chicken meats and fish of three study sites in Bangladesh.

Location	Chicken [mg/kg FW, mean (SD)]				Fish [mg/kg FW, mean (SD)]			
	n	Cr	Cd	Pb	*n*	Cr	Cd	Pb
Overall	359	0.66 ± 0.93	0.02 ± 0.03	0.09 ± 0.10	249	0.49 ± 0.62	0.02 ± 0.03	0.06 ± 0.09
Urban	170	0.78 ± 0.077[a]	0.02 ± 0.04[a]	0.03 ± 0.06[a]	123	0.68 ± 0.67[a]	0.02 ± 0.03[a]	0.02 ± 0.05[a]
Peri-urban	94	0.04 ± 0.05[b]	0.01 ± 0.04[b]	0.001 ± 0.01[b]	65	0.06 ± 0.09[b]	0.01 ± 0.01[a]	0.01 ± 0.01[a]
Rural	95	0.64 ± 1.24[a]	0.01 ± 0.02[b]	0.05 ± 0.11[a]	61	0.28 ± 0.50[b]	0.01 ± 0.02[b]	0.02 ± 0.08[a]
P-value*		0.001	0.001	0.001		0.001	0.02	0.09
Maximum allowable concentration[‡]		1[§]	0.05[†]	0.1[¶]		1[§]	0.05[†]	0.3[¶]

Different superscript in the same column indicated statistically significant difference, *ANOVA test, [‡]Maximum allowable concentration varies with countries, and here some examples are presented: maximum allowable concentration according to [†]Codex Alimentarius (2019), [§]Islam et al. (2015a), [¶]JECFA (2005). Different superscripts indicate statistically significant differences.

TABLE 4 Correlation coefficient among heavy metals in chicken and fish.

Sample type	Chicken (*n* = 249)			Fish (*n* = 359)		
Metals	Cr	Cd	Pb	Cr	Cd	Pb
Cr	1			1		
Cd	0.1553*	1		0.2816*	1	
Pb	−0.0776	−0.0400	1	−0.0374	0.0172	1

*Significant ($p < 0.05$) correlation between these heavy metals.

much higher Cr concentration have also been reported previously in Bangladesh, such as 6.92–12.23 mg/kg dry weight (Dhaleshwari river, Ahmed et al., 2009), 0.47–2.07 mg/kg dry weight (Bangshi River, Rahman et al., 2012), 5.27–7.38 mg/kg dry weight (Buriganga River, Ahmad et al., 2010). Our finding on Cr concentration was also lower compared to fish caught from urban rivers around the Dhaka city (0.75–4.8 mg/kg wet weight) or from cultured fishes (1.054–1.349 mg/kg wet weight, Islam et al., 2015b). The differences in contamination levels to our results could be explained by different study areas, but could potentially also indicate an improvement of food production conditions and environmental pollution compared to the earlier studies.

Cadmium is a highly toxic element capable of causing severe toxicity even when it is present at a very low concentration of ∼1 mg/kg (Friberg et al., 2018). The accumulation of Cd in the human body may give rise to hepatic, pulmonary, renal, skeletal, reproductive effects, and even cancer. The mean Cd concentration in both chicken and fish detected in this study were between 0.01 and 0.02 mg/kg amongst rural, peri-urban and urban areas. Islam et al. (2015a) reported 0.030 ± 0.032 mg/kg FW in chicken meat, whereas another study reported much higher levels 0.23 mg/kg FW in chicken meat (Islam, 2018). Cd concentration in fish in the present study were similar to the concentrations reported earlier in Bangladesh, which ranged from 0.51–0.73 mg/kg dry weight (fish from Dhaleshwari river, Ahmed et al., 2009), 0.09–0.87 mg/kg dry weight (fishes from Bangshi River, Rahman et al., 2012), 0.008–0.13 mg/kg wet weight (fish from urban rivers around Dhaka city, Islam et al., 2015b), 0.001–0.003 mg/kg wet weight in cultured fishes (Ahmed et al., 2015). In addition, the concentration of Cd found in the different study sites in this study was lower than the maximum allowable range (JECFA, 2005), indicating that consumption of these two commodities may rarely contribute to toxic effects of Cd.

Regarding the lead contamination in chicken meat, concentrations were 0.05 ± 0.11, 0.001 ± 0.01, and 0.03 ± 0.06 mg/kg in rural, peri-urban and urban areas, respectively. Average concentrations of lead in fish were 0.02 ± 0.08, 0.01 ± 0.01, and 0.02 ± 0.05 mg/kg collected in rural, peri-urban, and urban, respectively. Previous studies reported that lead in chicken meat was 0.17 ± 0.16 mg/kg (Islam et al., 2015a) which was higher compared to our finding. It was reported that the range of lead contamination in fish caught from different rivers were 4.25–8.17 mg/kg dry weight (Dhaleshwari river, Ahmed et al., 2009), 1.76–10.27 mg/kg dry weight (Bangshi river, Rahman et al., 2012), 8.03–13.52 mg/kg dry weight (Buriganga river, Ahmad et al., 2010), 0.052–2.7 mg/kg wet weight (urban rivers around Dhaka city, Islam et al., 2015b), 0.017–0.090 mg/kg wet weight (cultured fishes, Ahmed et al., 2015). Lead is a non-essential heavy metal and may cause many adverse health effects, including neurotoxicity and nephrotoxicity (García-Lestón Julia et al., 2010). Compared to the earlier studies, it may seem that the lead contamination in chicken meat and fish has been reduced, which shows a positive signal in improving the safety level related to lead.

In the present study, concentrations of the three heavy/toxic metals were lower than maximum tolerable daily intake in all three studied areas, which suggested that these food items alone are not contributing to significant health risks for consumer. However, the consumption amount revealed in this study was relatively low, with <100 g consumed per person per day. Growing income could lead to increased consumption of meat and fish, which, along with poor monitoring, surveillance,

Food type	Area	Consumption rate (g/day)*	Estimated daily intake (mg/kg/day BW)					
			Point estimate			Stochastic (mean, min-max)		
			Cr	Cd	Pb	Cr	Cd	Pb
Chicken meat	Urban	22.5	0.000293	0.000008	0.000011	0.000982 (0–0.0259)	0.000029 (0–0.0010)	0.000042 (0–0.0011)
	Periurban	22.5	0.000015	0.000004	0.000000	0.000054 (0–0.0026)	0.000007 (0–0.0002)	0.000004 (0–0.0001)
	Rural	15.1	0.000161	0.000003	0.000013	0.000809 (0–0.0184)	0.000015 (0–0.0007)	0.000064 (0–0.0023)
Fish	Urban	67.9	0.000770	0.000023	0.000023	0.000855 (0–0.0207)	0.000025 (0–0.0007)	0.000022 (0–0.0006)
	Periurban	67.9	0.000068	0.000011	0.000011	0.000077 (0–0.0017)	0.000011 (0–0.0003)	0.000003 (0–0.0001)
	Rural	60.6	0.000283	0.000010	0.000020	0.000527 (0–0.0131)	0.000031 (0–0.0007)	0.000023 (0–0.0006)
Both chicken meat and fish	Urban	90.4	0.001062	0.000030	0.000034	0.001836 (0–0.0266)	0.000054 (0–0.0011)	0.000065 (0–0.0011)
	Periurban	90.4	0.000083	0.000015	0.000012	0.000131 (0–0.0036)	0.000018 (0–0.0003)	0.000007 (0–0.0001)
	Rural	75.7	0.000444	0.000013	0.000033	0.001337 (0–0.0202)	0.000046 (0–0.0008)	0.000087 (0–0.0023)
Reference maximum tolerable daily intake (MTDI)**			0.0033[c]	0.00083[d]	0.0036[e]	0.0033[c]	0.00083[d]	0.0036[e]

*Consumption rate (g/day) was considered based on a national survey of 2016 (HIES, 2019).

**Reference maximum tolerable daily intake (MTDI) indicated by (c) recommended daily intake of 0.2 mg/kg divided by 60 kg as average body weight (Recommended Dietary Allowances, 1989) and (d) calculated based on provisional tolerably monthly intake given as 0.025 mg/kg bw (47)/30 days. (e) calculated based of the earlier provisional tolerably weekly intake (PTWI) given as 0.025 mg/kg bw (47)/7. The latter PTWI is no longer recommended since this level still may have negative health effects.

TABLE 6 Mean contamination levels, oral reference dose (RfD), estimated daily intake (EDI), target hazard quotient (THQ) and target carcinogenic risk (TR) of metals for adults (assumed 60 kg body weight) due to consumption of chicken meat and fish in Bangladesh.

Food type and Heavy metals	Mean concentration (mg/kg FW)	RfD (mg/kg/day)	Point estimate			Stochastic (mean, min-max)		
			EDI (mg/kg/day BW)	Target hazard quotient (THQ)	Target carcinogenic risk (TR)	Estimated daily intake (mg/kg/day BW)	Target hazard quotient (THQ)	Target carcinogenic risk (TR)
Chicken meat								
Urban								
Cr	0.78	1.5	0.0002925	0.00020	NA	0.000982 (0–0.0259)	0.00065 (0–0.0173)	NA
Cd	0.02	0.001	0.0000075	0.00750	NA	0.000029 (0–0.0010)	0.02945 (0–0.9986)	NA
Pb	0.03	0.0125	0.0000113	0.00090	9.6×10^{-8}	0.000042 (0–0.0011)	0.00339 (0–0.0866)	3.6×10^{-7} ($0–9.2 \times 10^{-6}$)
Peri-urban								
Cr	0.78	1.5	0.0000150	0.00001	NA	0.000054 (0–0.0026)	0.00004 (0–0.0017)	NA
Cd	0.02	0.001	0.0000038	0.00375	NA	0.000007 (0–0.0002)	0.00746 (0–0.2025)	NA
Pb	0.03	0.0125	0.0000004	0.00003	3.2×10^{-9}	0.000004 (0–0.0001)	0.00029 (0–0.0082)	3.1×10^{-8} ($0–8.7 \times 10^{-7}$)
Rural								
Cr	0.64	1.5	0.0001611	0.00011	NA	0.000809 (0–0.0184)	0.00054 (0–0.0123)	NA
Cd	0.01	0.001	0.0000025	0.00252	NA	0.000015 (0–0.0007)	0.01534 (0–0.7004)	NA
Pb	0.05	0.0125	0.0000126	0.00101	$1.0x10^{-7}$	0.000064 (0–0.0023)	0.00509 (0–0.1840)	5.4×10^{-7} ($0–2.0 \times 10^{-5}$)
Fish								
Urban								
Cr	0.68	1.5	0.0007695	0.00051	NA	0.000855 (0–0.0207)	0.00057 (0–0.0138)	NA
Cd	0.02	0.001	0.0000226	0.02263	NA	0.000025 (0–0.0007)	0.02481 (0–0.7032)	NA
Pb	0.02	0.0125	0.0000226	0.00181	$1.9x10^{-7}$	0.000022 (0–0.0006)	0.00179 (0–0.0442)	1.9×10^{-7} ($0–4.7 \times 10^{-6}$)
Peri-urban								
Cr	0.78	1.5	0.0000679	0.00005	NA	0.000077 (0–0.0017)	0.00005 (0–0.0011)	NA
Cd	0.02	0.001	0.0000113	0.01132	NA	0.000011 (0–0.0003)	0.01061 (0–0.2579)	NA
Pb	0.03	0.0125	0.0000113	0.00091	9.6×10^{-8}	0.000003 (0–0.0001)	0.00025 (0–0.0074)	2.6×10^{-8} ($0–7.9 \times 10^{-7}$)
Rural								
Cr	0.28	1.5	0.0002828	0.00019	NA	0.000527 (0–0.0131)	0.00035 (0–0.0088)	NA
Cd	0.02	0.001	0.0000101	0.01010	NA	0.000031 (0–0.0007)	0.03059 (0–0.7046)	NA
Pb	0.02	0.0125	0.0000202	0.00162	1.7×10^{-7}	0.000023 (0–0.0006)	0.00186 (0–0.0497)	2.0×10^{-7} ($0–5.3 \times 10^{-6}$)

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and implementation, could contribute to higher exposure in the future. In addition, a tendency of accumulation of metal in various organ may trigger serious health implication in a longer term. Positive correlation was shown between Cr and Cd signifying it might be common sources of these metals, such as contamination of feed (Hasan et al., 2013; Ullah et al., 2017).

The result on THQ values related Pb, Cr, and Cd in this study would suggest that on average, people would not be suffering negative health risks through the consumption of these food. In addition, TR for Pb was also lower than 10^{-6}, which indicating the risk of cancer due to exposure to Pb through chicken meat and fish consumption was negligible, given the present amount of consumption. Our results are in line with earlier assessments of the risks from heavy metals in fish (Ullah et al., 2017), which also concluded that there were negligible risks from fish consumption at the present levels. Given the potential health benefits of consuming animal-source foods, particularly by undernourished children (Alonso et al., 2019), it may be important that people are not afraid to increase their consumption from the presently very low levels. However, this study could only cover a few of all the potential heavy metal hazards in the food, and Pb, Cr, and Cd were selected based on the priorities of the country, according to the national stakeholders consulted. Future studies are needed to more accurately assess the risks of additional hazards present in the food.

This study had some limitations. First, the risk assessment based on the total amount of metals show actual exposure, that could not cover the nature of the metal metabolism in the human body, percentage of bio-accessible, e.g., not reaching the circulatory system. Secondly, during food production and cooking processes, part of the metal may be reduced which could also influence the final exposure dose. However, using the total dietary intake, the estimates provided here would likely be overestimations, and the real risk, taking bioavailability into account would then likely be even lower. Third, due to the laboratory capacity of the national laboratory BLRI, AAS was used, which is not as accurate as some other methods. Optimally ICP-OES or ICP-MS would have been used for confirmation, but these methods were not available. Instead all precautions were done to ensure validity of the results, including the use of multiple standards for calibration. In addition, the dietary intake was based on interviews, which also may be prone to errors in the estimate. Thus, the final risk assessments should be considered taking into account that the data is limited and there are major assumptions, that may not make even the stochastic estimates applicable to the entire population. Fourth, we only found related data of Pb which we could conduct a cancer risk assessment, while for other heavy metal (Cr and Cd), we have limited access to the information which could use to carry out the cancer risk assessment.

5. Conclusion

The results of this study revealed the prevalence and concentration of Pb, Cr, and Cd in fish and chicken meat in different retailed types and study areas. Their concentrations were below the maximum allowable range which indicates acceptable level for human consumption. However, heavy metal contamination in fish and chicken meat would imply that contaminated feed and water, potentially through environmental sources like polluted sewage water, intensive use of pesticide and rapid development of industry, which could affect the quality and safety of livestock and aquaculture products, and as consequence effect to human health. Therefore, it is important to keep monitoring and implement measures to reduce heavy metals contamination in feed and water along the food chain.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Ethical Review Committee of ILRI, approval number 2018–27. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Funding and conceptualization: JFL and DG. Study design: JFL, MAS, SD-X, and DG. Data collection and laboratory analyses: RB, SI, RA, NAS, ASMAU, MSS, and AM. Supervision: MAS and JFL. Data analyses: RB, SD-X, SI, and JFL. Drafting of manuscript: RB, SI, and JFL. Critically revising and approving the manuscript: All authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Structure and milk hygiene of dairy cooperative value chains in an intensive production area of Uganda—A bottleneck of intervention

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In Uganda, informal raw milk sales dominate for domestic dairy consumption. This study was implemented to identify the structure of the dairy value chain starting from farms that participated in the Japan International Cooperation Agency Safe Milk Promotion in Mbarara project conducted between 2016 and 2019, to assess the hygiene conditions along the chain, and thereby identify the bottleneck of dairy hygiene intervention. A longitudinal study was conducted in 30 dairy farms in Mbarara District to compare the practice, prevalence of sub-clinical mastitis, and level of milk hygiene in 2016–2017 and 2019, before and after the milking hygiene intervention in 2018. California Mastitis Test was used for diagnosis with sub-clinical mastitis. Bulk milk samples were collected and a checklist was used to examine hygiene practices by observation. A cross-sectional study was conducted in 15 milk collecting centers using a structured questionnaire to quantify the dairy value chain, and to sample milk from cooler tanks in 2020. Microbiological examinations of bulk milk from farms and collection centers were conducted using six-point blood agar scoring and 3M Petri film, respectively. Participatory online appraisals with farmers and dairy cooperatives union were conducted to better understand the overall dairy value chains. The cooperatives sold milk to both formal and informal chains, but the sale of raw milk to Kampala was conducted by independent private traders. Within-herd prevalence of sub-clinical mastitis significantly decreased from 72.3% before the intervention to 25.8% after ($p < 0.001$). However, the farm bulk milk score did not change (3.3 vs. 3.2, $p = 0.418$). A significant increase in the total bacterial count was observed in the milk from collection centers (mean: 6.50 $\log_{10}$ CFU/ml) when compared to farm bulk milk (mean: 3.79 $\log_{10}$ CFU/ml; $p < 0.001$). Only 13.3% of the samples from the centers met the microbiological criteria for processing for human consumption. Our findings suggest that intervention targeted only at mastitis does not lead to better public health due to the low level of hygiene in transportation and milk handling in milk collection centers. Systematic interventions are needed to improve post-harvest dairy hygiene in Uganda.

KEYWORDS

dairy value chain, milk hygiene, dairy cooperative, hygiene management, milk contamination

1. Introduction

Even during the coronavirus disease pandemic, Uganda's economy was estimated to have grown by 2.9% in the 2019/2020 fiscal year (Uganda Bureau of Statistics, 2021). Domestic milk production increased from 2.08 billion liters in 2015 to 2.81 billion liters in 2021 (Dairy Development Authority, 2021). Revenue from the export of dairy products has risen from United States Dollars (USD) 131.5 million [1 USD is ∼3,650 Uganda Shillings (Shs)] in 2018 to USD 205 million in 2020 (Ministry of Agriculture, 2021), and accounted for 5.0% of the total export revenue of Shs 15,126 billion in the 2019/2020 fiscal year (Uganda Revenue Authority, 2021). While dairy exports significantly contribute to Uganda's economy, dairy farming provides three main benefits to more than 2.5 million farming households, i.e., nutritious food, additional income, and a productive labor force, and it plays a significant role in reducing Uganda's food insecurity and poverty (Staal and Kaguongo, 2003; Balikowa, 2011; Wangalwa et al., 2016). Pasteurized milk can safely provide nutrition to the population, but the marketing of untreated milk is popular in Uganda; it has been reported that 72.1% of milk is sold through formal channels [cooperative union purchases (38.06%) and milk collection centers (MCCs) owned by licensed raw milk traders (34.05%)] while 27.9% of milk is sold through informal channels (vendors and restaurants) in Kiruhura District (Nkwasibwe et al., 2015).

Contaminated and spoiled raw milk affects the entire dairy industry, ultimately resulting in raw milk of reduced quality, dairy products with less flavor, and a shorter shelf life (Roberts, 1993; Barbano et al., 2006). In particular, raw milk may contain microorganisms that are dangerous to human health, and the consumption of unsafe milk is known to cause many milk-borne diseases (Dhanashekar et al., 2012). Poor animal husbandry and practices, such as udder and handwashing with unsafe water, adulteration, and poor equipment cleaning, have been identified to potentially cause the spread of harmful pathogens in milk (Oliver et al., 2009). It is crucial to ensure that high-quality raw milk is produced from healthy animals under hygienic conditions, and that quality control measures are applied in the value chain.

Mbarara District, located in southwest Uganda, is one of Uganda's most crucial milk production and market areas. In this region, a project called the "Japan International Cooperation Agency (JICA) Safe Milk Promotion in Mbarara (Safe Milk) Project" was implemented from September 2016 to September 2019 in the dairy herds of Mbarara District (Rakuno Gakuen University, 2019). The project aimed to improve the dairy productivity in intensive dairy production areas of Uganda. However, even though the quality of milk at the time of production has improved, dairy hygiene measures need to be maintained throughout the value chain to deliver good quality milk to consumers. The purposes of this study were to quantitatively understand the structure of the dairy cooperative value chains, starting from JICA project farms, in Mbarara, an intensive dairy production area of the country, and to assess the hygiene levels along the value chains, and thereby identify the bottleneck of dairy hygiene intervention in Uganda.

2. Materials and methods

2.1. Study area

The study was conducted in Mbarara District, the Republic of Uganda, located between 0°12′20.6″ and 0°50′36.5″ south in latitude, and between 30°18′47.5″ and 30°49′12.8″ east in longitude (Figure 1). The topography of the district includes a mixture of shallow valleys and flat land. The average annual rainfall is 1,200 mm, rainy seasons last from February to May and September to December, temperatures range from 17 to 30°C, and humidity levels range from 80 to 90% (Mbarara District Local Government, 2021). In Uganda, the Western Region, which includes Mbarara District, is the region with the second highest number of cattle in the country, following the Northern Region (Uganda Bureau of Statistics, 2021). According to the Mbarara District Veterinary Office, the estimated total number of cattle in Mbarara District at the time of project implementation was 185,680, and there were 10,200 dairy farmers (Miyama et al., 2020).

2.2. Study design

A longitudinal study was conducted in the 30 dairy farms that participated in the JICA Safe Milk project, to compare the practice and level of milk hygiene between in 2016–2017 and 2019, before and after the dairy hygiene intervention, which took place in 2018. The intervention involved frequent visits to the farms for demonstration of hygienic milking using proper hand milking and one towel for a cow. All the participating farms employed hand milking. The longitudinal study in the dairy farms also aimed to determine the association between farm bulk milk hygiene and hygiene practices.

