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Biosecurity and milk production among smallholder dairy farmers in Rwanda

Effects on child nutrition and prevention of zoonotic diseases

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Cover: The typical Rwandan crossbreed between Ankole cattle and Friesian dairy cow
(photo: Jean Pierre M. Mpatswenumugabo)

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Abstract

Animal-source foods like milk are important for the growth and development of young children, especially in developing countries. However, Rwanda faces challenges related to low milk yield and quality, impacting both dairy production and public health. This thesis investigated the factors affecting milk production and quality on smallholder dairy farms in Rwanda's Northern Province, aiming to improve food security, nutrition, and livelihoods of poor households.

The field research involved collecting cow's milk and children's faecal samples from 156 households with lactating cows for laboratory analysis. The results showed that 12.9% of milk samples contained antibiotic residues, and 34.2% had somatic cell counts exceeding 300,000 cells/ml, a marker of poor milk quality. *Salmonella* spp. was found in 25.6% of milk samples, while *E. coli* strains were most prevalent in children's faecal samples. No identical pathogens were found in both milk and faecal samples from the same households. Factors influencing milk yield included feeding practices, cattle breeds, milking frequency, body condition, and parity. Interestingly, farm management practices did not significantly affect the presence of pathogens in milk samples, though household hygiene practices were associated with pathogens in children's faecal samples. Anthropometric measurements of 601 children showed that 27% were stunted, with a higher prevalence among boys (33.8%) than girls (20.9%). Statistical analysis identified several factors contributing to stunting, including livestock ownership and household income. These findings emphasize the need for a multidisciplinary approach to improve milk production and quality. Such interventions could help address child malnutrition and enhance food security, nutrition, and livelihoods in Rwanda's rural areas.

Keywords: contamination, hygiene, unpasteurized, crossbreed, multiplex PCR, developing countries, grazing, height-for-age, food insecurity, East Africa.

Dedication

In loving memory of my mother.

"No amount of experimentation can prove me right; a single experiment can prove me wrong."

– Albert Einstein

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Mpatswenumugabo, J. P.,** Mukasafari, M. A., Ndahetuye, J. B., Wredle, E., and Båge, R. A systematic literature review of milk consumption and associated bacterial zoonoses in East Africa. *Journal of Applied Microbiology*, 2023, 134(4), 1–13.

II. Mukasafari, M. A*., **Mpatswenumugabo, J. P*.,** Ndahetuye, J. B., Wredle, E., and Båge, R. Management factors affecting milk yield, composition, and quality on smallholder dairy farms (*submitted manuscript*).

III. **Mpatswenumugabo, J. P.,** Ndahetuye, J. B., Andersson, M. E., Elfving, K., and Båge, R. Risk factors associated with the prevalence of zoonotic enteric pathogens in cow milk and children faecal samples coming from the same households in Rwanda (*manuscript*).

IV. Kagoyire, C., Ndagijimana, A., Nduwayezu, G., Utumatwishima, J.P., **Mpatswenumugabo, J.P**., Mukasafari, M.A., Rinda, D., Ndahindwa, V., Elfving,K., Krantz, G., Lindd, T., Mansourian, A., Båge, R., Wredle, E., Nyandwi, E., Umubyeyi, A., Ndahetuye, J.B., Pilesjö, P. Sampling and Analysing Spatial Determinants of Undernutrition in Northern Rwanda – A Multidisciplinary Approach (*submitted manuscript*)

*Shared first authorship

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The contribution of Jean Pierre M. Mpatswenumugabo to the papers included in this thesis was as follows:

I. Took part in the planning and organisation of the study. Performed data extraction from databases, data analysis and interpretation of results. Drafted the manuscript, revised it based on feedback from co-authors and corresponded with the journal.

II. Took part in the planning and organisation of the study and collected samples. Performed the laboratory analysis together with one co-author. Performed statistical analysis in collaboration with a statistician and interpretation of results. Drafted the manuscript together with one co-author, revised it based on feedback from co-authors and corresponded with the journal.

III. Took part in the planning and organisation of the study and collected samples. Performed the laboratory analysis together with one co-author. Performed statistical analysis in collaboration with a statistician and interpretation of results. Drafted the manuscript, revised it based on feedback from co-authors.

IV. Took part in the planning and organisation of the study and collected data. Performed data cleaning together with some co-authors. Participated in drafting the manuscript.

The following articles were published during Jean Pierre M. Mpatswenumugabo PhD project with his contribution, but are not included in this thesis:

I. Garcia, S.N., **Mpatswenumugabo, J.P.**, Ntampaka, P., and Nandi, S., (2023). A one health framework to advance food safety and security : An on- farm case study in the Rwandan dairy sector. One Health 16, 1–12. https://doi.org/10.1016/j.onehlt.2023.100531

II. Iraguha, I., **Mpatswenumugabo, J.P.**, Gasana, N.M., Åsbjer, E., (2024). Mitigating antibiotics misuse in dairy farming systems and milk value chain market: Insights into practices, factors, and farmers education in Nyabihu district, Rwanda, One Health, 19, 1–7. https://doi.org/10.1016/j.onehlt.2024.100843

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Abbreviations

µl	Microliter
AI	Artificial insemination
ASF	Animal-source food
DDM	Dairy dynamic management
DNA	Deoxyribonucleic acid
FAO	Food agriculture organization of the United Nations
LMIC	Low and middle-income country
MINAGRI	Ministry of agriculture and animal resources
ml	Milliliter
MT	