The target farms were the 30 farms in Mbarara District that participated in the JICA project. For the JICA project, farmers were purposively selected in collaboration with the Mbarara District Veterinary Officer (DVO) according to the following criteria: (a) herd size, five farms with a small herd (<10 adult cows, including milking and dry cows), 20 farms with a medium herd (10–40 adult cows), and five farms with a large herd (>40 adult cows); (b) herd management type; (c) accessibility, the farms had to be located within driving distance for regular visits; (d) farm distribution, two–five farms per sub-county and five–six farms per MCC; and (e) commitment, the farms were anticipated to continue participating in the JICA project. The criteria a, b, and d were decided, so that the selected farms are representative of the dairy farms in Mbarara District. The 30 study farms were selected from 10 sub-counties in the district, namely Biharwe, Bubaare, Bukiro, Kagongi, Kakiika, Kakoba, Kashare, Rubaya, Rubindi, and Rwanyamahembe.

In 2020, a cross-sectional study was conducted in cooperatives' MCCs to better understand the structure of dairy value chains, starting from the JICA project participating dairy farms in Mbarara District, and the level of hygiene in bulk milk at the MCCs. The targeted MCCs were all of the 17 milk collection stations of the

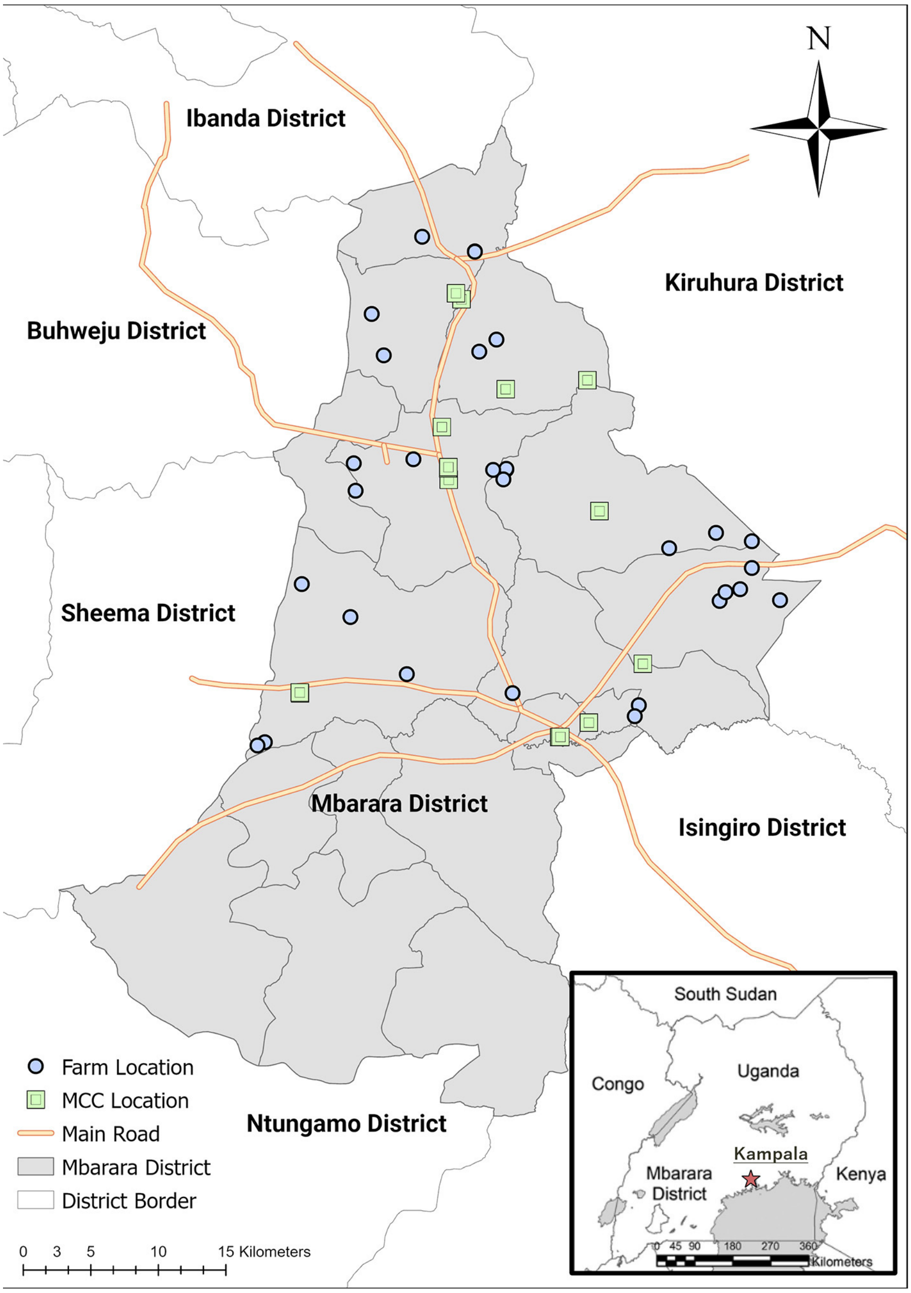

FIGURE 1
Map of Mbarara District with the locations of the JICA project farms and milk collection centers (MCCs) studied.

producers' cooperatives in Mbarara District; they were located in eight sub-counties: Bubaare, Kakiika, Kakoba, Kamukuzi, Kashare, Rubaya, Rubindi, and Rwanyamahembe.

In addition, online participatory appraisals with dairy stakeholders were carried out to ensure the representativeness of this study in relation to wider dairy value chains.

The research protocol was approved by the School of Veterinary Medicine and Animal Resources (SVAR) Research Ethics Committee of Makerere University (reference number SVARREC/09/2018). Informed consent was obtained from all farms studied.

2.3. Field work

Field studies of the milking hygiene practices and the level of hygiene on the 30 dairy farms was conducted between October 2016 and May 2017, and in January 2019. The milking hygiene practices were checked during afternoon milking on each farm in both studies using a check list (Table 1). For the seven farms that did not milk cows in the afternoon, milking practice information was collected through interviews with the owners/managers in both periods. The checklist questioning, accompanied by observation, was performed in English, the official language of Uganda, and the

TABLE 1 A comparison in the frequency and percentage of milking hygiene practices conducted at 30 dairy farms in Mbarara District, Uganda, between before and after the intervention.

Items	Before (2017)	After (2019)
Before going to the milking parlor		
1. A proper concentration of disinfectant was prepared (200 ppm of sodium hypochlorite)	1 (3.3%)	21 (70.0%)
2. Dry towels were dipped into the disinfectant, and kept there for 10 min	0 (0.0%)	21 (70.0%)
3. Towels were squeezed with clean hands, and kept in a bucket	0 (0.0%)	20 (67.0%)
4. Another empty bucket was prepared for used towels	1 (3.3%)	18 (60.0%)
5. A bucket of water was prepared for washing hands	1 (3.3%)	22 (73.0%)
At the parlor		
1. Cows with mastitis were milked last	4 (13.3%)	26 (87.0%)
2. Hands were washed before milking each cow	3 (10.0%)	23 (77.0%)
3. Hands were disinfected	0 (0.0%)	26 (87.0%)
4. Pre-dipping was conducted using a strip cup	2 (6.7%)	12 (40.0%)
5. One towel was picked up from the bucket, and one towel was used for each cow when wiping the teats	1 (3.3%)	19 (63.0%)
6. Each teat was wiped using a hand-twisting motion to remove dirt	0 (0.0%)	22 (73.0%)
7. A clean part of the towel was used for each teat	1 (3.3%)	20 (67.0%)
8. After finishing all teats, the teat tips were wiped using the other side of the towel	0 (0.0%)	20 (67.0%)
9. The used towel was put into the other bucket	1 (3.3%)	19 (63.0%)
When milking		
1. Milking was done gently (hand milking) WITHOUT hurting the teats (Finger milking was NOT performed)	3 (10.0%)	25 (83.0%)
After milking		
1. Post-dipping with iodine was conducted	1 (3.3%)	14 (47.0%)
2. Teat coverage by the iodine was 75%–100%	1 (3.3%)	14 (47.0%)
Preparation for the next milking		
1. All of the used towels were washed with soap and rinsed cleanly	2 (6.7%)	21 (70.0%)
2. Towels were dried in sunlight	2 (6.7%)	21 (70.0%)

judgment criteria for the contents were confirmed among assessors before the surveys, to avoid information bias during checklist administration. During the surveys in 2016–2017 and 2019, the California Mastitis Test (CMT) was performed for individual milking cows to detect sub-clinical mastitis. The results of CMT were classified as negative, trace, or score 1, 2, or 3, depending on the amount of gel formed (Ruegg, 2005). A quarter was defined as CMT positive if it had a score 1 or above, and a cow was defined as CMT positive if it had at least one CMT-positive quarter. In addition, during the both surveys, farm bulk milk was aseptically sampled for microbiological tests. In addition, swabs of empty cans before milking (dry swabs), empty cans after washing (wet swabs), and samples of the water used to clean milk cans were collected from the farms in 2019. Information on the milk yield at each farm and the shipping destination was collected in 2019. These surveys were conducted during the JICA project, and data on the geographical locations of the farms were obtained from the project.

In January 2020, a field study of the value chain and milk hygiene was conducted in all of the 17 MCCs that were owned by dairy cooperatives and registered in Mbarara District. Of the 17 MCCs, two MCCs had stopped doing business. A structured questionnaire regarding the testing of milk upon the receipt of raw milk, the sources and sales destinations of milk, and the volumes of milk was administered through interviews with MCC managers. The purchase of milk from the 30 dairy farms participating in the JICA project was also investigated in the MCCs. Bulk milk was aseptically sampled from milk coolers in these MCCs.

All milk, water, and swab samples were transported to the Mbarara DVO laboratory in a cooling box immediately after sampling.

2.4. Microbiological tests

In the JICA project, the microbiological level of milk hygiene in dairy farm bulk milk was semi-quantitatively assessed by the observation of 5% sheep blood agar with a six-point scoring system (Supplementary Figure 1). This test was selected by the project because it is sustainable for use in the field for identification of causal bacteria for sub-clinical mastitis at the teat level; this scoring system has also been used by the Japanese Dairy Association (Ministry of Agriculture, 1997). For this method, 0.1 ml of raw

milk was inoculated onto 5% sheep blood agar and incubated at 37°C for 48 h. The bacterial counts were estimated on a scale of 1–6: (1) <3,000 colony forming units (CFU)/ml; (2) 3,000 to <4,500 CFU/ml; (3) 4,500 to <6,000 CFU/ml; (4) 6,000 to <13,000 CFU/ml; (5) 13,000 to <20,000 CFU/ml; and (6) ≥20,000 CFU/ml (Ministry of Agriculture, 1997).

For the bulk milk samples collected from the MCCs, and the wet and dry swabs and tap water samples from the farms, the total bacterial counts were determine using 3M Petri film according to the manufacturer's instructions (3M Japan Limited. Shinagawa, Japan). Serial dilutions of 10, 10^3, and 10^5 were prepared, and the total bacterial count (CFU/ml) was determined by direct counting. Plates with ≥400 colonies were categorized as "too numerous to count" (TNTC), indicating a high level of bacterial contamination in the sample.

All of the data collected from the survey, scoring, and diagnostic tests were digitized using Microsoft Excel spreadsheets (Microsoft Office 365, USA) and imported into Microsoft Access (Microsoft Office 365) for assembly; datasets for the farm level and MCC level were then built. ArcGIS software (ESRI Japan, Tokyo, Japan) was used to map the milk contamination levels, milk handling volumes, and value chain status.

2.5. Statistical analysis

Descriptive analysis was conducted for all data collected at the farms and the MCCs. The means, medians, and ranges were calculated for numerical variables, and the number of responses and percentages were calculated for categorical variables. The dairy value chain through cooperatives was described by summarizing the questionnaire survey results from the farms and the MCCs. The Wilcoxon rank sum test was used to compare the milk sales volume between MCCs that accepted milk from JICA project farms and those that did not, and between MCCs that sold milk to processing plants and those that did not.

The within-farm prevalence of sub-clinical mastitis diagnosed by CMT was compared between before and after the intervention using mixed-effects model with binomial errors, selecting identity of farms as a random effect, in lme4 package (Bates et al., 2023). The six-point farm bulk milk hygiene scores were compared between before and after the intervention using Wilcoxon signed rank test, matching the scores of respective farms. The proportions of the farms with bulk milk score <4 were compared between before and after the intervention using a chi-squared test.

Sub-clinical mastitis is generally caused by bacterial infection, which can increase the risk of bacterial contamination in farm bulk milk. Spearman's correlation test was performed for the six-point farm bulk milk hygiene score and the within-farm prevalence of sub-clinical mastitis by CMT, for before and after the intervention. A detailed study on the risk factors for sub-clinical mastitis on these 30 farms has been published elsewhere (Miyama et al., 2020).

To evaluate the effect of hygiene practices during milking on the milk quality in farm bulk milk, first, the six-point bulk milk hygiene scores were compared using the Wilcoxon rank sum test between the farms that conducted or did not conduct each of the hygiene practice items on the checklist. Second, the number of hygiene practice items on the checklist that were conducted was counted as the hygiene practice score. Spearman's correlation test was performed for the six-point farm bulk milk hygiene score and the hygiene practice score. These analyses for the effect of hygiene practices on the quality of farm bulk milk were conducted for before and after the intervention. The proportions of hygiene practice items conducted were compared between before and after the intervention using mixed-effects model with binomial errors, selecting identity of farms as a random effect.

The relationship between the hygiene practice score and herd size was analyzed using a generalized linear model with Poisson errors with the hygiene practice score as an outcome variable and herd size and cattle breed as explanatory variables to test the hypothesis that milking hygiene is higher on farms with intensified milk production, for before and after the intervention. The relationship between the herd size and average milk yield per cow was analyzed using linear regression after checking the normality of the milk yield distribution, selecting the average milk yield as an outcome variable.

As many dry swabs and tap water samples had TNTC total bacterial counts (see Section 3), the farm bulk milk hygiene scores were compared using the Wilcoxon rank sum test between the farms with and without heavy contamination (TNTC) in the empty cans, and between the farms with or without heavy contamination (TNTC) in tap water.

To characterize the level of hygiene of bulk cooler milk in MCCs, a Spearman's correlation test was performed for the relationship between the average daily milk volume shipped from the MCCs and the logarithm of the total bacterial count ($\log_{10}$ CFU/ml). To test the hypothesis that milk quality is higher if a MCC sells milk to a milk processing plant, the mean $\log_{10}$ total bacterial counts were compared using a t-test between MCCs that sold milk to processing plants and those that did not (i.e., they sold milk to milk shops and milk vendors in towns only).

To evaluate the magnitude of post-harvest increases in the level of bacterial contamination in raw milk, a series of simulation was applied. This was because (1) the level of bacterial contamination in farm bulk milk was measured with the six-point hygiene score while that of MCCs was enumerated using 3M Petri film, and (2) the sample size of MCCs was only 15, and resampling of these values would provide robust view by Bayesian approach. To implement this analysis, the $\log_{10}$ total bacterial counts of farm bulk milk and bulk cooler milk in MCCs were first simulated, then the simulated $\log_{10}$ total bacterial counts were compared. For the simulation of the $\log_{10}$ total bacterial counts in farm bulk milk, a random number was sampled from a uniform distribution of the logarithm range of the bacterial counts of the six-point scores for each of 29 farms (a farm was not included since farm bulk milk was not sampled from that farm), and one value was resampled from these 29 values to obtain a total sample number of 30. To estimate the mean $\log_{10}$ total bacterial count in farm bulk milk, calculation of a mean of randomly sampled these 30 values was iterated 1,000 times. For the simulation of the $\log_{10}$ total bacterial count in bulk cooler milk of MCCs, 30 values were randomly sampled from a set of $\log_{10}$ total bacterial counts from the 15 MCCs studied. A comparison using a t-test of these 30 simulated values each from farm bulk milk and MCCs was repeated for 1,000 iterations to obtain a Bayesian simulation of the p-values. This comparison between farm bulk

milk and MCCs was performed for the farm bulk milk samples both before and after the intervention.

The simulated $\log_{10}$ total bacterial count in farm bulk milk and MCC bulk milk were compared with the $\log_{10}$ acceptable limit of bacterial count for human consumption, 10^5 CFU/ml, according to the International Microbiological Criteria for Dairy Products for raw bovine milk intended for processing for human consumption [Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, 2003].

All statistical analyses were conducted using R statistical software version 4.2.1 (R Core Team, Vienna, Austria).

2.6. Participatory value chain analysis

To understand the relationships between dairy cooperative value chains in Mbarara and wider dairy value chains interconnecting with the capital, Kampala, and other formal and informal dairy value chains, online participatory appraisals were conducted with the 30 farmers who participated in the JICA project, the Uganda Crane Creameries Cooperatives Union (UCCCU), and the Mbarara DVO via social networking service and online meetings in November 2022. The research team created an online chat group in a social networking service in 2017, and invited the 30 farmers, UCCCU personnel and Mbarara DVO officers to enhance the project communication. The farmers still regularly use the online group chat to exchange information on dairy farming.

For the online participatory appraisal, first, the research team had an online meeting using Webex system (Cisco, San Jose, USA) with a representative farmer, to construct the first draft of the dairy value chain. There were disruptions in connection several times, but this exercise successfully produced a draft figure in 40 min. Second, the draft figure was shared in the online chat group, and the group members were invited for comments. A number of comments were provided by the group members in a few days. The draft figure was improved based on the comments, and was shared in the group chat again for additional comments. This procedure was repeated for three times, and the figure was finalized at the third round with the approval from the members.

3. Results

3.1. Structure of dairy cooperative value chains

Figure 2 shows the dairy cooperative value chains connecting the 30 dairy farms studied. The mean herd size of the 30 farms was 73.6 cows (median: 60.5, quartile range: 30–79, range: 7–547). The mean milk yield per cow per day at the farms was 9.2 l (median = 8.3, range: 1.5–20.1), and the mean farm milk yield per day was 193.2 l (median = 162.5, quartile range: 67.0–247.2, range: 3.0–808.5) in 2019. There was no significant linear relationship between the log herd size and average milk yield per cow [slope = −0.01, standard error (SE) = 0.01, p = 0.270].

The mean daily milk sales volume at 15 MCCs was 1,669 l (median = 900, quartile range: 450–2,250, range: 100–6,850). Among the 14 MCCs that provided a response, 6 MCCs (42.9%) bought milk from JICA project farms, and the mean number of JICA project farms that sold milk to an MCC was 3.2 (median = 2.5, range: 1–9, Figure 2). Out of the 30 JICA project farms, 16 farms (53.3%) sold milk to the six MCCs. The mean milk sales volume was significantly higher in the MCCs that accepted milk from JICA project farms (2,933.3 l) than in those that did not (866.3 l, p = 0.033). Out of 15 MCCs, nine MCCs sold milk to processing plants (60.0%), and the mean milk sales volume was marginally higher in the MCCs that sold milk to processing plants (2,386.7 l) than in those that specialized in selling raw milk to local towns (591.7 l, p = 0.052). Of the nine MCCs that sold milk to processing plants, seven MCCs (77.8%) also sold milk to local raw milk sales: milk shops and vendors, and even to local individual customers. Among the MCCs that sold milk to both processing plants and local customers, the mean proportion of local raw milk sales was 25.8% (median = 10.0%, range: 1.8%−70.6%).

Figure 2 shows the geographical structure of the dairy cooperative value chains in Mbarara District. There were three hub dairy cooperative MCCs that accepted milk from JICA project farms. Farmers did not always sell milk to neighboring MCCs; they sometimes chose to transport the milk over a long distance to the hub collection centers. Regardless the sales destinations (processing plants or milk shops/vendors), the majority (12/15, 80.0%) were located on the main tarmac roads.

Figure 3 shows the structure of dairy value chains starting from farms in Mbarara District that were identified in the participatory online appraisals. The field surveys in this study targeted the dairy value chains of dairy cooperatives belonging to the UCCCU. The participatory appraisals identified two other types of MCCs: MCCs owned by dairy processors and those by independent centers of traders. Dairy cooperatives distributed raw milk to both formal and informal value chains, as can be described quantitatively, but MCCs of independent traders distributed entirely to information value chains, which included a large volume of long distance supply to Kampala, the capital of Uganda. The milk collected by foreign investors was processed for export. There were several MCCs of domestic dairy processors of pasteurized and packaged milk, which were distributed to both Mbarara town and other cities, including Kampala. Ghee, cheese, yogurt, and butter were produced by foreign and domestic processors, dairy cooperatives, and home-based cottage industries. Consumers in Mbarara District bought raw milk directly from farm gates, MCCs, milk shops, and milk vendors.

3.2. Milk hygiene

The mean farm bulk milk contamination score was not significantly different between before (3.3, median: 4; range: 1–4) and after (3.2, median: 3; range: 2–5) the intervention (p =

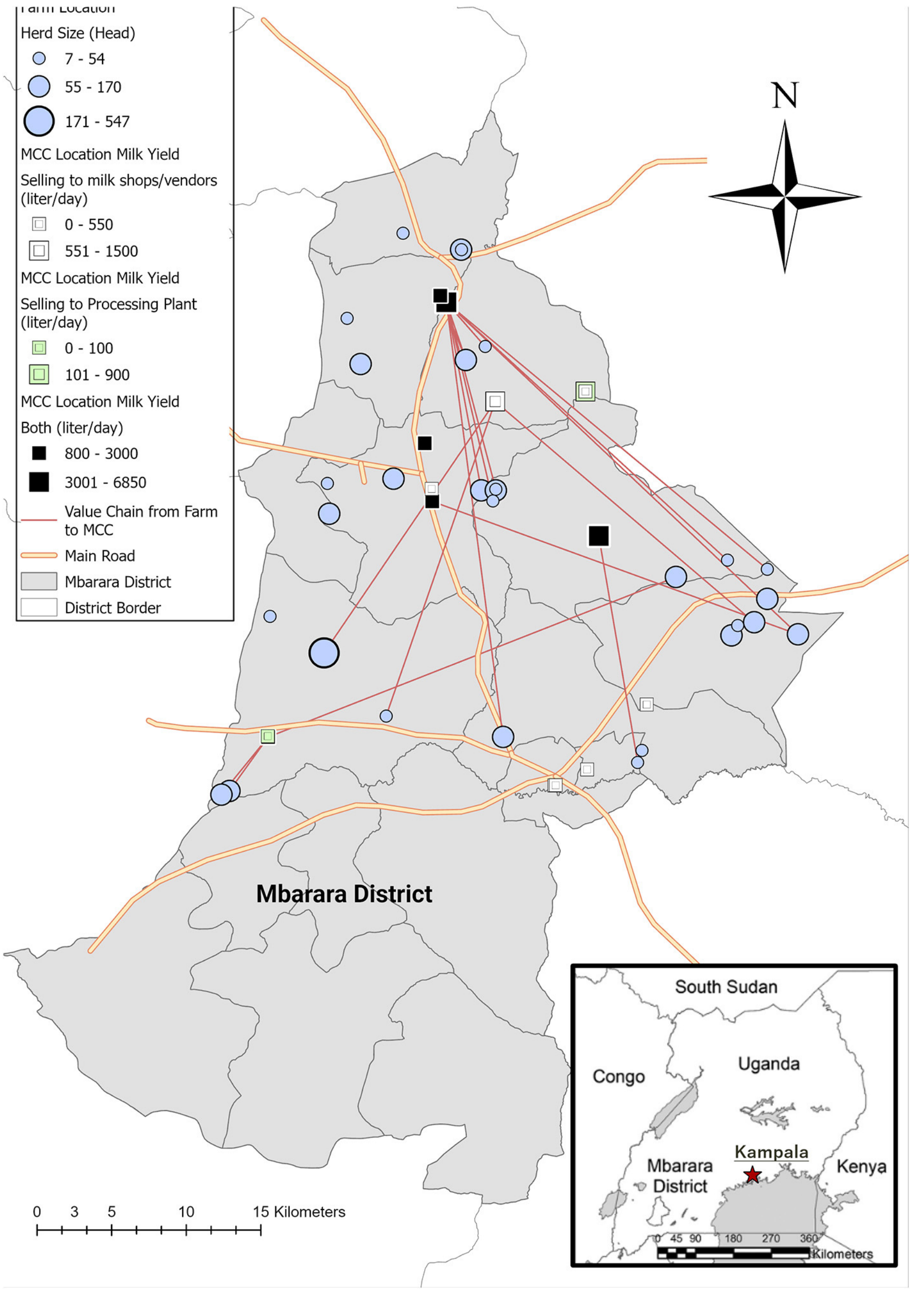

FIGURE 2
The structure of dairy cooperative value chains from the studied dairy farms to milk collection centers (MCCs) in Mbarara District. The blue circles indicate the locations and herd sizes of the dairy farms. The green squares indicate the locations and milk yields in liters of the MCCs that sold milk to processing plants only. The black squares indicate the locations and milk yields in liters of the MCCs that sold milk to both processing plants and milk shops/vendors in town. The white squares indicate the locations and milk yields in liters of the MCCs that sold milk to shops/vendors in town only.

0.418). However, the proportion of hygienic farm bulk milk samples with score <4 significantly increased from before the intervention (37.9%, 11/29 farms) to after (69.0%, 20/29 farms, $x^2 = 4.4$, df $= 1$, $p = 0.035$). Based on the scores, the mean $\log_{10}$ total bacterial count of farm bulk milk was simulated to be 3.69 (95% credible interval: 3.60–3.78) and 3.79 (95% credible interval: 3.76–3.81), before and after the intervention, respectively. Both total bacterial counts of farm bulk milk before and after the intervention were below the acceptable limit for human consumption as source milk intended for processing ($p < 0.001$).

The mean within-herd prevalence of sub-clinical mastitis significantly reduced from 72.3% before the intervention to 25.8% after [difference in logit $= -2.29$, SE $= 0.160$, $p < 0.001$, random effect: standard deviation (SD) $= 0.775$]. Based on the CMT results, there was no significant relationship between the farm bulk milk score and the within-farm prevalence of sub-clinical mastitis either before the intervention (rho $= 0.09$, $p = 0.646$) or after (rho $= 0.25$, $p = 0.199$).

Table 1 shows a comparison of the frequency and percentages of milking hygiene practice items conducted before and after the

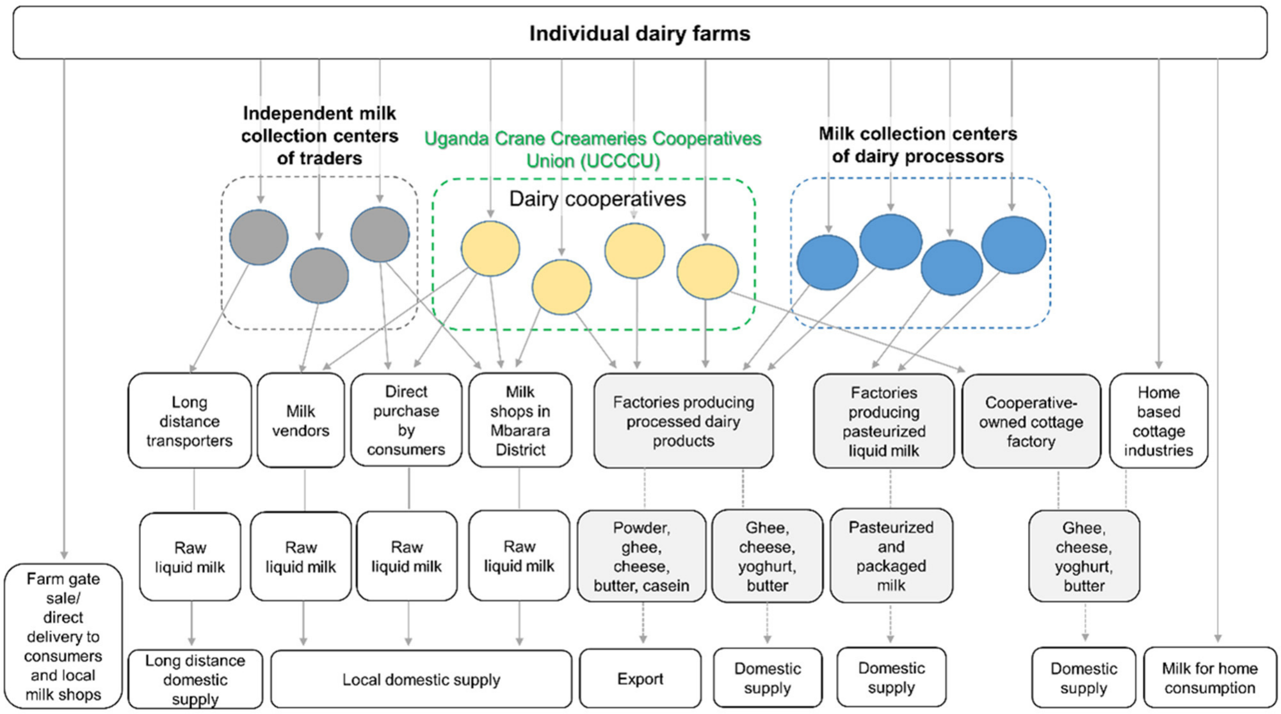

FIGURE 3
The structure of dairy value chains starting from individual dairy farms in Mbarara District, Uganda. Gray squares show the formal value chains, which export or sell treated products, and white squares show the informal value chains of raw liquid cow milk.

intervention. The mean proportion of the 19 items on the milking hygiene practice conducted significantly increased from before the intervention (4.2%) to after (67.4%, difference in logit = 7.13, SE = 0.636, $p < 0.001$, random effect SD = 2.498). The practices most conducted after the intervention were the milking of cows with mastitis last, and the disinfection of hands at the parlor (87.0%); the least conducted was pre-dipping using a strip cup (40.0%). Post-milking care (post-dipping, 47.0%) was often not conducted.

Table 2 shows the results of comparisons of mean farm bulk milk hygiene scores between the farms conducting milk hygienic items and not conducting among 29 dairy farms before the intervention. The mean bulk milk hygiene score was significantly or marginally lower (less bacteria contamination) in the farms conducting gentle hand milking (2.33), washing used towel with soap and rinsing cleanly (2.00), and drying a towel in sunlight (2.00) than the farms not conducting (3.36, $p = 0.036$; 3.41, $p = 0.053$, and 3.41, $p = 0.053$). In contrast, there was no milk hygiene practice item with significant difference in milk hygiene score between the farms conducting and not conducting after the intervention (Table 3). There was no significant relationship between the milk hygiene practice score and the farm bulk milk hygiene score both before the intervention (rho = −0.29, $p = 0.121$) and after (rho = 0.06, $p = 0.787$). The number of hygiene practice items conducted was not associated with the herd size either before the intervention (slope in log = −0.008, SE = 0.017, $p = 0.656$) or after (slope in log = 0.003, SE = 0.017, $p = 0.859$).

The proportions of samples with a TNTC bacterial count among the dry swab and wet swab samples were 73.3% (22/30) and 80.0% (24/30), respectively. Water used for cleaning was also contaminated, with 56.7% (17/30) of the samples having a TNTC bacterial count. There were no significant differences in the mean bulk milk hygiene scores between farms with or without TNTC contamination in the pre-milking dry collecting cans (3.1 and 3.3, respectively, $p = 0.957$), between farms with or without TNTC contamination in the collecting cans after washing (3.3 and 2.8, respectively, $p = 0.467$), and between farms with or without TNTC contamination in the water used for cleaning (3.4 and 2.9, respectively, $p = 0.234$).

The $\log_{10}$ mean total bacterial count of bulk cooler milk in MCCs was estimated to be 6.50 (95% credible interval: 4.15–7.68). The proportion of milk samples with a total bacterial count below 10^5 CFU/ml, which is the limit for human consumption [Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, 2003], was 13.3% (2/15). When receiving milk, 100.0% (14/14), 92.8% (13/14), and 7.1% (1/14) of the MCCs ($n = 14$) conducted regular alcohol tests, used a lactometer, and conducted resazurin tests. There was no significant correlation between the $\log_{10}$ total bacterial count in sold bulk cooler milk and the milk sales volume in MCCs (rho = 0.30, $p = 0.277$). The $\log_{10}$ total bacterial counts of bulk cooler milk were not significantly different between the MCCs that shipped milk to the processing plants of foreign investors (mean = 6.6) and those that shipped milk to local milk shops and vendors (mean = 6.3, $t = 0.51$, df = 13, $p = 0.621$).

TABLE 2 Comparisons of mean farm bulk milk hygiene scores between the farms conducting milk hygiene practice items and not conducting among 29 dairy farms before the intervention.

Hygiene practice items	Mean hygiene score in farms conducting	Mean hygiene score in farms not conducting	p-value
Before going to the milking parlor			
1. A proper concentration of disinfectant was prepared	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
2. Dry towels were dipped into the disinfectant, and kept there for 10 min	– ($n = 0$)	3.31 ($n = 29$)	–
3. Towels were squeezed with clean hands, and kept in a bucket	– ($n = 0$)	3.31 ($n = 29$)	–
4. Another empty bucket was prepared for used towels	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
5. A bucket of water was prepared for washing hands	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
At the parlor			
1. Cows with mastitis were milked last	2.75 ($n = 4$)	3.40 ($n = 25$)	0.145
2. Hands were washed before milking each cow	2.67 ($n = 3$)	3.38 ($n = 26$)	0.248
3. Hands were disinfected	– ($n = 0$)	3.31 ($n = 29$)	–
4. Pre-dipping was conducted using a strip cup	3.00 ($n = 2$)	3.33 ($n = 27$)	0.692
5. One towel was picked up from the bucket, and one towel was used for each cow when wiping the teats	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
6. Each teat was wiped using a hand-twisting motion to remove dirt	– ($n = 0$)	3.31 ($n = 29$)	–
7. A clean part of the towel was used for each teat	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
8. After finishing all teats, the teat tips were wiped using the other side of the towel	– ($n = 0$)	3.31 ($n = 29$)	–
9. The used towel was put into the other bucket	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
When you milk			
1. Milking was done gently (hand milking) WITHOUT hurting the teats	2.33 ($n = 3$)	3.42 ($n = 26$)	0.036
After milking			
1. Post-dipping with iodine was conducted	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
2. Teat coverage by the iodine was 75% to 100%	2.00 ($n = 1$)	3.36 ($n = 28$)	0.191
Preparation for the next milking			
1. All of the used towels were washed with soap and rinsed cleanly	2.00 ($n = 2$)	3.41 ($n = 27$)	0.053
2. Towels were dried in sunlight	2.00 ($n = 2$)	3.41 ($n = 27$)	0.053

In the evaluation of the magnitude of post-harvest contamination/bacterial multiplication, all of the 1,000 iterations of Wilcoxon rank sum tests comparing the $\log_{10}$ total bacterial counts between farm bulk milk (mean, before the intervention: 3.69, and after: 3.79, respectively) and bulk cooler milk in MCCs (mean: 6.50) produced p-values <0.001, suggesting that the level of bacterial contamination is significantly higher in MCCs than in farm bulk milk.

4. Discussion

This study was conducted to quantitatively understand the structure of the dairy cooperative value chains in an intensive dairy production area of Uganda, Mbarara District, and to assess the hygiene along the chain, and thereby identify the bottleneck of dairy hygiene intervention.

The JICA project targeted dairy farms that belong to the UCCCU, because the union comprised dairy cooperatives involving 18,000 farmers as of 2016, and was the largest dairy farmers' association in Uganda (Rakuno Gakuen University, 2019). Southwestern Uganda, including its historical center, Mbarara, the capital of the Ankole Kingdom, has been reported to be the largest source of raw milk in the value chain in Kampala, the capital of Uganda (Makita et al., 2010). However, the present study found that raw milk collected at the MCCs of dairy cooperatives under UCCCU was not transported to Kampala, but was targeted at formal value chains connected to export and domestic trades and the local domestic raw milk supply in Mbarara District. Although the relative contributions of entire dairy value chains were not quantified, the additional

TABLE 3 Comparisons of mean farm bulk milk hygiene scores between the farms conducting milk hygiene practice items and not conducting among 29 dairy farms after the intervention.

Hygiene practice items	Mean hygiene score in farms conducting	Mean hygiene score in farms not conducting	p-value
Before going to the milking parlor			
1. A proper concentration of disinfectant was prepared	3.14 ($n = 21$)	3.25 ($n = 8$)	0.739
2. Dry towels were dipped into the disinfectant, and kept there for 10 min	3.14 ($n = 21$)	3.25 ($n = 8$)	0.739
3. Towels were squeezed with clean hands, and kept in a bucket	3.20 ($n = 20$)	3.11 ($n = 9$)	0.901
4. Another empty bucket was prepared for used towels	3.11 ($n = 18$)	3.27 ($n = 11$)	0.688
5. A bucket of water was prepared for washing hands	3.36 ($n = 22$)	2.57 ($n = 21$)	0.114
At the parlor			
1. Cows with mastitis were milked last	3.12 ($n = 25$)	3.50 ($n = 4$)	0.336
2. Hands were washed before milking each cow	3.27 ($n = 22$)	2.85 ($n = 7$)	0.521
3. Hands were disinfected	3.24 ($n = 25$)	2.75 ($n = 4$)	0.528
4. Pre-dipping was conducted using a strip cup	3.16 ($n = 12$)	3.17 ($n = 17$)	0.780
5. One towel was picked up from the bucket, and one towel was used for each cow when wiping the teats	3.21 ($n = 19$)	3.10 ($n = 10$)	0.904
6. Each teat was wiped using a hand-twisting motion to remove dirt	3.18 ($n = 22$)	3.14 ($n = 7$)	1.000
7. A clean part of the towel was used for each teat	3.20 ($n = 20$)	3.11 ($n = 9$)	0.901
8. After finishing all teats, the teat tips were wiped using the other side of the towel	3.20 ($n = 20$)	3.11 ($n = 9$)	0.901
9. The used towel was put into the other bucket	3.21 ($n = 19$)	3.10 ($n = 10$)	0.904
When you milk			
1. Milking was done gently (hand milking) WITHOUT hurting the teats	3.29 ($n = 24$)	2.60 ($n = 5$)	0.237
After milking			
1. Post-dipping with iodine was conducted	3.21 ($n = 14$)	3.13 ($n = 15$)	0.945
2. Teat coverage by the iodine was 75%—100%	3.21 (n =14)	3.13 ($n = 15$)	0.945
Preparation for the next milking			
1. All of the used towels were washed with soap and rinsed cleanly	3.14 ($n = 21$)	3.25 ($n = 8$)	0.739
2. Towels were dried in sunlight	3.14 ($n = 21$)	3.25 ($n = 8$)	0.739

participatory online appraisals characterized entire formal and informal dairy value chains starting from Mbarara District. The entire picture indicated that the findings of the present study are representative of the milk hygiene situation only in dairy cooperative value chains. Therefore, the hygiene of dairy value chains of MCCs owned by independent traders and dairy processors should be separately studied. The UCCCU is preparing to open its own factory that produces pasteurized and packaged milk for domestic supply in Uganda (personal communications). This factory will bring direct revenue from consumers to the UCCCU, and will contribute to formalizing dairy value chains, and improve public health in Uganda. It has been estimated that the pasteurization of milk in intensive dairy production areas would have the greatest impact on reducing milk-borne diseases, such as brucellosis, in Kampala, if introduced (Makita et al., 2010).

The JICA project participating farms adopted the milk hygiene practice items very well, and the behavioral changes may have occurred because of the provision of epidemiological evidence and frequent visits for demonstration of intervention packages (Rakuno Gakuen University, 2019; Miyama et al., 2020). Before the intervention, there were significant associations between conduct of gentle hand milking and hygiene in the towels used for wiping teat and better farm bulk milk quality. The findings on the effect of these hygiene practices were similar to those on sub-clinical mastitis in these farms (Miyama et al., 2020), suggesting some potential effect of reducing sub-clinical mastitis on reducing bacterial contamination in bulk milk. However, our results showed that the level of bulk milk hygiene was not improved by the intervention in the health of cows. After the intervention, there was no milk hygiene practice item or handling of equipment which was associated with bulk milk hygiene. This may be due

to the effects of several other hygiene practices introduced, and also contamination by the failure in hygienic handling of milk and equipment. The high levels of contamination of the water and milk cans before and after milking were of concern, and they indicated that the cleaning process was ineffective. The dry swab culture results suggested that sun-drying may have little effect against bacterial contamination if the washing process was inadequate inside a can. Regarding the issue of contaminated water, there have been reports of microbial contamination during the handling and storage after water collection in sub-Saharan Africa (Harris et al., 2013; Owusu-Kwarteng et al., 2020). It has also been reported that water contamination can occur when water storage containers are placed outdoors (Amenu et al., 2016). Although such contamination may be present, the level of hygiene in farm bulk milk is acceptable as a source milk intended for processing for human consumption [Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, 2003].

A critical challenge identified was that post-harvest dairy hygiene still needs to be improved even though milk hygiene at the farm level is below the acceptable limit for processing. This study showed a significant increase in the bacterial counts at the MCCs when compared to the dairy farms. Only 13.3% of the MCCs in Mbarara District met this standard of the International Microbiological Criteria for Dairy Products [Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, 2003]. Inadequate dairy hygiene practices by dairy farmers may contribute to increases in the bacterial counts in the value chain from dairy farmers to MCCs (Food and Agriculture Organization, 2019; Majalija et al., 2020; Miyama et al., 2020). Although the farms examined in the present study were trained for hygiene in the JICA project and used metal milk cans, there were still farmers using plastic buckets for milking in Mbarara District (Daburon and Ndambi, 2019). Dairy farms with poor hygiene management may cause more contamination in the MCCs. Even more problematic is the use of plastic jerry cans for transport. These cans cannot be adequately cleaned, and their surfaces are easily scratched, which promotes bacterial contamination. Although it has been reported that more farmers and MCCs in Mbarara District use milk cans when compared to other districts (Van Campenhout et al., 2019), the high contamination level of bulk milk in the MCCs studied may have been due to less hygienic milking equipment and contaminated water from farms that were not trained for hygiene in the JICA project (Van Campenhout et al., 2019; Majalija et al., 2020). The present study did not examine other factors associated with the transportation of milk from farms to MCCs, such as the milk temperature, mode of transportation, and the amount of time in transport, nor the environmental conditions at the dairy farms and MCCs. As shown in Figure 2, sometimes, farms did not transport milk to the nearest MCCs, but to distant ones. Some farms used a truck, motorcycle, or even a bicycle for transporting milk without cooling equipment. A prolonged transportation period without cooling may lead to increased bacterial counts (Mogotu et al., 2022). All of these factors might have contributed to the high bacterial count in bulk milk in the MCCs. As this study has shown, milk is transported to Kampala in both formal (packaged pasteurized milk) and informal value chains. A report has suggested that informal marketing systems generally lack proper sanitation, and raw milk is traded without regard to international standards for quality, pasteurization, or cold chain facilities (Majalija et al., 2020). Another study in Kampala reported that all of the 50 milk samples collected from milk outlets had total aerobic counts exceeding the limits of the World Health Organization and Uganda National Bureau of Standards (Kateregga et al., 2019). There is another limitation of the study—the sampling framework of dairy farms. To capture entire dairy value chain starting from Mbarara, larger sample size involving the dairy farms which do not belong to UCCCU would be necessary.

To improve the quality of milk in dairy value chains, the Netherlands Development Organization conducted a pilot project on a Quality Based Milk Payment System (QBMPS) in Mbarara between 2016 and 2019 (Daburon and Ndambi, 2019). The project introduced QBMPS to three processors (a large, a medium, and a small processor), and the large processor succeeded in developing QBMPS in the sourcing network, while the medium and small processors have yet to finish the pilot project (Daburon and Ndambi, 2019). The QBMPS needs to be introduced at least at the MCC level, as the processor examines the milk quality at that level. A pilot QBMPS project in Kenya has shown that the milk quality improved as well (Njiru and 3R Kenya Project, 2018). Sub-clinical mastitis can decrease the milk yield without being noticed (Blowey and Edmondson, 2010), and rejection of low-quality milk affects both dairy farmers and the MCCs. The present study showed the bottleneck of dairy hygiene intervention in Uganda. Awareness of the importance of milk quality must be urgently raised among health and agricultural authorities and private sectors to shift to quality-based thinking along entire dairy value chains throughout the country.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KM, DB, JBu, WM, and AA conceptualized the study. KM, JBu, DB, JBy, MA, TK, and YM designed the study. JBu, DB, JBy, MA, TS, TK, and KM conducted field surveys. WM and AA facilitated the field surveys. YS, HS, JBy, and KM conducted the formal analyses. YS and KM drafted the manuscript. All authors edited and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1110915/full#supplementary-material

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Describing food safety perceptions among growers and vendors in Cambodian informal vegetable markets

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Improving food safety often requires individuals or groups to adopt new food safety behaviors. Understanding individuals' perceptions of food safety is an important step in creating programs that enable the adoption of such behaviors. To inform the design of food safety programs in Cambodia, this study measured perceptions of vegetable safety among vegetable growers ($n = 69$; Battambang, Siem Reap) and vendors ($n = 31$; Phnom Penh) involved in Cambodian informal vegetable markets using a quantitative questionnaire. The majority of respondents (≥ 62.7%, lower bounds of 95% confidence intervals at least 46.2%) across all groups (growers and vendors) were at least "moderately concerned" (scale: "extremely concerned," "moderately concerned," "slightly concerned," "not concerned at all") about the safety of vegetables sold in Cambodia. However, the mean estimated probability of respondents reporting that chemical contamination was of greater concern than microbial contamination was 84.9% [76.0, 90.9]%. Most respondents reported familiarity with the health effects of consuming chemically (71.4% [61.5, 79.6]%) or microbially (57.3% [47.2, 66.9]%) contaminated vegetables. However, less than half (between 7.3% and 48.4%) of all respondents provided a commonly recognized example of such health effects. Across all groups, respondents most frequently perceived contamination of vegetables as occurring primarily "at the farm" (≥ 76.7%, lower bounds of 95% confidence intervals at least 61.5%, and ≥ 39.3%, lower bounds of 95% confidence intervals at least 21.2%, respectively). Additionally, most respondents (≥ 51.6%, lower bounds of 95% confidence intervals at least 34.0%) perceived "vegetable farmers" as primarily responsible for preventing chemical contamination. Perceptions of responsibility for preventing microbial contamination varied across groups ($p = 0.02$). Of the vendors in Phnom Penh, growers in Battambang, and growers in Siem Reap involved in this research, 22.6%, 39.0%, and 53.6%, respectively, described at least one commonly accepted contamination prevention practice. These results suggest that food safety programs for each of the described groups should include efforts to increase participants' understanding of the health impacts of consuming contaminated vegetables. However, specific emphasis should be placed on increasing awareness on the health impacts of consuming vegetables contaminated with microbial pathogens as respondents were generally less aware and concerned with microbial vs. chemical contamination of vegetables. Additionally, programs targeting vegetable growers could leverage growers' sense

of personal responsibility for both contamination and contamination prevention, while programs for vegetable vendors may need to emphasize the importance of vendors in ensuring vegetable safety.

KEYWORDS

Cambodia, food safety, informal markets, perceptions, vegetables

Introduction

Cambodia, with a growing population of ~16.7 million (Central Intelligence Agency (CIA), 2022), has experienced significant economic and social progress in recent years. Poverty rates have declined from 47.8% in 2007 to 13.5% in 2014, and in 2015 the World Bank altered its classification of Cambodia from a low income to a lower-middle income economy (World Bank Group, 2017). Agriculture continues to play a central role in the Cambodian economy. Over 60% of the Cambodian population lives in rural areas, where approximately half of those employed between the ages of 15 and 64 work primarily in production agriculture (National Institute of Statistics & Ministry of Planning (NIS, MOP), 2020). Furthermore, the agricultural sector is estimated to represent 22% of Cambodia's GDP, supplying both international and domestic markets with fresh fruits and vegetables among many other products and commodities (U.S. Mission Cambodia, 2020; Agricultural Marketing Office (AMO), 2022; National Institute of Statistics and Ministry of Planning (NIS, MOP), n.d.).

Vegetables in particular are an important part of the Cambodian diet, both in terms of quantity and frequency of consumption (Windus et al., 2022). Many Cambodians purchase their vegetables in informal, open-air markets, which are comprised of networks of vegetable producers, collectors, distributors, and vendors (Sokhen et al., 2004; Desiree et al., 2020). These informal vegetable markets exist largely outside of governmental oversight (e.g., regulatory monitoring/surveillance, taxation, etc.), and numerous food safety gaps have been identified in these markets, including poor sanitation of vegetable transport vehicles, improper vegetable handling, insufficient composting time, inadequate cold storage, lack of sanitation facilities and infrastructure (e.g., toilets, handwashing stations, adequate drainage, etc.), presence of pests, use of poor quality water for irrigation or vegetable washing, and ample opportunity for cross-contamination between raw animal-source foods and raw vegetables, among others (Desiree et al., 2020). In addition, several groups have isolated *E. coli*, coliforms, and *Salmonella* spp. from raw vegetables and vegetable contact surfaces in informal vegetable markets in Cambodia (Phoeurk et al., 2019; Desiree et al., 2021; Schwan et al., 2021). Considering these food safety gaps along with the substantial role of informal vegetable markets in meeting the high demand for vegetables in Cambodia, improving food safety practices in these markets is clearly an essential component of reducing the risk of foodborne illness in Cambodia.

Foodborne illnesses are most frequently the result of diarrheagenic pathogens (Havelaar et al., 2015). For low and lower-middle income countries, information on the exact proportion of all diarrheal diseases that are caused by foodborne pathogens is generally not available. However, even conservative estimates indicate that the negative health impacts of diarrhea associated with the consumption of contaminated food are considerable in these contexts (Grace, 2015). Diarrhea may be either acute or chronic and can result in dehydration, malabsorption of nutrients, economic losses, and, in extreme cases, death (National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2016; World Health Organization (WHO), 2017; Nemeth and Pfleghaar, 2021; Niyibitegeka et al., 2021). Children are especially impacted by diarrheal disease: according to the World Health Organization (WHO), diarrhea is the second most common cause of death in children under 5 years old around the world (World Health Organization (WHO), 2017). In Cambodia, an estimated 7.4 and 15% of deaths in children 0–4 years old and 1–59 months old, respectively, are attributed to diarrhea (World Health Organization (WHO), 2018). In addition, inflammation of the GIT resulting from diarrhea inhibits the absorption of nutrients and can contribute to malnutrition. This is of particular concern in Cambodia, where rates of stunting (21.9%) and wasting (9.6%) in children under 5 years of age remain medium to high according to the prevalence thresholds set by the WHO (de Onis et al., 2018; National Institute of Statistics, Ministry of Health, and ICF (NIS, MoH, and ICF), 2022). Frequent exposure to foodborne pathogens may also contribute to the development of environmental enteropathy (environmental enteric dysfunction) in both children and adults, a condition associated with intestinal villus blunting, increased intestinal permeability, and nutrient malabsorption (Kelly et al., 2016; Louis-Auguste and Kelly, 2017).

Several Cambodian government policies and development organization initiatives have sought to improve nutritional outcomes in Cambodia by supporting increased production and consumption of nutrient-dense foods, including fresh fruits and vegetables (Ministry of Agriculture, Forestry and Fisheries, the European Union, and the United States Agency for International Development (MAFF, EU, and USAID), 2015; Royal Government of Cambodia, 2019; Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification, 2020; Save the Children, 2020). However, fruits and vegetables are considered high-risk foods in terms of food safety. Microbial contamination is of especial concern, as vegetables are frequently consumed raw and therefore are often not exposed to high temperatures or other control measures that would reduce or eliminate microbial loads. Given that vegetables in informal markets in Cambodia may be frequently contaminated with microbes, consuming fresh vegetables purchased from these markets may carry a particularly high risk of foodborne illness. As such, the malabsorption of nutrients caused by diarrheal diseases and other foodborne illnesses contracted through the consumption of fresh vegetables may ultimately negate the nutritional benefits of the produce itself.

To improve food safety in informal vegetable markets in Cambodia, those involved in the informal vegetable value chain must begin implementing positive food safety practices. In general, in order

to design programs that effectively facilitate the adoption of a new practice it is important to understand and account for the target audiences' existing perceptions about the problem that the new practice would address. Identifying the level of concern among members of the target audience regarding that problem, for instance, could help program developers determine whether sensitization to the issue itself is needed prior to the implementation of a program that promotes the practice in question. Evaluations of audience members' perceptions of who is responsible for the particular problem, how frequently the problem occurs, and the ramifications of the problem are needed to inform program development and implementation. For instance, information regarding these perceptions may facilitate recognition of potential barriers to and enablers of behavior change embedded in the mindset of the target audience. Once identified, both enablers and barriers can be harnessed or reduced, thereby improving the efficacy of engagement programs and increasing the likelihood of adoption of the behavior.

Research objectives

Assessing perceptions of food safety among actors involved in informal vegetable markets in Cambodia is a relatively new area of research and is not comprehensively understood. The purpose of this research was to explore and describe perceptions of vegetable safety among vegetable growers and vegetable vendors involved in informal vegetable markets in Cambodia. Specifically, the study aimed to measure participants' perceptions of: (1) the importance of vegetable safety; (2) the frequency of vegetable contamination; and (3) the health impacts of consuming contaminated vegetables. Additionally, the study aimed to understand participants' current practices for preventing vegetable contamination and their perceptions of where contamination primarily occurs and who is most responsible for preventing contamination. Describing these perceptions, as well as the differences in perceptions across participant groups (i.e., vegetable vendors in Phnom Penh and vegetable growers in Battambang and Siem Reap), was intended to facilitate the development of food safety programs tailored to specific participant groups that effectively promote the adoption of positive food safety behaviors within each group.

Methods

Ethical approval

All research protocols described here were reviewed by the Institutional Review Board of Purdue University (IRB-2020-383; West Lafayette, IN) and the Royal University of Agriculture (Phnom Penh, Cambodia).

Questionnaire design and pilot study

In order to inform the design of future food safety programs, the researchers designed a quantitative questionnaire intended to measure perceptions of food safety among vegetable growers and vegetable vendors involved in informal vegetable markets in Cambodia. Due to travel constraints between and within countries related to the COVID-19 pandemic, however, the questionnaire could not be piloted with targeted groups (i.e., vegetable producers, vendors, etc.) as that audience required in-person recruitment and in-person questionnaire completion. Thus, the questionnaire was modified to an online version and piloted with undergraduate students in food technology and related majors at Cambodian universities as connecting with this audience and completing the questionnaire could take place entirely online and did not require any travel. The pilot questionnaire sought to explore and describe participants' general familiarity with vegetable food safety practices, perceptions of the frequency and consequences of chemical and microbial contamination of vegetables, willingness to learn about food safety practices, and perceptions of the importance of implementing specific food safety practices. As prior research has indicated that there is greater awareness and concern for chemical contamination compared to microbial contamination among Cambodians (Consumers International, 2013; Ebner et al., 2020; Brown et al., 2022), the questionnaire explicitly distinguished these two types of contamination. The pilot questionnaire was composed of 32 content (i.e., non-demographic) questions, all of which were formatted in five-point Likert answer scales. These questions were translated into Khmer, reviewed by Cambodian members of the research team for cultural appropriateness and comprehensibility, and digitized using KoBo Toolbox (Kobo Inc, n.d.).

Potential participants in the pilot study were contacted via email. The first round of pilot data collection took place in March and April of 2021, yielding responses from a total of 215 participants. After removing responses from individuals who declined to participate ($n = 2$), responses that were less than 50% complete ($n = 14$), and responses for which it was not possible to confirm that the respondent was part of the target audience ($n = 87$), the data set included responses from 112 participants. After a qualitative assessment of the data, the questionnaire was revised for identifiable errors and for clarity, resulting in a subsequent pilot questionnaire containing 34 content questions.

A second round of pilot data collection was conducted in May and June of 2021 using the revised 34-question questionnaire. This data collection event yielded responses from 62 participants. Excluding two responses from individuals who did not consent to participate, six responses that were less than 50% complete, and four from respondents whose status as part of the targeted audience was unclear, the resulting data set contained responses from 50 participants.

Following preliminary analyses of descriptive statistics based on response data from the two rounds of pilot data collection, it became apparent that the scope of the questionnaire was too broad. For this reason, the questionnaire was once again revised. During this revision, the questionnaire was focused on respondents' general levels of concern regarding vegetable safety in Cambodia, their current contamination prevention practices, and their perceptions of the frequency of chemical and microbial contamination of vegetables, the potential health impacts of chemical and microbial contamination, the production stage at which most contamination occurs, and the participant group primarily responsible for preventing contamination.

The revised questionnaire (Supplementary material) consisted of a total of 17 content questions, including 14 quantitative questions and three free response questions. Free response questions were included to qualitatively explore participants' perceptions of the potential health impacts of chemical and microbial contamination and to

determine participants' current food safety practices without limiting responses to scaled answers. Questions that were modified or added as the questionnaire was revised were translated into Khmer by native Khmer-speaking members of the research team. At the end of the revisions process, the questionnaire was reviewed by Cambodian members of the research team in order to ensure that the questions were easily and consistently understood in Khmer and that the questions were worded in a culturally appropriate manner. The questionnaire was then digitized using KoBo Toolbox in preparation for data collection (Kobo Inc, n.d.).

Data collection with vendors and growers involved in informal vegetable markets

The revised questionnaire consisting of 17 content questions was first implemented in informal vegetable markets in Phnom Penh, Cambodia in August 2021. Phnom Penh was chosen as the location for initial data collection because informal vegetable markets in Phnom Penh were accessible to the research team when COVID-19-related travel restrictions were in effect. Participants were selected using availability sampling techniques (Daniel, 2012) and, as before, all participants were over 18 years of age and consented to participate in the research. Questionnaire enumerators implemented the questionnaire in person, approaching 31 vegetable vendors regarding participation in the study. All 31 individuals agreed to be surveyed and answered at least 50% of the questions on the questionnaire. To facilitate conceptualization of key terms in the questionnaire, each enumerator read the following statement aloud in Khmer to each participant prior to initiating questions:

Sometimes, foods can contain unwanted chemicals, microbes, and other substances that can make people sick. These unwanted chemicals, microbes, and other substances are often called "contaminants". Today, we will be talking about two types of harmful contaminants that are sometimes found in vegetables:

1. ***Chemical contaminants:*** *chemical contaminants can include pesticides or herbicides used to grow the vegetables, leftover cleaning chemicals, chemicals that form in the food on accident, or chemicals that are transferred from the soil to the vegetables, among others.*
2. ***Microbial contaminants:*** *microbial contaminants include bacteria (e.g., E. coli), viruses, fungi, among others.*

The term ***"Food safety"*** *refers to practices that each of us does to make sure the food we eat does not contain harmful chemicals or microbes.* ***"Safe"*** *food is* ***safe*** *to eat and does not make us sick.*

In order to ensure that participants had a clear and consistent understanding of the questionnaire's content regardless of literacy level, each question and its corresponding answer scale was read aloud in Khmer during questionnaire implementation. To note, although both men and women were eligible to participate in the research, a large majority (93.5%) of respondents were women, reflecting the fact that most vegetable vendors in Cambodia are women.

Once COVID-19 related travel restrictions were relaxed, enumerators traveled to the provinces of Battambang and Siem Reap in February 2022 for data collection. Battambang and Siem Reap were the geographic focus for data collection because these provinces are included in the current USAID Feed the Future Zone of Influence in Cambodia (United States Agency for International Development (USAID), n.d.). As before, availability sampling was used to identify potential participants (Daniel, 2012), all of whom voluntarily agreed to participate in the research and were over 18 years of age. The questionnaire was again implemented in person, with questionnaire enumerators reading each question and the appropriate answer scale aloud in Khmer to facilitate an accurate and consistent understanding of the questionnaire. During data collection in Battambang and Siem Reap, 69 vegetable growers ($n = 41$, Battambang; $n = 28$, Siem Reap) were invited to participate. All those who were approached agreed to take part and gave responses to at least 50% of the questions on the questionnaire. As a result, the data set presented and analyzed here included responses from all 69 producers in addition to the aforementioned 31 responses from vendors. There were again more female than male respondents (Siem Reap: 21.4% male, 78.6% female; Battambang: 36.6% male, 63.4% female).

Data analysis

Questionnaire responses from vendors in Phnom Penh, growers in Siem Reap, and growers in Battambang were retrieved from KoBo Toolbox (Kobo Inc, n.d.) and compiled into a single dataset in Excel. For all quantitative questions, preliminary descriptive statistics consisting of frequency tables were used to identify missing response values and to anticipate modeling problems related to quasi-complete separation of data points (i.e., extreme category). Problems with quasi-complete separation of data points were observed for one question (regarding the frequency of microbial contamination on domestically grown vegetables) and were addressed using Firth's penalized maximum likelihood estimation for bias reduction.

A logistic regression model was fitted separately to response data from each quantitative question. For response data from questions with a binary yes/no or other two-level categorical answer scale (i.e., questions 1, 3, 8, 9, and 16; Supplementary material), the model assumed a Bernoulli distribution fitted with a canonical logit link function. For response data from questions evaluated on a five-point Likert scale (i.e., questions 2, 4, 5, 6, and 7; Supplementary material), the model assumed a multinomial distribution of the conditional response fitted with a cumulative logit link function so as to recognize the ordered categorical nature of the response. Finally, for response data from questions with nominal four-level answer scales (i.e., questions 12, 13, 14, and 15; Supplementary material), the model assumed a multinomial distribution and implemented a generalized logit link function. In all cases, the linear predictor for the model included the fixed effect of participant group. This fixed effect consisted of three levels defined by the combination of occupation and location, namely, vegetable vendors in Phnom Penh, vegetable growers in Siem Reap, and vegetable growers in Battambang. The data generation process did not support the inclusion of random effects in the linear predictor in any instance. All statistical models were fitted using maximum likelihood estimation as implemented by the LOGISTIC and GLIMMIX procedures of SAS® data analysis software (SAS Version 9.4, SAS Institute, Cary, NC).

Following model fitting, overdispersion was evaluated for questions with a binary answer scale using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. No evidence for overdispersion was apparent for any of the questions modeled. For

models fitted to ordinal responses, the proportional odds assumption was checked using a chi-square test statistic. In the one case in which the proportional odds assumption was violated, analysis proceeded using a generalized logit link function as described for data from questions with nominal answer scales.

For each question, response probability estimates and corresponding 95% confidence intervals were calculated based on fitted models and were reported for each participant group. Wald-based type III tests were performed to assess differences in the probability of the response outcomes between participant groups. Relevant pairwise comparisons were then conducted using Tukey–Kramer adjustment to avoid inflation of the Type I error rate.

Participants who reported that they were familiar with the health effects of consuming vegetables contaminated with chemicals (question 8; Supplementary material) or microbes (question 9; Supplementary material) were subsequently asked to provide examples of such effects (questions 10 and 11; Supplementary material). Response data were evaluated for clarity and relevance. Responses containing examples of health impacts that were unlikely to occur as a result of consuming contaminated vegetables, exceedingly vague, or irrelevant were not considered commonly recognized health effects. The number of respondents in each group who provided at least one commonly recognized example (e.g., diarrhea, vomiting, etc.) of the health effects of consuming vegetables contaminated with microbes or chemicals was compared to the total number of respondents reporting familiarity with these health effects. When >50% of the respondents who provided at least one commonly recognized example also gave an example that was not considered a commonly recognized health effect, the number of respondents who gave a combination of commonly recognized and not commonly recognized examples was noted. In addition, the percentage of respondents in each group who gave at least one commonly recognized example of the health effects of consuming contaminated vegetables was calculated in order to provide insight into respondents' level of familiarity with such effects. The most frequent responses to these questions were then identified for each group. Responses that were given by three or more respondents in a participant group were also noted, but examples mentioned by only one or two respondents are not presented unless those examples were the most frequently given examples in that participant group.

The third free response question asked participants who indicated that they were employing chemical or microbial contamination prevention practices to describe these practices (question 17; see Supplementary material). Response data from this question were evaluated for clarity and relevance. Responses that were exceedingly vague or unrelated to food safety were not considered to represent commonly accepted contamination prevention practices. The number of respondents in each group who described at least one commonly accepted contamination prevention practice was compared to the number of respondents who stated that they employed practices to prevent contamination. The percentage of respondents in each group who described at least one commonly accepted contamination prevention practice was also determined in order to investigate the prevalence of commonly accepted food safety practices within each participant group. The various contamination prevention practices described by respondents were grouped by practice type and the most frequently mentioned practices or practice types in each participant group were identified and presented. Practices mentioned by three or more respondents were also specifically noted.

Results

Concern about vegetable safety

Overall, the estimated mean probability of respondents reporting concern (i.e., extreme, moderate, or slight concern; scale: extremely concerned, moderately concerned, slightly concerned, not at all concerned) about the safety of vegetables sold in Cambodia was 97.4% (95% CI = [89.7, 99.4]%), with no evidence for significant differences observed between group (growers, vendors)-specific estimates ($p = 0.76$). Among respondents reporting concern ($n = 98$), an estimated 62.7% [46.2, 76.7]%, 79.5 [66.7, 88.2]%, and 79.1 [63.7, 89.1]% of vendors in Phnom Penh, growers in Battambang, and growers in Siem Reap, respectively, indicated being either "extremely" or "moderately" concerned about the safety of vegetables in Cambodia (out of a range of answer choices that further included "somewhat concerned," "slightly concerned," and "not at all concerned"; Figure 1). There was no evidence of between-group differences in respondents' levels of concern regarding the safety of vegetables in Cambodia ($p = 0.12$). The mean estimated probability of respondents reporting that chemical contamination was of greater concern than microbial contamination was 84.9% [76.0, 90.9]% (data not shown). No evidence for significant differences in relative perceptions was apparent between groups ($p = 0.36$).

Perceived contamination frequency

The estimated cumulative probabilities of respondents reporting that domestically grown or imported vegetables are "always," "very often," "sometimes," "rarely" or "never" contaminated with chemicals for each participant group are presented in Figure 2. For domestically grown vegetables, no evidence for differences between participant groups was apparent with regards to perceived frequency of chemical contamination ($p = 0.24$). There was a fairly even distribution of responses between "always" and "rarely" in all participant groups (Figure 2A).

Meanwhile, perceptions of the frequency of chemical contamination on imported, rather than domestically grown, vegetables, differed significantly between participant groups ($p = 0.008$). Specifically, differences were observed between vendors in Phnom Penh and growers in Siem Reap ($p = 0.006$; Figure 2B). No evidence for differences was apparent between either of these groups and growers in Battambang ($p \geq 0.13$), however. Among vendors in Phnom Penh, the estimated probability of imported vegetables being perceived as "always" chemically contaminated was 46.0% [30.1, 62.6]%, while the same estimate was 85.9% [67.8, 94.6]% among growers in Siem Reap (Figure 2B). Among growers in Battambang, this estimate was 64.3% [49.0, 77.2]% (Figure 2B). Estimated probabilities of respondents reporting that imported vegetables were at least "very often" chemically contaminated followed a similar pattern, with cumulative probability estimates of 75.4% [59.5, 86.4] among vendors in Phnom Penh, 95.6% [87.2, 98.6]% among growers in Siem Reap, and 86.6% [75.3, 93.2]% among growers in Battambang (Figure 2B).

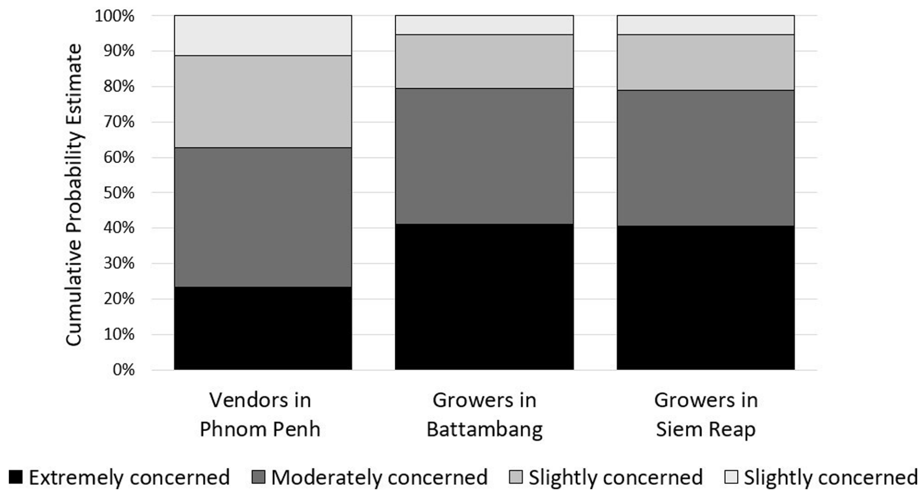

FIGURE 1
Assessment of the level of concern among Cambodian vegetable growers and vendors regarding the safety of vegetables consumed in Cambodia. Cumulative probability estimates for responses to the question "How concerned are you about the safety of vegetables sold in Cambodia?" amongst participants from each group that indicated concern. There was no evidence for differences in the ordinal distribution of responses across participant groups ($p = 0.12$).

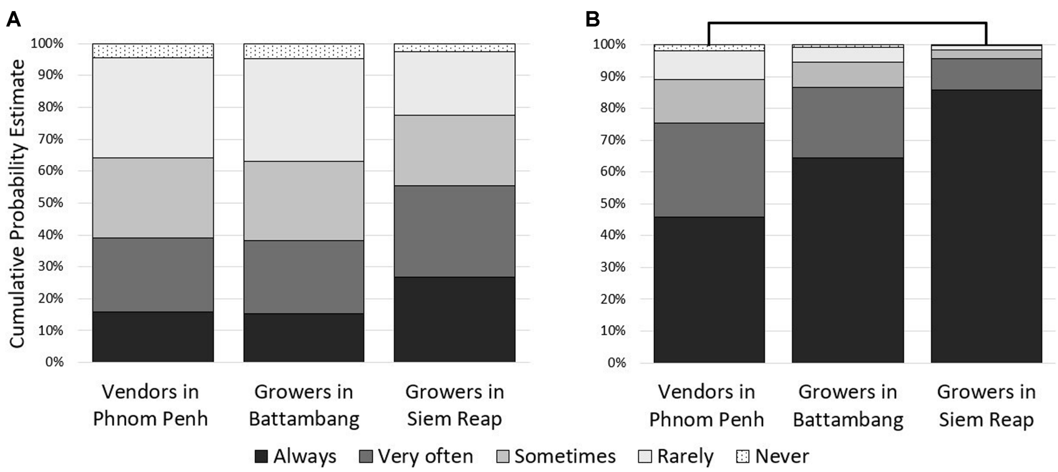

FIGURE 2
Perceptions among Cambodian vegetable growers and vendors regarding the level of chemical contamination of vegetables consumed in Cambodia. Cumulative probability estimates for responses to question **(A)** "Regarding chemical contamination, how often do you think vegetables **grown in Cambodia** are contaminated with chemicals?" and **(B)** "Regarding chemical contamination, how often do you think vegetables **imported to Cambodia from other countries** are contaminated with chemicals?" for each participant group. For question **(A)**, there was no evidence for differences in the ordinal distribution of responses across participant groups ($p = 0.24$). For question **(B)**, significant between-group differences ($p < 0.05$) in the ordinal distribution of responses were apparent. Significant differences have been indicated by a line connecting groups that significantly differ.

The estimated cumulative probabilities of respondents reporting the frequency of microbial contamination on domestically and internationally grown vegetables as "always," "very often," "sometimes," "rarely" or "never" for each participant group are presented found in Figure 3. Differences across participant groups were observed with regards to perceptions of the frequency of microbial contamination on domestically grown vegetables ($p = 0.004$). Specifically, the probability of growers in Siem Reap perceiving that domestically grown vegetables were "always" contaminated with microbes (42.9% [24.5, 61.2]) was significantly higher than said probability for both vendors in Phnom Penh (3.2% [0.0, 9.4]%; $p = 0.004$) and growers in Battambang (4.9% [0.0, 11.5]%; $p = 0.004$; Figure 3A). No evidence for significant between-group differences in probability estimates for the remaining response choices (i.e., "very often," "sometimes," "rarely," and "never") was observed, however ($p \geq 0.12$ in all cases; Figure 3A).

When participants were asked about microbial contamination on imported vegetables, differences in perceived frequency were observed between growers in Siem Reap and each of the two other groups, namely vendors in Phnom Penh ($p < 0.001$) and growers in Battambang ($p = 0.01$) (Figure 3B). Growers in Siem Reap had an 88.2% [75.8, 94.7]% estimated cumulative probability of perceiving

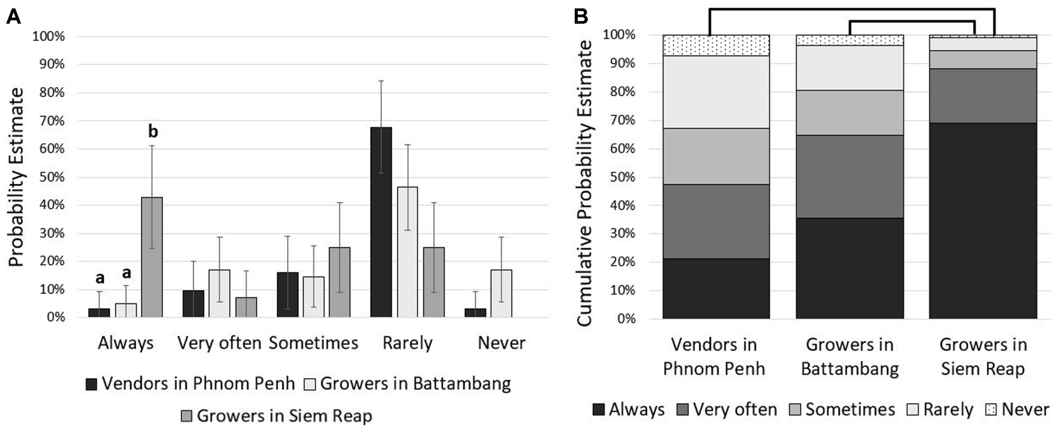

FIGURE 3
Perceptions among Cambodian vegetable growers and vendors regarding the level of microbial contamination of vegetables consumed in Cambodia. Cumulative probability estimates for responses to question **(A)** "Regarding microbial contamination, how often do you think vegetables **grown in Cambodia** are contaminated with microbes?" and question **(B)** "Regarding microbial contamination, how often do you think vegetables **imported to Cambodia from other countries** are contaminated with microbes?" for each participant group. Significant between-group differences were apparent for question **(A)**, as indicated by different letters **(A,B)**. Significant between-group differences ($p < 0.05$) in the ordinal distribution of responses to question **(B)** were apparent. Significant differences have been indicated by lines connecting groups that significantly differ.

imported vegetables as at least "very often" contaminated with microbes; this estimate was 64.9% [50.3, 77.2]% for vendors in Phnom Penh and 47.4% [31.8, 63.5]%) for growers in Battambang (Figure 3B). Perceptions of imported vegetables as either "rarely" or "never" contaminated with microbes were minimal among growers in Siem Reap, but non-negligible among vendors in Phnom Penh and growers in Battambang (Figure 3B).

Perceptions of the potential health effects of contamination

No evidence for group differences in reported familiarity with the health effects associated with consuming vegetables contaminated with chemicals ($p = 0.86$) or microbes ($p = 0.60$) was observed. For chemical contamination, the mean estimated probability of respondents reporting familiarity with such health effects was 71.4% [61.5, 79.6]% across occupations and locations. Meanwhile, the overall probability of respondents reporting familiarity with the health impacts of consuming microbially contaminated vegetables was estimated at 57.3% [47.2, 66.9]%.

In order to further investigate respondents' familiarity with the health effects of consuming contaminated vegetables, respondents who reported that they were familiar with these health effects were subsequently asked to provide examples of such effects. Of the vendors in Phnom Penh who reported familiarity with the impacts of chemical contamination ($n = 23$), 15 gave at least one commonly recognized example (e.g., vomiting, diarrhea, cancer, chronic disease, and gastrointestinal problems) of the health effects of consuming chemically contaminated vegetables. To note, however, nine of these vendors also gave examples that were vague or otherwise not considered as commonly recognized health effects (e.g., itchiness, poisoning, headache). Vomiting (mentioned by six respondents) and diarrhea (mentioned by five respondents) were most commonly cited as potential health effects of consuming chemically contaminated vegetables among vendors in Phnom Penh. Three or more vendors mentioned cancer, headache, or poisoning as examples of the potential health effects of consuming chemically contaminated vegetables. Of the 20 vendors indicating familiarity with the health effects of consuming microbially contaminated vegetables, 11 provided at least one commonly recognized example (e.g., diarrhea, vomiting, chronic disease, and fever) of the health effects of consuming vegetables contaminated with microbes. In this group, diarrhea (mentioned by nine respondents) was the most commonly given example of a potential health effect of consuming microbially contaminated vegetables. Taken together, these results indicate that 15 of the 31 vendors surveyed in Phnom Penh (48.4%) provided at least one commonly recognized example of the health effects of consuming chemically contaminated vegetables, while 11 of these 31 vendors (35.5%) gave at least one commonly recognized example of the health effects of consuming microbially contaminated vegetables.

Among growers in Battambang who indicated that they were familiar with the health effects of consuming chemically contaminated vegetables ($n = 28$), six gave at least one example of a commonly recognized health effect of consuming chemically contaminated vegetables. Diarrhea and headache (both mentioned by four respondents) were the two most common examples of potential health impacts of consuming vegetables contaminated with chemicals. In addition, three growers mentioned either nonspecific negative health effects or illness as an example of a potential health impact of chemical contamination. Of those growers in Battambang who reported familiarity with the health impacts of consuming microbially contaminated vegetables ($n = 22$), three provided at least one example of a commonly recognized health effect of the consuming of microbially contaminated vegetables. Diarrhea, vomiting, headache, and skin reactions were all mentioned twice as examples of potential health impacts of consuming microbially contaminated vegetables (to note, headache and skin reactions were not considered examples of commonly recognized health impacts of

consuming microbially contaminated vegetables). Overall, six of the 41 growers surveyed in Battambang (14.6%) provided at least one example of a commonly recognized health effect of consuming vegetables contaminated with chemicals, and three of these 41 growers (7.3%) provided at least one example of a commonly recognized health effect of consuming vegetables contaminated with microbes.

Of the 20 growers in Siem Reap who perceived themselves as familiar with the health impacts of the consumption of vegetables contaminated with chemicals, eight gave at least one example of a commonly recognized health effect of consuming chemically contaminated vegetables. However, five of these eight growers also gave examples that were not considered to be commonly recognized health effects (e.g., nonspecific negative health effects or illness, fatigue, and headache). Growers in Siem Reap most commonly cited diarrhea, headache, or nonspecific negative health effects or illness (mentioned by three respondents each) as examples of potential health impacts of consuming chemically contaminated vegetables. Among those growers in Siem Reap who indicated familiarity with the health effects of consuming vegetables contaminated with microbes ($n = 15$), four provided at least one example of a commonly recognized effect. Diarrhea (mentioned by four respondents) was the most commonly cited example of a health effect of consuming microbially contaminated vegetables. Considered as a whole, these results indicate that eight of the 28 growers surveyed in Siem Reap (28.6%) provided at least one commonly recognized example of the health impacts of consuming chemically contaminated vegetables, while four of these 28 growers (14.3%) provided at least one example of a commonly recognized health impact of consuming microbially contaminated vegetables.

Perceived primary source of contamination

Estimated probabilities (and corresponding 95% confidence intervals) for respondent perceptions of the primary source of chemical and microbial contamination of vegetables for each participant group are presented in Figure 4. When asked whether chemical contamination primarily occurs "at the farm," "during transportation from farm to market," "at the market," or "during food preparation," most respondents, regardless of occupation and location ($p = 0.84$), indicated that chemical contamination occurs primarily "at the farm." The estimated probability of this response was 76.7% [61.5, 91.8]% for vendors in Phnom Penh, 77.5% [64.6, 90.4]% for growers in Battambang, and 78.6% [63.4, 93.8]% for growers in Siem Reap. Probability estimates for the remaining responses were small (≤ 16.7%, with upper bounds of corresponding 95% confidence intervals at most 30.0%) in all cases (Figure 4A).

Respondents were also asked to identify the step in the vegetable value chain at which they perceived most microbial contamination to occur. Across all groups, respondents most frequently perceived that microbial contamination primarily occurs "at the farm," with probability estimates of 39.3% [21.2, 57.4]%, 42.5% [27.2, 57.8]%, and 57.1 [38.8, 75.5]% for this response among vendors in Phnom Penh, growers in Battambang, and growers in Siem Reap, respectively (Figure 4B). Probability estimates for the responses "during transportation from farm to market," "at the market," and "during food preparation" were less than or equal to 25.0% across all participant groups, with upper bounds of corresponding 95% confidence intervals at most 41.0% (Figure 4B). To note, there was no evidence for significant differences between participant groups with regards to perceptions of the primary source of microbial contamination ($p = 0.67$).

Perceived responsibility for contamination prevention

For each participant group, estimated probabilities (corresponding 95% confidence intervals) for respondent perceptions of which vegetable value chain actor (i.e., "vegetable farmers," "vegetable transporters," "vegetable marketers," or "food preparers") has primary responsibility for preventing chemical and microbial contamination of vegetables are presented in Figure 5. When respondents were asked to identify which vegetable value chain actor they perceived as most responsible for preventing chemical contamination of vegetables, no evidence for between-group differences was apparent in their responses ($p = 0.20$). Across all groups, "vegetable farmers" were most frequently perceived as having primary responsibility for preventing chemical contamination, with probability estimates of 51.6% [34.0, 69.2]%, 60.0 [44.8, 75.2]%, and 62.3 [44.8, 81.2]% for this response among vendors in Phnom Penh, growers in Battambang, and growers in Siem Reap, respectively (Figure 5A). "Vegetable transporters," "vegetable marketers," and "food preparers" were perceived as most responsible for chemical contamination prevention somewhat less frequently, with probabilities of these responses estimated at 12.9% [1.1, 24.7]%, 3.2 [0.0, 9.4]%, and 32.3 [15.8, 48.7]% for vendors in Phnom Penh, 5.0% [0.0, 11.8]%, 15.0 [3.9, 26.1]%, and 20.0 [7.6, 32.4]% for growers in Battambang, and 7.4% [0.0, 17.3]%, 22.2 [6.5, 37.9]%, and 7.4 [0.0, 17.3]% for growers in Siem Reap, respectively (Figure 5A).

Respondents' perceptions of which actor in the vegetable value chain was most responsible for preventing vegetables from becoming microbially contaminated were found to significantly differ across participant groups ($p = 0.02$). Specifically, vendors in Phnom Penh were estimated to have a significantly lower probability (17.2% [3.5, 31.0]%) of perceiving "vegetable farmers" as primarily responsible for preventing microbial contamination compared to growers in both Battambang ($p = 0.008$, 53.9% [38.2, 69.5]%) and Siem Reap ($p = 0.002$, 55.6% [36.8, 74.3]%) (Figure 5B). Conversely, vendors in Phnom Penh had a significantly higher probability of perceiving "food preparers" as most responsible for microbial contamination prevention compared to growers in either Battambang ($p = 0.008$) or Siem Reap ($p = 0.002$), with probability estimates at 58.6% [40.7, 76.6]%, 28.2 [14.1, 42.3]%, and 14.8 [1.4, 28.2]%, respectively (Figure 5B). There was no evidence for significant differences in the estimated probabilities of "vegetable transporters" or "vegetable marketers" being perceived as primarily responsible for preventing microbial contamination of vegetables across participant groups ($p \geq 0.08$ in all cases); probability estimates for these responses were ≤ 14.8% in all groups, with upper bounds of corresponding 95% confidence intervals 28.2% at most (Figure 5B).

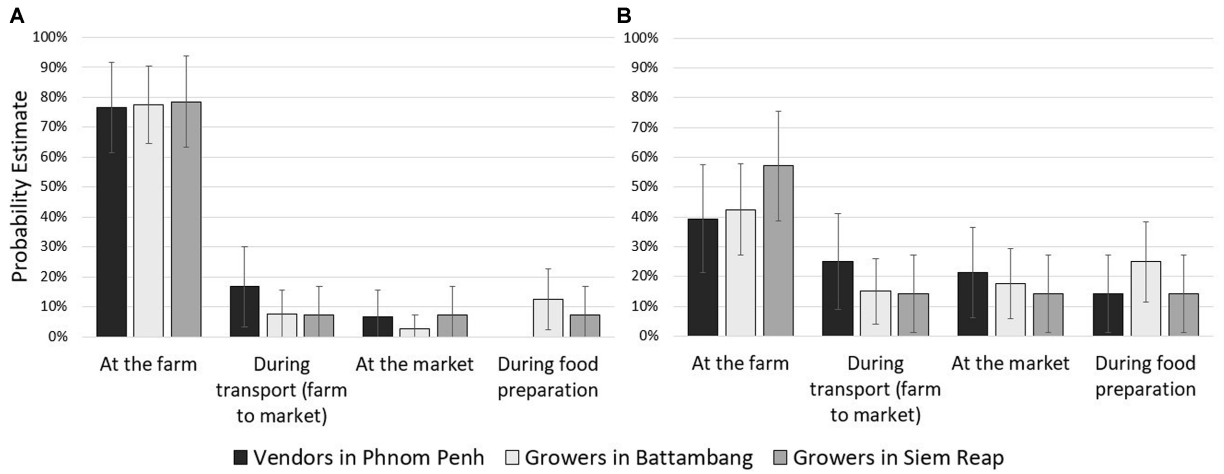

FIGURE 4
Perceptions among Cambodian vegetable growers and vendors regarding the sources of contamination in vegetables consumed in Cambodia. Probability estimates (and corresponding 95% confidence intervals) for responses to question **(A)** "When considering the different steps in vegetable production from the farm to the consumer, where do you think most **chemical** contamination of vegetables occurs?" and **(B)** "When considering the different steps in vegetable production from the farm to the consumer, where do you think most **microbial** contamination of vegetables occurs?" for each participant group. There was no evidence for differences between participant groups in the distribution of responses for either question **(A)** ($p = 0.84$) or question **(B)** ($p = 0.67$).

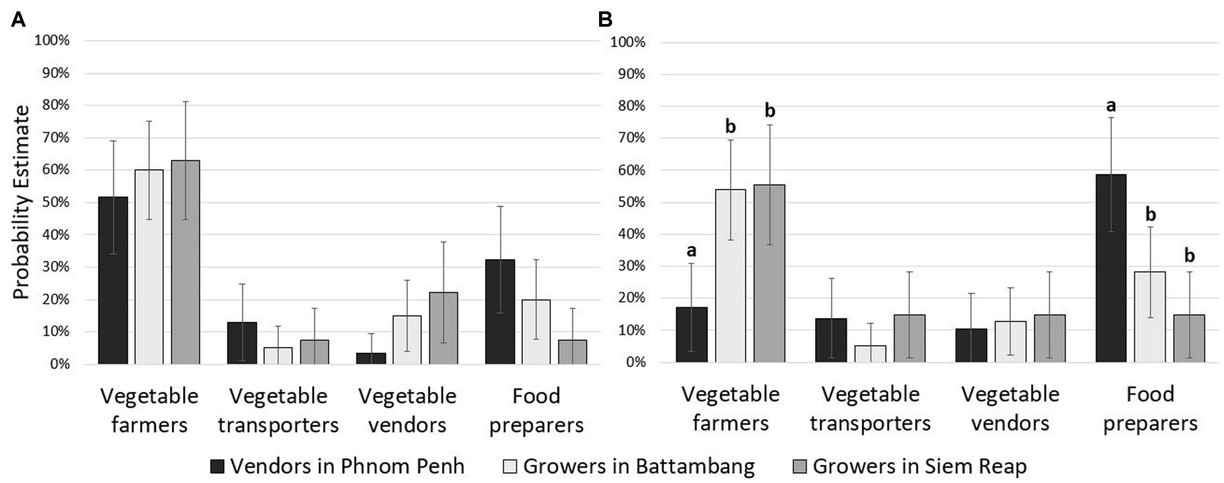

FIGURE 5
Perceptions among Cambodian vegetable growers and vendors regarding the responsibility for preventing contamination of vegetables consumed in Cambodia. Probability estimates (and corresponding 95% confidence intervals) for responses to question **(A)** "Thinking about the different steps of vegetable production from the farm to the consumer that we talked about earlier, who do you think has the most responsibility for preventing **chemical** contamination of vegetables?" and **(B)** "Thinking about the different steps of vegetable production from the farm to the consumer that we talked about earlier, who do you think has the most responsibility for preventing **microbial** contamination of vegetables?" for each participant group. For question **(A)**, there was no evidence for differences between participant groups in the distribution of responses ($p = 0.20$). For question **(B)**, significant between-group differences in probability estimates for individual response categories are indicated by different letters **(A,B)**.

Current contamination prevention practices

In addition to being asked about who had the primary responsibility for contamination prevention, respondents were also asked whether or not they themselves were doing anything to prevent vegetables from becoming contaminated with chemicals or microbes. The majority of growers in Battambang and Siem Reap indicated that they were taking steps to prevent contamination, with a 75.6% [60.1, 86.5]% and 75.0 [55.8, 87.7]% probability of a "yes" response to this question, respectively, in these groups ($p > 0.99$). However, vendors in Phnom Penh had a significantly lower estimated probability (35.5% [20.7, 53.7]%) of reporting that they performed practices intended to prevent contamination in comparison to both growers in Battambang ($p = 0.004$) and growers in Siem Reap ($p = 0.01$).

When vendors in Phnom Penh were asked to describe the practices that they were employing to prevent vegetables from

becoming contaminated with chemicals or microbes, seven of the 11 vendors in Phnom Penh who had previously reported employing such a practice clearly described at least one commonly accepted contamination prevention practice. All seven of these vendors indicated that they washed their vegetables, with six vendors mentioning washing their vegetables with water specifically. Among growers in Battambang who indicated that they were implementing practices to prevent chemical or microbial contamination ($n = 31$), 16 growers clearly described at least one commonly accepted contamination prevention practice. In this group, practices involving decreasing or eliminating pesticide use were the most commonly described practices that were considered commonly accepted contamination prevention practices (mentioned by six growers), though washing was the most frequently cited practice overall (mentioned by seven growers). In addition, three to four growers in Battambang gave responses involving knowledge gathering strategies, decreasing or eliminating chemical use, or using natural pesticides. Of the growers in Siem Reap who reported that they were employing practices to prevent chemical or microbial contamination ($n = 21$), 15 described at least one commonly accepted contamination prevention practice. Responses describing the use of natural or organic pesticides were the most common responses given by growers in Siem Reap (mentioned by eight growers). Other common responses involved decreasing or limiting pesticide use (mentioned by five growers) and variations of "washing" (mentioned by three growers). Taken together, these results indicate that seven of the 31 surveyed vendors in Phnom Penh (22.6%), 16 of the 41 surveyed growers in Battambang (39.0%), and 15 of the 28 surveyed growers in Siem Reap (53.6%) described at least one commonly accepted contamination prevention practice. To note, there was no group in which >50% of the respondents who described at least one commonly accepted contamination prevention practice also described a practice that is not commonly accepted as a contamination prevention practice. For this reason, the number of respondents who described a combination of commonly accepted and not commonly accepted practices has not been presented here.

Discussion

The aim of this study was to measure perceptions of food safety among vegetable vendors and vegetable growers involved in informal vegetable markets in Battambang, Siem Reap, and Phnom Penh in order to inform future food safety programs in Cambodia. The findings presented here provide a basis for understanding food safety perceptions among these audiences, thereby addressing a substantial gap in knowledge regarding Cambodians' perceptions of food safety. In turn, these results provide food safety program designers with information that can enable them to more effectively tailor food safety programs to specific Cambodian audiences and promote the adoption of positive food safety practices.

The existing body of literature on food safety perceptions in Cambodia is limited, being primarily composed of research and reports regarding common food safety concerns (Consumers International, 2013), the perceived consequences of unsafe foods and perceived barriers to consuming safe foods (Roesel et al., 2018), perceptions of the presence of arsenic in rice (Pravalprukskul et al., 2018), drivers of vegetable risk perceptions (Nguyen et al., 2020), the perceived health effects of pesticide contamination (Bureau-Point, 2021), perceived access to safe, high quality foods (Duong et al., 2021), and general perceptions of food safety and nutrition among particular Cambodian audiences (Brown et al., 2022). Only one study that we are aware of has included participants from the Province of Battambang (Bureau-Point, 2021). In addition, previous research on food safety perceptions in Cambodia has typically focused on assessing the perceptions of Cambodians in their role as consumers (Consumers International, 2013; Nguyen et al., 2020; Duong et al., 2021; Brown et al., 2022) to our knowledge, no study has focused solely or primarily on perceptions of food safety among any of the other actors involved in informal vegetable markets in Cambodia. Many prior studies have also either chosen not to distinguish between chemical and microbial food safety concerns (Roesel et al., 2018) or have focused exclusively on participants' perceptions of chemical contamination (Consumers International, 2013; Pravalprukskul et al., 2018; Bureau-Point, 2021; Duong et al., 2021).

The findings of this research address several of the limitations of previous work and suggest that food safety program designers have ample opportunity to increase the implementation of food safety practices among actors in the informal vegetable value chain in Cambodia, as only 22.6% of the vendors interviewed in Phnom Penh, 39.0% of growers interviewed in Battambang, and 53.6% of growers interviewed in Siem Reap reported implementing even one commonly accepted contamination prevention practice. In particular, there seems to be substantial opportunity for increased adoption of microbial contamination prevention practices, as evidenced by a scarcity of responses describing practices related to microbial contamination prevention. Encouragingly, many respondents reported being moderately or extremely concerned about the safety of vegetables sold in Cambodia, suggesting that, in general, vegetable safety is recognized as an important issue by those positioned to improve it. However, in alignment with previous research (Consumers International, 2013; Ebner et al., 2020; Brown et al., 2022), respondents were 84% likely to be more concerned with chemical contamination vs. microbial contamination of vegetables. This prioritization of chemical contamination over and above microbial contamination suggests that future food safety programs may need to include an emphasis on the significance of microbial contamination for food safety, particularly with regards to the severity of the health impacts that result from consuming vegetables contaminated with microbial pathogens. Food safety programs for vendors in Phnom Penh and growers in Battambang may especially benefit from an emphasis on the importance of microbial contamination, as respondents in these groups perceived vegetables as being less often contaminated with microbes than did growers in Siem Reap. Conversely, program designers targeting growers in Siem Reap may be able to draw on the existing perception within this group that vegetables in Cambodia are frequently contaminated with chemicals or microbes, using this perception as support for both the importance of microbial contamination and, more generally, of implementing effective food safety practices.

Program designers should also consider that only a small percentage of vegetable vendors in Phnom Penh identified "vegetable marketers" (i.e., vegetable vendors) as having the primary responsibility for preventing either chemical or microbial

contamination of vegetables. The vendors' response in this case was significantly different from the response of growers. The difference in responses could be due to location (all vendors were located in the capital, Phnom Penh), previous exposure of either vendors or growers to food safety programs, or as of yet unknown reasons. Regardless, while it is possible that, given the opportunity, vegetable vendors would have indicated that all actors in the vegetable supply chain had equal responsibility for contamination prevention, food safety programs for this group may need to highlight the importance of the role of vegetable vendors in ensuring the safety of vegetables in Cambodia. As the majority of growers in Battambang and Siem Reap perceived "vegetable farmers" as primarily responsible for preventing vegetable contamination, food safety programs that explain how the adoption of food safety practices can allow growers to fulfill this perceived responsibility may effectively encourage the adoption of such practices among these audiences. Such programs must also consider growers' capacity for implementing new food safety practices, however, as it is possible that growers are motivated to change but lack, or perceive that they lack, the resources or knowledge to do so. Additionally, while growers in Siem Reap and Battambang most often identified "vegetable farmers" as principally responsible for preventing contamination, further research is needed to investigate whether growers are identifying themselves as most responsible for contamination prevention or if they are identifying other growers as principally responsible for contamination prevention. Similarly, research exploring whether growers in Siem Reap and Battambang perceive their own farms or other growers' farms as the primary location of contamination is needed.

The majority of respondents in all groups reported being familiar with the health effects of consuming chemically contaminated vegetables, and many respondents indicated familiarity with the health effects of consuming microbially contaminated vegetables. However, regardless of contamination type, less than half of the respondents in each participant group had enough familiarity to be able to articulate at least one commonly recognized example of such effects. Additionally, participants' responses seemed narrow in scope and did not reflect the full spectrum of the potential health impacts of consuming chemically or microbially contaminated vegetables. More severe, yet commonly understood, health outcomes (e.g., severe malnutrition, organ failure, death, etc.) were either not mentioned often or were not mentioned at all (Bhunia, 2018). These low levels of familiarity and the limited scope of participants' responses indicate respondents may not recognize food safety as a pressing problem and, thus, may be less likely to undertake efforts to address the problem. As such, program designers should integrate educational components to clearly articulate the array of negative health effects associated with the consumption of contaminated vegetables into food safety programs. Additionally, the dischord in perceptions as to who has the most responsibility to improving food safety may indicate that respondents are not clear as to how food safety can be improved. As such, food safety education programs should also promote food safety practices that are both adoptable and effective in the Cambodian informal vegetable market context.

The findings of this study are primarily meant to inform the design and implementation of food safety programs for vegetable growers and sellers involved in informal vegetable markets in Battambang, Siem Reap, and Phnom Penh. Several findings that were consistent across all three groups, namely the perception of chemical contamination as a greater concern than microbial contamination and low levels of familiarity with the health effects of consuming contaminated vegetables, may also serve to inform programs across a wider context. Program designers working with vegetable growers and sellers involved in informal markets in other provinces, for instance, may also find these results useful. Generalizing about Cambodians' perceptions of food safety should be done with caution, however, as the differences observed across the three participant groups included in this research indicate that food safety perceptions can be context specific.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Purdue University (IRB-2020-383; West Lafayette, IN) and the Royal University of Agriculture (Phnom Penh, Cambodia). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SM: responsible for questionnaire design, testing, revision, data analysis, and manuscript preparation. KO: responsible for questionnaire design, testing, revision, and data collection. NB: responsible for data analysis and manuscript preparation. MC: responsible for questionnaire design, testing, revision, and data collection. LT: responsible for questionnaire design, testing, and revision. JV: responsible for experimental design and manuscript preparation. LH: responsible for experimental design and manuscript preparation. PE: responsible for experimental design, questionnaire design, testing, revision, data analysis, and manuscript preparation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Effect of nixtamalization of maize and heat treatment of soybean on the nutrient, antinutrient, and mycotoxin levels of maize-soybean-based composite flour

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Poor energy and nutrient-dense complementary food for infants have resulted in malnutrition and poor growth. Some processes are known to improve the nutritional value while reducing the antinutrient factors in food formulations. Maize-soybean-based composite flours from nixtamalized maize and heat-treated soybean were used to formulate six composite flours (CFs). The proximate composition, mineral content, antinutritional effect, mineral molar ratios, and mycotoxin level were investigated. The nixtamalization of maize and the heat treatment of soybean substantially improved the nutritional properties of the CFs. The pH value varied with the nixtamalization process, from 6.82 ± 0.7 to 9.32 ± 0.4. The energy content of the complementary foods was in the range of 354.77 ± 3.2 to 429.56 ± 4 kcal, meeting the minimum standard set for a cereal-based *CF*, which is 400 kcal. The protein values of the CFs (9.48 ± 0.3 to 13.92 ± 1.92%) on a dry weight basis were slightly below 15% of the recommended levels set by Codex. The antinutrient levels were reduced with nixtamalization and heat treatment. The molar ratio of phytate to minerals (calcium, zinc, and magnesium) was lower than the reported critical value, except for phytate to iron, where only whole maize, nixtamalized maize, and composite flour 3 (CF3) had a molar ratio that exceeded the reported critical value of 1. Although all composite flours were contaminated with mycotoxin, aflatoxin content in CFs ranged from 1.35 ± 0.67 to 13.8 ± 0.29 g/kg and from 0.3 ± 0.07 to 0.63 ± 0.015 mg/kg for fumonisin. Only the control and CFs made from the untreated maize did not meet the EU regulatory threshold (4.0 g/kg) for total aflatoxins and total fumonisin (4 mg/kg). The soybeans were not contaminated with the mycotoxins. The composite flour 5 (Composite flour 25% HS + 75% NixM) can be considered the best composite flour with regard to its nutritional properties, mineral, antinutrient, and mycotoxin content. These findings have shown that nixtamalization and heat treatment can improve the nutritional properties and food safety of composite flours.

KEYWORDS

nixtamalization, heat treatment, mineral availability, antinutrients, composite flour

1. Introduction

Malnutrition is a situation in which there is a deficiency or excess of nutrients in the population's diet, causing the deaths of more than half of the children in the world (FAO, 2022). Although the entire world population suffers from various nutritional deficiencies, people with low incomes are the most affected, particularly in developing countries where the majority have to compromise on the nutritional quality of the food they consume as well as reduce consumption (Erokhin et al., 2021).

When there is a lack of nutrients like protein, carbohydrates, and vitamins, malnutrition can also impede intellectual development and reduce working capacity in adults (Arimond et al., 2008; UNICEF, 2020). Regarding the statistics in the Democratic Republic of Congo (DRC), from 2006 to 2016, Kuamba (2016) reported that the proportion of children who suffer from stunting is constant and affects at least 42% of children in the country. The most affected regions are South Kivu and Kasai provinces, with a rate of 48 and 52% malnourished children, respectively (Kuamba, 2016). Only three crops—wheat, rice, and corn—provide half of the world's calories, but these grains are also deficient in protein and numerous minerals (Campbell et al., 2016). In diets, legumes supplement cereals; they provide significant protein, necessary amino acids, and minerals and can be used in place of meat when it is unavailable or pricey (Singh et al., 2022). Due to its high protein content, soybean is a common legume used for enriching porridges served at breakfast for the whole family and as a complementary food for infants and young children in sub-Saharan Africa (Flax et al., 2010; Mangani et al., 2015). However, the uncooked soybean flavors are often described as "painful and unpleasant" and make the legume unpalatable (Wibke et al., 2017). Soybeans are usually roasted or extruded with maize to get rid of antinutritional factors and unpleasant tastes. This helps reduce the time it takes to make porridge (Kalumbi et al., 2019). In sub-Saharan Africa, people eat maize-based porridges that are mostly flat and tasteless, and any change from this norm is likely to be rejected (Stevens and Winter-Nelson, 2008; Khumalo et al., 2011; Pillay et al., 2011; De Groote and Kimenju, 2012). Similarly, it is also impractical to use a raw corn-soybean meal mixture, as it takes a long time to cook and this form of preparation retains unpleasant flavors (Kalumbi et al., 2019).

Maize is a staple food and has the most significant calorie contribution in South Kivu (Smale et al., 2013; CTA, 2015). However, it is one of the commodities that is prone to contamination by mycotoxins. The studies conducted by Matendo et al. (2022) and Udomkun et al. (2018) show that the prevalence of aflatoxin in maize in South Kivu was very high, with a range between 3.1–2,806.5 μg/kg. Aflatoxins are toxins that occur naturally due to *Aspergillus* spp. metabolism (Ayo et al., 2018). Therefore, aflatoxin-contaminated maize poses a serious problem, rendering it unsuitable for human and livestock consumption (Zain, 2011). Soybean has been reported to be less contaminated by mycotoxins. Niyibituronsa et al. (2018) reported an aflatoxin prevalence of 0.023% in soybean in Rwanda, with only 0.003% of samples above the most stringent EU maximum permitted limit of 4 μg/kg. Even when contaminated with *A. flavus,* previous research has shown that soybeans are not a good substrate for aflatoxin production. This means that soybeans can be promoted as a healthy and aflatoxin-free food (Niyibituronsa et al., 2018).

The relationship between aflatoxins and the childhood disease kwashiorkor is unclear. Although kwashiorkor is widely thought to be a form of protein-energy malnutrition, there are hypotheses suggesting that some characteristic features of the disease are known to be among the pathological effects caused by aflatoxins in animals (Williams et al., 2004, 2010). Also, specific antinutritional factors present in foods limit the bioavailability of micronutrients (Zimmermann and Hurrell, 2007). These antinutrients tend to limit mineral delivery by interfering with intake, digestion, and absorption. Of particular concern are the relatively heat-stable phytates and tannins (Popova and Mihaylova, 2019).

Soybeans and maize can be subjected to technologies that could improve their nutritional value and acceptability. Nixtamalization is a traditional maize preparation process in which dried maize is cooked and steeped in an alkaline solution, usually water and food-grade lime (calcium hydroxide) (Odukoya et al., 2021). This process is used to prepare some maize-based products like tortillas, masa, and snacks. This technique can cause several physicochemical modifications to maize kernels, contribute to flavor, and reduce mycotoxins in the final product (Odukoya et al., 2021).

Even though hydrothermal deactivation of lipoxygenase is used in the soymilk industry, there is no report on evaluating the hydrothermal treatment of soybean in the preparation of odorless products for the enrichment of protein-deficient maize slurry treated by nixtamalization techniques to reduce mycotoxin incidence and antinutrient factors. Therefore, the objective of this study was to evaluate the nutrient, antinutrient, mineral molar ratios, and mycotoxin contents of cereal-legume-based composite flours that undergo nixtamalization and heat treatment.

2. Materials and methods

2.1. Source of raw materials

The soybeans (variety SB24) and maize (Bambou) were obtained at INERA (Institut National d'Etude et de Recherche Agronomique/Mulungu station). Fifteen kilograms of the maize sample were packed in a one-kilogram polyethylene plastic bag (biohazard peligroso) and labeled with the following information: day and month, source, and district. For soybeans, only five kilograms were bought from INERA. The samples were transported and kept at room temperature prior to processing. Before processing, the grains were first sorted to remove impurities. A total of eight experimental flours were prepared from maize and maize-soybean mixtures, following a 3×2 factorial design involving two factors, namely soybean type (roasted, torrefied, and hydrothermal soybean) and maize flour type (whole maize and nixtamalized maize), with two controls as summarized in Figure 1.

2.2. Preparation of composite flours

After sorting, soybeans were heated to remove the bean and grass taste, as follow:

1. For hydrothermal treatment, soybeans were washed, then the clean soybeans were gradually introduced into boiling water and held for 1 h (one part soybean to three parts water). After

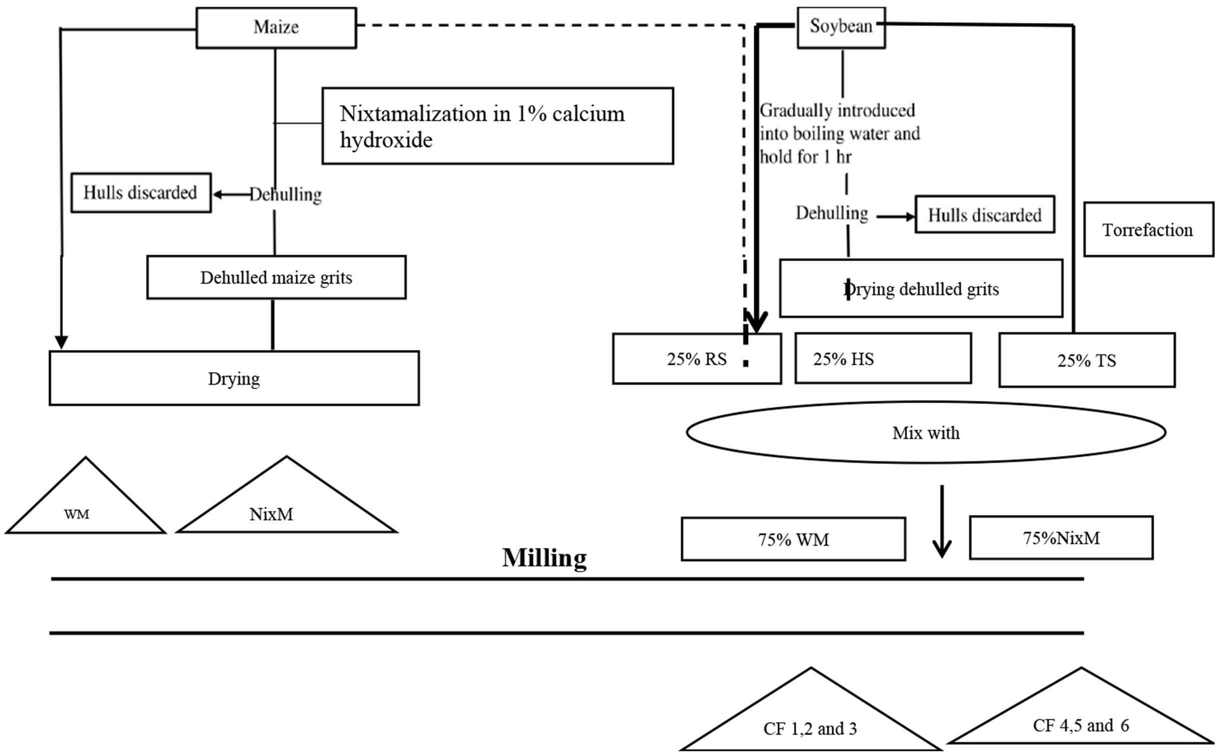

FIGURE 1
Diagram of the preparation of composite flours of flour preparation. W.M.: Whole maize. NixM: Nixtamalized Maize. R.S.: Roasted Soybean. H.S.: Hydrothermal soybean T.S.: Torrefied Soybean. CF1: (Composite flour 25%RS + 75%WM), CF2: (Composite flour 2: 25%HS + 75%WM). CF3: (Composite flour 3: 25%TS + 75%WM),CF4:(Composite flour 25%RS +75% NixM) CF5:(Composite flour 25%HS + 75% NixM), CF6: (Composite flour 25%TS + 75% NixM).

cooling the soybean in water, the hulls were removed manually by rubbing the seeds between the palms of the hands. The shelled soybeans were then washed and let dry.

2. For roasted soybean: Sorted soybean was maintained at 100°C for 15 min in an oven according to the methods described by WFP (WFP, 2004).
3. For torrefied or toasted soybeans: Sorted soybeans were maintained at 180°C in a roaster for 10 min.

Nixtamalized maize: 400 g of maize kernels were boiled or cooked in 2.000 mL of a 1% calcium hydroxide solution for 40 min at 92°C. After steeping the cooked maize for 16 h at room temperature, the cooking liquor was collected, and the nixtamal was rinsed. Wet nixtamal was dried for 24 h at 65°C using an air oven, then mixed with different soybean types and ground to a fine flour. In order to ensure homogeneity of the maize meal mixtures, the dried and heat-treated soybeans were carefully mixed with the maize portion (whole maize or nixtamalized maize) before milling. Figure 1 shows a diagram of the preparation of different composite flours. From the (NixM) and non-nixtamalized maize (W.M.) and different types of soybean (Roasted, hydrothermal, and torrefied). We formulated six different types of composite flour (*CF*). The composite flour 1 was a mixture of 25% roasted soybean and 75% whole maize; the composite flour 2 was a combination of 25% hydrothermal soybean and 75% whole maize; and the composite flour 3 was a mixture of 25% roasted soybean and 75% whole maize. The composite flour 4 was a mixture of 25% roasted soybean and 75% nixtamalized maize; the composite flour 5 was a mixture of 25% hydrothermal soybean and 75% nixtamalized maize; and finally, the composite flour 6 was a mixture of 25% torrefied soybean and 75% nixtamalized maize.

2.3. Evaluation of different parameters

2.3.1. Proximate analysis

The moisture content was determined using the method [Association of Official Analytical Chemists; AOAC 952.08, (AOAC, 2016)]. About 2 grams of each sample were kept overnight in an air oven (model UF55, Memmert Oven, Germany) at 105°C for 16 h and weighed. The loss in weight was regarded as a measure of moisture content. The crude protein was determined by the micro Kjeldahl method (AOAC 992.23, 2016) and the total protein was calculated by conversion from the total nitrogen. A 6.25 value was used as the conversion factor.

For ash content, the method AOAC 930.30, (AOAC, 2016) was used. A clean, dry crucible was weighed, and about 2 grams of sample were weighed into it. The crucibles were placed on a hot plate under a fume hood, and the temperature increased slowly until smoking stopped and the samples were thoroughly charred. They were then put in a muffle furnace, where the temperature increased gradually to 250°C and was heated for an hour. The temperature was increased to 550°C and incinerated for about 5 h in a VULCAN™ furnace (model 3–1750, Cole- Parmer, Illinoi, United States). The temperature was

then decreased to 300°C, and the crucibles were removed and cooled to room temperature.

The fat content was determined by the AOAC 948.15, 2016 method; approximately two grams of moisture-free samples were placed in a Soxhlet cartridge and covered tightly with oil-free cotton. After extraction using petroleum ether at 60–80°C in a Soxhlet apparatus (model FOSS Soxtec™ extraction, Sweden) for about 6–8 h, The extract was evaporated in a pre-weighed beaker. The increase in weight of the beaker gave the crude fat content of the samples, and the petroleum ether was collected in a separate balloon. The crude fiber content was determined using fiber extraction equipment (model FOSS Fibertec™ 2010). The percentage of carbohydrate was calculated using the formula: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein). The pH was determined using a digital pH meter (Nahita Model 903, Auxilab S.L., Beriáin, Spain) equipped with a glass electrode (XS Sensor Food S7, XS Instruments, Carpi, Italy). Energy was calculated using the Atwater's calorie conversion factors: 4 kcal/g for crude protein, 9 kcal/g for crude fat and 4 kcal/g for available carbohydrate and adding up the values. Each sample was measured in triplicate.

2.3.2. Determination of minerals

Mineral analysis were determined according to AOAC 985.35, 2016 for calcium while for iron the AOAC 984.27, 2016 was used. Analyses were conducted using dry ashing and an atomic absorption spectrophotometer (AAS). The ash was transferred quantitatively to a 100-ml beaker using 20 mL of 1 N HCl, then heated at 80°C on a hot plate for 5 min. This was then transferred to a 100-ml volumetric flask and filled to the mark using 0.5 N HNO_3. Insoluble matter was filtered, and the filtrate was kept in a labeled polyethylene bottle. Minerals were quantified using an atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000 series, Japan). The AAS was equipped with an automatic atomizer changer, a graphite furnace atomizer, an auto-sampler, and a dual atomizer system. Mineral determinations were undertaken in triplicate for each composite flour. The various mineral standards were also prepared for the calibration curve.

2.4. Quantification of antinutrients

2.4.1. Phytate quantification

The high-performance liquid chromatography (HPLC) method of phytic acid was used to analyze phytates (Ochanda et al., 2010). Approximately 0.5 g of sample was accurately weighed using an analytical balance and placed into a 100-ml Erlenmeyer flask. Samples were extracted with 10 mL of 3% H_2S0_4 shaken and centrifuged at 1500 rpm at 25°C for 30 min. Contents were filtered using a filter paper of the Whatman series, and 50 mL of the filtrate was transferred into centrifuge tubes and placed in a boiling water bath for 5 min, followed by the addition of 3 mL of a $FeCl_3$ solution containing 6 mg ferric iron per ml in 3% H_2S0_4.

The contents were heated for 45 min to complete the precipitation of the ferric phytate complex. They were then centrifuged at 2500 rpm for 10 min, and the supernatant was discarded. The precipitate was washed with 30 mL of distilled water, centrifuged, and the supernatant discarded. 3 mL of 1.5 N NaOH was added to the residues, and the volume was brought to 30 mL with distilled water. The contents were heated for 30 min in a boiling water bath to precipitate the ferric hydroxide. Cooled samples were centrifuged, and the supernatant was transferred into a 50-ml volumetric flask. The precipitate was rinsed with 10 mL of distilled water, centrifuged, and the supernatant added to the contents of the volumetric flask. This was microfiltered (with the microfilter of 0.45 μm porosity) and kept awaiting HPLC analysis. HPLC analysis was done using a Shimadzu Refractive Index Detector (RID-10A). The standards were serial dilutions prepared from a stock solution of 10 mg/mL of sodium phytate (inositol hexaphosphoric acid C_6H_6 (OPO_3Na_2) $6H_2O$) in distilled water. The mobile phase had a flow rate of 1 mL/min at 30°C and contained 0.005 N sodium acetate. The column used was C18 ODS, 5 μM size, 250x46mm. The analysis was carried out in triplicate for each composite flour.

2.4.2. Tannin quantification

The vanillin-HCl method of Ochanda et al. (2010) served to estimate tannin quantification. The first extraction was performed from approximately 0.5 g of the composite flours for the extraction of tannins by using acidic methanol. Ten mL of 4% HCl in methanol was pipetted into each flask, shaken for 20 min, using a shaker (Labortechnik KS 250b, Germany), then separation done using a refrigerator cemtrifuge (Kokusan, Type H-2000C, Japan) at 4500 rpm and 25°C for 10 min. The supernatant was then transferred to 25-ml flasks. Then, the second extraction was done by adding 5 mL of 1% HCl in methanol to the residue of the first extraction. The supernatants from both extractions were combined and made up to 25 mL using methanol. The standards were serial dilutions prepared from a stock solution of catechin. 1 mL of the suitably diluted extract was taken in a test tube, and 5 mL of freshly prepared vanillin-HCl reagent was slowly added to the samples extracts and standards. The absorbance of the standards, CFs, and blanks was read in a UV–VIS spectrophotometer (Shimadzu, UV mini 1,240, Japan) at 500 nm. The quantification of tannins was expressed in mg of catechin equivalent per gram of sample. All measurements were carried out in triplicate. The measurement was recorded in an Excel sheet, and the content of tannin was calculated as percent catechin equivalent (CE) using the standard calibration curve.

2.5. Determination of the molar ratio of antinutrients to minerals

To predict mineral bioavailability *in vitro*, the molar ratio between antinutrients and minerals has been used (Zhang et al., 2020). The study has explored four to two antinutrient-to-mineral molar ratio categories. The molar ratios of phytate and tannins to iron, calcium, zinc, and magnesium were calculated by dividing the weight of those chemicals by their atomic weight. The molar mass ratio between antinutrient and mineral was obtained by dividing the mole of the antinutrient by the mole of the mineral, as demonstrated in the equation below.

$$\text{Antinutrient:mineral molar ratio} = \frac{\dfrac{\text{Conc of antinutrient } \mu g/g}{\text{Molar mass of antinutrient } \mu g/mol}}{\dfrac{\text{Conc of mineral } \mu g/g}{\text{Molar mass of mineral } \mu g/mol}} \quad (1)$$

With the following values for the molar masses: phytic acid = 660.04 g/mol, tannins = 636.5 g/mol, iron = 55.845 g/mol, zinc = 65.38 g/mol, Mg =24.305 g/mol and Calcium =40.078 g/mole (Proietti et al., 2013).

TABLE 1 Proximate analysis of the composite flour in % dry weight basis.

Samples	pH	Moisture (%)	Protein (%)	Lipids (%)	CHO (%)	Fibre (%)	Ash (%)	Energy (Kcal/100 g)
W.M.	6.82 ± 0.7[c]	4.2 ± 0.17[b]	9.48 ± 0.3[d]	2.57 ± 0.2[d]	74.43 ± 6.2[c]	1.48 ± 0.2[a]	1.29 ± 0.2[d]	354.7[e] ± 3.2
NixM	9.32 ± 0.4[a]	5.6 ± 0.34[a]	9.01 ± 0.7[d]	3.77 ± 0.1[d]	77.06 ± 7.0[b]	1.86 ± 0.5[a]	1.97 ± 0.0[c]	366.2[d] ± 2.7
CF1	6.69 ± 0.6[c]	3.8 ± 0.14[c]	12.6 ± 1.2[b]	6.21 ± 0.9[c]	78.6 ± 8.43[a]	0.34 ± 0.0[c]	2.0 ± 0.3[c]	421.6[c] ± 3.5
CF2	6.73 ± 0.7[c]	4.8 ± 0.4[b]	13.9 ± 1.9[a]	7.9 ± 1.1[a]	75.01 ± 6.2[b]	0.28 ± 0.0[d]	2.8 ± 0.3[a]	426.9 [b] ± 4.0
CF3	6.7 ± 0.3[c]	4 ± 0.3[b]	12.4 ± 1.2[b]	6.19 ± 0.4[c]	78.86 ± 7.3[a]	0.3 ± 0.03[c]	2.23 ± 0.3[b]	420.7[c] ± 3.8
CF4	9.2 ± 0.7[ab]	5.01 ± 0.3[a]	11.74 ± 1.4[c]	6.7 ± 0.2[bc]	74.7 ± 5[bc]	0.5 ± 0.06[b]	1.9 ± 0.1[c]	424.0[b] ± 4.6
CF5	9.1 ± 0.9[ab]	4.9 ± 0.4[a]	13.24 ± 1.3[a]	8.4 ± 0.7[a]	74.75 ± 6.1[bc]	0.23 ± 0.0[d]	2.8 ± 0.33[a]	429.5[a] ± 4
CF6	8.8 ± 0.6[b]	5.1 ± 0.7[a]	11.98 ± 0.9[c]	7.1 ± 0.5[b]	78.5 ± 6.21[a]	0.4 ± 0.0[c]	2.05 ± 0.2[c]	425.7[b] ± 3.6
Codex standard			15	10–25	60–75	<3	<5	400–425

Values are means ± standard deviation of triplicate determinations. Means values within a row with different superscript letters are significantly different ($p < 0.05$).
CF1: (Composite flour 25%RS + 75%WM), CF2: (Composite flour 2: 25%HS + 75%WM).
CF3: (Composite flour 3: 25%TS + 75%WM),CF4:(Composite flour 25%RS + 75% NixM).
CF5:(Composite flour 25%HS + 75% NixM), CF6: (Composite flour 25%TS + 75% NixM).

2.6. Mycotoxins analysis

Aflatoxins and fumonisins analyses were done at the IITA Kalambo laboratory. The samples were analyzed using Neogen Reveal® Q+ kits to determine mycotoxins. A 10 g subsample was weighed with a balance and transferred into a 100 mL beaker. Then 100 mL of 65% ethanol was added. The mixture was stirred for 3 min using an orbital shaker at 200 rpm. After that, the mixture rest for 3 min before filtering with Whatman No. 1 filter paper. Five hundred microliters of sample diluent for aflatoxin and 400 μL for fumonisin were transferred to a sample dish, and 100 μL of sample extract for aflatoxin and 200 μL for fumonisin were added. Finally, a 400 μL aliquot of sample extract and diluent was added to the Neogen Raptor cartridge. The reading was done on the Neogen Raptor® display screen after 6 min. Aflatoxin concentration was quantified in parts per billion (ppb; μg/kg), while fumonisin concentration was quantified in parts per million (ppm; mg/kg). The Reveal Q + assay for aflatoxin is a quantitative assay for total aflatoxins. The linear range of detection is 2–150 μg/Kg. The Reveal Q + assay for fumonisin is also a quantitative assay for the quantification of B1 plus B2. The linear range of detection for B1 plus B2 is 1–7 mg/kg. Maize-soybean composite flour samples were analyzed in triplicate.

2.7. Data analysis

The data was recorded using Microsoft Office Excel, and R software was used for the analysis. A two-way ANOVA was used to test the main effects and interactions among the independent variables (maize-soybean composite flour). *Post-hoc* mean separations were performed using Tukey's honestly significant difference test at a significance level of 5%.

3. Results

3.1. Proximate analysis

The proximate analysis of the composite flour is shown in Table 1 and Figure 2. Results show that heat treatment and nixtamalization processing influence the proximate composition of different flours. The moisture content did not vary significantly with heat treatment of soybean. Meanwhile, hydrothermal treatment of soybean led to a slightly increase of moisture. The total protein and carbohydrate contents ranged from 74.8 to 78.68% dry weight (dwt) and 12.19 to 13.57% dwt, respectively. The total carbohydrate content did vary significantly with the heat treatment of soybean with $p < 0.04$. The heat treatment also influences the protein content ($p < 0.05$), with the hydrothermal soybean flours generally exhibiting higher protein content than roasted and toasted. The heat inactivation of the trypsin inhibitors and the heat denaturation of soyabean globulins, makes them more susceptible to proteolysis, thereby improving soy protein bioavailability for human.

The *CF*'s moisture content was in the range of 3.8 to 5.6% on a dry weight basis; this is thought to be contributed by the moisture content of the ingredient flours. Among the proximate values, moisture content is an indicator of quality and an important factor that impacts the storage and safety of food (Gemede, 2020; Ibeabuchi et al., 2020). When the moisture is low, we assume an extended shelf life. Furthermore, the pH value varied with the nixtamalization process, from 6.82 to 9.32.

This increase is due to the presence and absorption of calcium ions (Ca^{2+}). Protein content was slightly reduced after nixtamalization. Protein content in raw maize was 9.48% on a dry weight basis, and when nixtamalized, the protein was reduced to 9.01% on a dry weight basis. However, in the composite flour, the protein content increased due to the fortification of maize flour with soybean. CF2 and CF5 had the highest protein content. This indicates that in an environment where maize and maize products are often consumed and where protein deficiencies prevail, enrichment of maize by foods rich in protein is required. Additionally, the energy values obtained from CFs were in accordance with the recommendations. For carbohydrate content values in the range of (74.43–78.86%) and gross energy value (354.1–429.56 kcal/100 g), when we compare the codex requirement for baby's flours, some of the CFs did not meet the requirement of 400 Kcal/100 g, suggesting that there is a need to fortify maize so that it can provide the required energy.

The lipid content ranged from 2.57 to 8.4%. The fiber value varies from 0.23 to 1.86%, with nixtamalized maize having the highest-fiber

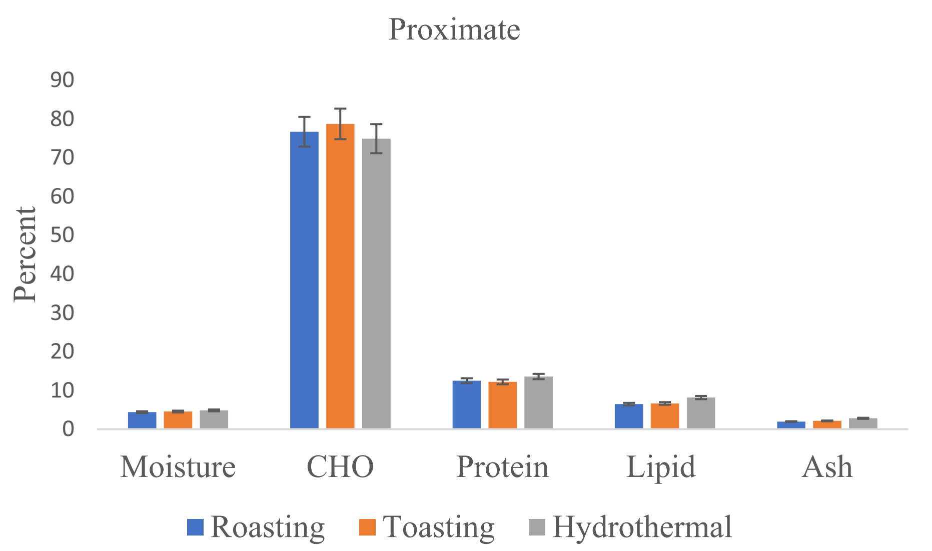

FIGURE 2
Proximate analysis of the composite flour in % dwt basis.

TABLE 2 The mineral and antinutrient contents.

Samples	Mg (mg/100 g)	Ca (mg/100 g)	Zn (mg/100 g)	Iron (mg/100 g)	Phytate (mg/g)	Tannins (mg/g)
W.M.	100.6 ± 8.5^{e}	1383.0 ± 118^{f}	3.4 ± 0.3^{f}	3.3 ± 0.04^{f}	1.5 ± 0.15^{a}	0.066 ± 0.005^{b}
NixM	129.9 ± 14.6 ^{c}	1449.4 ± 132.4^{e}	3.8 ± 0.4^{f}	4.9 ± 0.02^{e}	0.8 ± 0.05^{c}	0.017 ± 0.001^{d}
CF1	118.6 ± 6.8^{d}	1624.8 ± 91.1^{c}	6.7 ± 0.8^{d}	6.5 ± 0.05^{d}	0.7 ± 0.06^{c}	0.04 ± 0.008^{a}
CF2	121.6 ± 9.9^{c}	1565.2 ± 53.3^{d}	6.1 ± 0.7^{e}	7.3 ± 0.03^{c}	1.5 ± 0.1^{a}	0.05 ± 0.005^{b}
CF3	105.2 ± 10.8^{e}	1445.5 ± 138.5^{e}	6.9 ± 0.5^{c}	7.9 ± 0.0^{bc}	1.04 ± 0.06^{b}	0.034 ± 0.003^{c}
CF4	142.9 ± 11.7^{a}	1949.6 ± 110.8^{a}	7.7 ± 0.8^{b}	8.7 ± 0.0^{b}	0.57 ± 0.01^{d}	0.033 ± 0.002^{c}
CF5	123.4 ± 12.1^{c}	1888.7 ± 178^{b}	8.7 ± 0.8^{a}	9.6 ± 0.02^{a}	0.35 ± 0.012^{e}	0.014 ± 0.001^{d}
CF6	135.6 ± 4.6^{b}	1851.8 ± 62^{b}	7.1 ± 0.7^{c}	8.6 ± 0.03^{b}	0.64 ± 0.04^{d}	0.013 ± 0.001^{d}
Critical value	350	1,000	12–15	10–15		

Values are means ± standard deviation of triplicate determinations. Means values within a row with different superscript letters are significantly different ($p < 0.05$).
CF1: (Composite flour 25%RS + 75%WM), CF2: (Composite flour 2: 25%HS + 75%WM).
CF3: (Composite flour 3: 25%TS + 75%WM),CF4:(Composite flour 25%RS + 75% NixM).
CF5:(Composite flour 25%HS + 75% NixM), CF6: (Composite flour 25%TS + 75% NixM).

value. The main component of nixtamalized flour is carbohydrates. The difference between maize is owing to maize variety. Pigmented maize generally has a soft endosperm, whereas crystalline endosperm is present in white maize. The ash content also presented significant differences among treatments.

The mineral and antinutrient contents of CFs of maize-soybean based flours are shown in Table 2 and Figure 3. Generally, significant differences ($p < 0.05$) existed among the CFs. The mineral content increased with the use of technology in making the CFs. The calcium content obtained ranged between 1383.07 and 1949.64 mg/100 g. The highest value was found in the sample containing the nixtamalized maize and the roasted soybean, while the least value occurred in the sample with 100% untreated maize. The contents of magnesium were in the range of 100.66–142.92 mg/100 g and iron (3.32–9.63 mg/100 g). The highest value was found ($p < 0.05$) in CFs made of nixtamalized flour. Among composite flours, the lowest amount of magnesium (100.66 mg/100 g) was found in maize flour; whereas the highest content of iron was found in CF4, CF5, and CF6. The content of iron in CFs with nixtamalized maize led to a significant increase in iron content. CF5 contained the highest amount of zinc (8.78 mg/100 g).

Minerals are substances that are used in small amounts in the human body, but their deficiency can lead to the development of some disorders and diseases. A deficiency often happens slowly over time and can be caused by a number of reasons. An increased need for the mineral, a lack of the mineral in the diet, or difficulty absorbing the mineral from food are some of the more common reasons. Mineral deficiencies can lead to a variety of health problems, such as weak bones, fatigue, or a decreased immune system (Aysha et al., 2022).

The phytate content varied from 0.357 to 1.540 mg/g; the treated CF5 exhibited the lowest phytate content, which was 0.357 ± 0.012 mg/g, while whole maize and CF2 had the highest content, which was 1.5 mg/g (Table 2). The result showed that there is a great loss of phytic acid in nixtamalized maize and the CFs formulated by the treated maize. This reduction in phytic acid was comparable to that previously published by different authors, where we found losses of 17.4 to 35% when the maize was nixtamalized

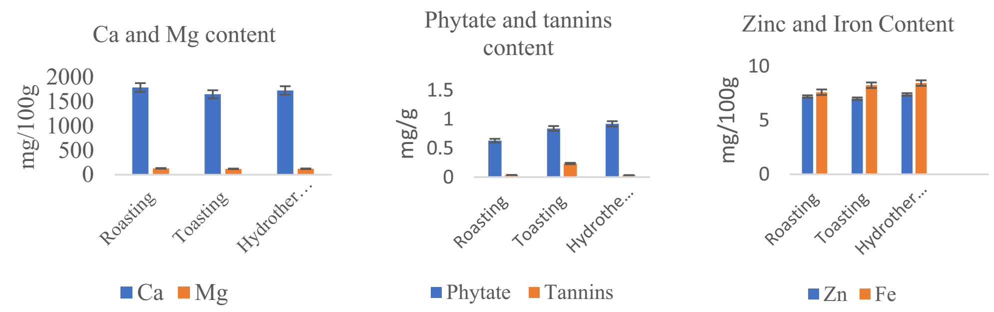

FIGURE 3
Mineral and antinutrient content of composite flour.

TABLE 3 Antinutrient to mineral molar ratios of different composite flour.

Samples	Phy: Ca	Phy: Zn	Phy: Fe	Phy: Mg	Tan: Fe	Phy + Tan: Fe	Phytate x calcium/zinc
W.M.	0.0067 ± 0.004^{a}	4.47 ± 0.43^{a}	3.92 ± 0.56^{a}	0.056 ± 0.004^{a}	1.75 ± 0.24^{a}	0.56 ± 0.24^{a}	0.035 ± 0.002^{a}
NixM	0.0047 ± 0.0002^{c}	2.89 ± 0.21^{b}	1.93 ± 0.17^{b}	0.032 ± 0.005^{c}	0.20 ± 0.03^{d}	0.21 ± 0.03^{b}	0.011 ± 0.001^{c}
CF1	0.0027 ± 0.0001^{d}	1.06 ± 0.23^{c}	0.92 ± 0.08^{d}	0.022 ± 0.001^{d}	1.2 ± 0.18^{a}	0.21 ± 0.04^{b}	0.024 ± 0.000^{a}
CF2	0.0054 ± 0.0005^{b}	2.7 ± 0.24^{b}	0.97 ± 0.01^{d}	0.045 ± 0.003^{b}	0.74 ± 0.06^{b}	0.14 ± 0.003^{c}	0.029 ± 0.0024^{a}
CF3	0.0043 ± 0.0003^{c}	1.34 ± 0.23^{c}	1.11 ± 0.21^{c}	0.036 ± 0.002^{c}	0.38 ± 0.02^{c}	0.32 ± 0.027^{b}	0.004 ± 0.00^{d}
CF4	0.0017 ± 0.0001^{e}	0.65 ± 0.45^{d}	0.55 ± 0.04^{e}	0.014 ± 0.001^{e}	0.15 ± 0.04^{d}	0.08 ± 0.063^{d}	0.003 ± 0.000^{d}
CF5	0.0012 ± 0.0011^{e}	0.40 ± 0.34^{e}	0.31 ± 0.03^{f}	0.01 ± 0.001^{e}	0.13 ± 0.01^{d}	0.04 ± 0.003^{d}	0.001 ± 0.0001^{d}
CF6	0.0021 ± 0.0001^{d}	0.89 ± 0.64^{d}	0.63 ± 0.05^{e}	0.017 ± 0.01^{e}	0.135 ± 0.012^{d}	0.07 ± 0.002^{d}	0.004 ± 0.000^{d}

Values are means ± standard deviation of triplicate determinations. Means values within a row with different superscript letters are significantly different ($p < 0.05$).
CF1: (Composite flour 25%RS + 75%WM), CF2: (Composite flour 2: 25%HS + 75%WM).
CF3: (Composite flour 3: 25%TS + 75%WM),CF4:(Composite flour 25%RS + 75% NixM).
CF5:(Composite flour 25%HS + 75% NixM), CF6: (Composite flour 25%TS + 75% NixM).

with 1.2% lime concentration (Bressani et al., 2004; Rong and Wang, 2009).

Phytic acid is an antinutrient that interacts with minerals and other substances like amino acids to form insoluble complexes that can chelate, decreasing the bioavailability of minerals and amino acids. It has been put forward that the fractional absorption of iron and zinc is much lower in high-phytate diets compared to low phytate diets (Gibson et al., 2010). Consumption of CFs with high phytate content, such as CF1 and CF3, identified in the current work, may be discouraged among populations with a high prevalence of micronutrient deficiencies (Popova and Mihaylova, 2019); although some findings have revealed phytic acid to have anti-carcinogenic and antioxidant properties as it can easily chelate some metal ions.

The tannin content in the CFs ranged from 0.0117 ± 0.001 to 0.066 ± 0.005 mg/g in maize flour (Table 2). These findings are in line with earlier studies in which the ranges of tannin contents reported were 9.4 mg/100 g when the maize was not nixtamalized and were reduced to 2.3 mg/100 g when it was fortified with 20% hydrothermally cooked soybean (Igbua et al., 2020).

In this study, because the concentrations of phytate in some CFs were high, we had to analyze their effect on the bioavailability of some minerals. The molar ratios predicting the bioavailability of calcium, iron, magnesium, and zinc are presented in Table 3.

The phytate to calcium (Phy: Ca), zinc (Phy: Zn), iron (Phy: Fe), and to magnesium (Phy: Mg) was evaluated. Also listed in Table 3 are tannins to iron (Tan: Fe), phytate plus tannins to iron (Phy + Tan: Fe), and phytate multiplied by calcium to zinc molar ratios (MRs) obtained for the different composite flours. The findings of this study indicated that the MRs varied within the different antinutrient to mineral categories. The Phy:Ca MRs ranged from 0.0012 ± 0.0011 in composite flour CF5 to 0.0067 ± 0.004 in whole maize. Interestingly, the Phy:Zn MRs ranged from 0.40 ± 0.34 in CF5, with the highest zinc content, to phytate of 2.7 ± 0.24 in CF2. The average values of Phy: Ca, Phy: Fe, and Phy:Zn MRs were in line with earlier reported values of 0.00049, 0.01, and 0.025, respectively, for composite flour made of maize and soybean (Igbua et al., 2020). It has been established that Phy:Ca, Phy:Fe molar ratios above 1, and Phy:Zn molar ratios above 15 negatively impact the bioavailability of the respective minerals (Castro-Alba et al., 2019). The Phy: Ca, Phy: Fe, and Phy: Zn MRs of the CFs in this study were below the critical limits, which means that the iron and zinc bioavailability was effective. It should be noted that it has been previously demonstrated that different processing techniques, such as nixtamalization and heat treatment, can increase mineral bioavailability. The Tan: Fe MRs ranged from 0.1 in composite flours 5 and 6 to 1.75 ± 0.24 in whole maize, whereas the Phy + Tan: Fe MRs ranged from 0.02 ± 0.003 to 0.56 ± 0.24. To the best of our knowledge, the negative influence of tannic acid, a hydrolysable tannin, on iron bioavailability has been explored, but

TABLE 4 Analysis of aflatoxins and fumonisins in composite flours.

Samples	Aflatoxins (μg/kg)	Fumonisins (mg/kg)
Soybean	ND	ND
WM	13.8 ± 0.29[a]	0.63 ± 0.015[a]
NixM	3.10 ± 0.30[e]	0.38 ± 0.015[b]
CF1	10.75 ± 3.6[c]	0.61 ± 0.01[a]
CF2	11.7 ± 1.67[b]	0.59 ± 0.07[a]
CF3	4.65 ± 0.22[d]	0.6 ± 0.01[a]
CF4	2.73 ± 0.9[f]	0.36 ± 0.03[b]
CF5	1.98 ± 0.7[g]	0.34 ± 0.8[b]
CF6	1.35 ± 0.67[g]	0.3 ± 0.07[b]
Codex Standard	<4	<4

Values are means ± standard deviation of triplicate determinations. Means values within a row with different superscript letters are significantly different ($p < 0.05$).
ND: Non-detected.
CF1: (Composite flour 25%RS + 75%WM), CF2: (Composite flour 2: 25%HS + 75%WM).
CF3: (Composite flour 3: 25%TS + 75%WM),CF4:(Composite flour 25%RS + 75% NixM).
CF5: (Composite flour 25%HS + 75% NixM), CF6: (Composite flour 25%TS + 75% NixM).

very little is known on Tan: Fe MRs and Phy + Tan:Fe MRs are associated with condensed tannins, which are the most common tannins in foods (Delimont et al., 2017).

The aflatoxin level in the composite flour varied according to the type of composition in the mixture. Thus, the results show that the high aflatoxin level was obtained from whole maize flour (13.8 μg/kg) while fumonisin and aflatoxin were not detected in soybean. All composite flours in this study were contaminated with aflatoxins and fumonisin; and levels ranged from 1.36 to 13.8 μg/kg and 0.3 to 0.6 mg/kg, respectively (Table 4).

Although most of the samples met the proposed East Africa regulatory threshold of 10 μg/kg, whole maize, the composite flours 1, 2, and 3 did not meet the EU regulatory threshold for total aflatoxins of 4.0 μg/kg (EU, 2010). In general, aflatoxin contamination in agricultural commodities can be compounded by a wide range of pre- and post-harvest factors such as high temperatures and drought conditions, poor farm practices, a lack of drying facilities, and inappropriate storage practices (Udomkun et al., 2018; Matendo et al., 2022). The highest aflatoxin contamination in this study was found in all samples composed of untreated whole maize. This might be attributed to poor storage of maize as well as poor governmental regulations and legislation on aflatoxin contamination in the country.

In addition, we also observe a variation in fumonisin levels depending on the composition and type of composite flour analyzed. The high fumonisin values of 0.63 mg/kg were obtained for the whole maize. On the other hand, the nixtamalized flour found its aflatoxin and fumonisin content lowered.

4. Discussion

There is a high prevalence of malnutrition in South Kivu Province, and a search for sustainable solutions by using available foods to improve the nutrient densities of staple diets in the region is crucial. The study reports on the effect of incorporating nixtamalized maize with different heat-treated soybeans on the nutritional and safety of the consumer. The results have shown that it is possible to enrich nixtamalized maize with a substantial proportion of heat-treated soybeans, thus enhancing the food's nutritional status.

So, the results of this study suggest that roasting, toasting, and hydrothermal treatment of soybeans add other flavors and get rid of some anti-nutritional factors like phytates, tannins, and mycotoxin that can make some nutrients unavailable and cause diseases.

4.1. Proximate analysis

Moisture is used as an indicator of quality. Food with a low moisture content lasts longer and is less susceptible to microorganism attack. Even the production of mycotoxins requires high moisture content and temperature, as it allows the fungi to grow and produce the secondary metabolite (Gemede, 2020; Ibeabuchi et al., 2020; Valencia-Quintana et al., 2020). Although the composite flour's moisture content was slightly higher ($p < 0.05$) than the codex standard's, other studies recommend the specified limits (14% or less), which means that the moisture of the composite flour in this study was significantly lower ($p < 0.05$). With low moisture, we expect extended shelf stability (Osuji et al., 2019; Ibeabuchi et al., 2020). The ash content obtained from this study correlates with the findings reported by Gemede (2020) for pea, maize, and anchor flours. Ash is the mineral constituent in flour; a composite flour with a high ash content could meet the minimum requirements of limiting minerals in complementary foods made of locally available commodities. The value of fiber content in composite flours was close to the value of 0.50% reported by Osuji et al. (2019) for rice flour. The fiber value of the whole maize and nixtamalized maize was slightly low compared to the 2.51% reported by Gemede (2020) for maize flours. The nixtamalization process appears to be the main contributor to fiber content in maize, as the interaction between the ions (Ca^{2+} and OH^-) present in the solution and the different constituents of the maize grains can produce some non-digestible products and thus increase the fiber content during the process. A higher amount of insoluble dietary fiber was found in nixtamalized flours (Liu et al., 2015). Fiber is known to cleanse the digestive tract (Emebu and Anyika, 2011) and facilitate food's passage quickly through the gut, which helps prevent constipation (Igwe et al., 2015; Odimegwu et al., 2019). *In vivo* and *in vitro* studies have demonstrated the ability of insoluble dietary fiber to absorb carcinogens (Naumann et al., 2019).

Soybean flour is a good source of protein (Agbemafle et al., 2020). Previous reports indicate that protein is lost during the nixtamalization process, probably due to the solubilization of some protein fractions (Bello-Pérez et al., 2003). Enrichment of nixtamalized maize with soybeans indicates that incorporating legumes treated by appropriate processes improves the nutritional value of infant flours. Marcel et al. (2022) show that increasing the proportion of soybean, amaranthus, and pumpkin in complementary flours increases their protein content, depending on the treatment. Thus, their utilization and consumption after inactivating the antinutritional component may be used to mitigate protein-energy malnutrition in South Kivu. Importantly, the overall effect on protein quality depends on the ingredients and processing methods used. Some processing methods, like germination and extrusion, are known to improve protein quality and digestibility by lowering the amount of antinutrients in food (Ofoedu et al., 2019, 2020, 2021). The lipid content of different composites was low; these

CFs can be enhanced to a higher level by adding a small quantity of fat or oil during the preparation of the porridge (Walker et al., 2003). In this study, the carbohydrate content was high due to starch's appreciable degradation into simple sugars during processing. During steeping, the intermolecular hydrogen bond breaks, which causes the starch to lose its crystalline structure. This can cause the starch granule to partially gel (Pena-Ryes et al., 2016). However, this is advantageous to infants, as the sugars produced can help minimize the addition of table sugar during the preparation of the porridge. The energy of complementary foods determines the quantity of food needed to meet the energy needs of infants.

4.2. Mineral, antinutrient and molar ratios

Minerals are used in small amounts but play a crucial role during metabolism. Among others, calcium is an essential nutrient that serves a critical role in bone structure, particularly in stages of growth such as infancy and childhood. Inadequate calcium intake during childhood may increase the risk of fractures and rickets and prevent the achievement of maximal peak bone mass later in life. Findings from Udomkun et al. (2019) show that the calcium content varies between 1580.3 and 1623.6 mg/100 g for a partial or full substitution of wheat flour with cassava flour in composite flours, respectively. The findings of this study show that we can easily improve the calcium content of maize by practicing nixtamalization. It's therefore implying that there is no need to fortify with calcium the maize that undergoes the nixtamalization process. In most studies where the CFs are formulated, they encourage substituting the calcium deficiency with other calcium-rich foodstuffs, probably of animal origin or entomophagy (the utilization of insects as food). However, the challenge would be affordability due to the low-income status of most rural and peri-urban households; and the unreliable option due to the seasonal availability of the insects in diverse global geographical regions. So, the nixtamalization process of maize might be one of the most promising solutions for low-income households. Previous findings show that calcium content significantly increased after the nixtamalization with lime (Bressani et al., 2020).

In terms of zinc content, the results showed that all CFs were within the Dietary Reference Intakes (DRIs) range (3.4–8.78 mg/100 g) set by the World Food Program (2018). However, the zinc content in the CFs was much higher than in the control samples. The CFs developed in this study are providing a good source of zinc as 50% of the zinc DRI requirement is met,which can be considered sufficient (Codex Alimentarius Comission, 2006; Adisetu et al., 2017). Zinc deficiency is associated with stunting, anemia, and higher disease susceptibility (Agbemafle et al., 2020). The magnesium content of formulated diets is evidently due to the raw materials utilized in CFs. Previous studies emphasized that 10% soybeans, in addition to orange-fleshed sweet potatoes, increased the magnesium content, indicating that soybeans are a good source of the mineral. Magnesium is essential as it helps strengthen the bones and maintain the immune system (Ndife et al., 2020). The findings show a high reduction of phytate when we combine the nixtamalization and hydrothermal treatment of soybean; this is in line with the findings of Rong and Wang (2009), who show that processes like nixtamalization degrade phytic acid in crops. Phytic acid should be lower in baby porridge to minimize mineral losses.

The phytate/calcium molar ratios in all the samples were less than 0.24, which is regarded as favorable for calcium absorption; predicting that among all the CFs, the calcium bioavailability could be achieved by using the local available crops like maize and soybean to make a good *CF.* The finding shows that CFs have phytate/iron molar ratios ranging between 0.3 and 3.92; the critical value of the [phytate]:[iron] molar ratio of <1.0 is accepted as phytate begins to lose its inhibitory effect on iron absorption at this level. The molar ratio of phytate to zinc is within the range of 0.40–7.485; this is lower than the range of the suggested critical level < 15 regarded as favorable for zinc absorption. A significant increase in [phytate]: [zinc] molar ratios was observed in CF2 and WM samples when compared with other CFs, while CF5 showed the lowest values. Research has shown that high dietary calcium impairs zinc absorption in the presence of a high intake of phytate (Gemede et al., 2020). Thus, the molar ratio of [phytate][calcium]/[zinc] might be better used as an indicator of zinc bioavailability than the molar ratio of [phytate]:[zinc] alone. In this study, the molar ratios of [phytate], [calcium], and [zinc] ranged from 0.001–0.031 (which is <0.5).

4.3. Mycotoxins contamination

These results are similar to what Agbetiameh et al. (2018) found, which shows that the high level of aflatoxin in untreated products would be caused by the growth of aflatoxigenic fungi in the seeds while they are being stored. Previous studies have shown that applying the nixtamalization process can reduce aflatoxin contamination by up to 70%. According to Price and Jorgensen (1985), cooking, extended steeping, and washing of nixtamal can help reduce the amount of aflatoxin in nixtamal, masa, and tortillas. When compared to the naturally infected, unprocessed maize (starting aflatoxin level of approximately 140 μg/kg), aflatoxin levels in tortillas were lowered by 50 to 70%.

The alkaline treatment led to the formation of at least two unidentified degradation or transformation products: one of 301.25 Da (molecular formula: $C_{17}H_{16}O_{15}$) and another of 325.33 Da (molecular formula: $C_{17}H_{18}O_5$) (Moreno-Pedraza et al., 2015). Lower mutagenicity and oxidative stress *in vitro* were observed in nixtamalized maize products, together with decreased aflatoxin contents (Price and Jorgensen, 1985; Moreno-Pedraza et al., 2015).

Other studies that have analyzed children's foods in various African countries have found there is a significant risk to children's health due to high levels of aflatoxins in foods (Kang'ethe et al., 2017). A study by Egal et al. (2005) on the dietary exposure to aflatoxin from maize and groundnut in under-five-year-old children from Benin and Togo concluded that maize was an important source of aflatoxin exposure among the children. This was explained by the fact that maize is consumed more frequently than other types of crops. Maize is the primary ingredient, even in food mixtures with additional ingredients. In South Kivu, the case is similar, where 98% of children consume foods made of grains, roots, and tubers, mostly maize, and 85% of infants consume foods made of legumes (Kambale et al., 2022).

In addition, the reduced levels of total fumonisins indicate that they are water-soluble and are easily washed away from the outer layer of the maize grains (Karlovsky et al., 2016). On the

other hand, some findings have shown that fumonisins are soluble and prone to leaching from grains into steeping and cooking solutions (Schaarschmidt and Fauhl-Hassek, 2019). In experimental studies, De La Campa et al. (2004) further investigated the impact of lime concentration and cooking time at different initial FB1 levels using fungal-inoculated maize. In doing so, they found a positive impact of lime concentration (when testing lime solutions of around 0.25–1.6%) on FB1 reduction. Nixtamalization significantly reduced the amount of fumonisin in raw corn.

5. Conclusion and recommendation

This work was done to figure out the nutrients, antinutrients, molar ratios of the antinutritional factor to minerals (mineral availability), and mycotoxin content of maize-soybean-based composite flours. Results show that the technologies used lead to CFs rich in some macro- and micronutrients and low in mycotoxin contamination. Also, the level of the antinutrients was reduced, and it was found to influence the bioavailability of minerals, especially zinc, iron, calcium, and magnesium. These CFs are recommended as a practical and sustainable approach for improving macro- and micronutrients and mineral availability for the resource-poor in low-income countries. The findings of this study will play an integral role in promoting the utilization of local crops and enhancing agricultural systems. Thus, we recommend using this process in our environment to obtain well-enriched and healthy infant flour.

However, as both the right amino acid composition and high digestibility and PDCAAS are required for proteins to meet the requirements of the human body, the ability of dietary proteins to meet these requirements varies widely. Such abilities are often quantitatively expressed in so-called protein quality metrics, which include amino acid composition and digestibility.

Thus, this could be done in further research, as such information would help optimize nutrient use and improve nutrient use efficiency. Finally, it is worth noting that all CFs have different levels of mycotoxin, with the treated CFs having their contamination reduced. Thus, these technologies can be promoted as strategies to manage mycotoxin contamination.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RM: conceptualization, data collection, formal analysis, and drafting paper. RM and PU: methodology. All authors review, editing of the manuscript, contributed to the article, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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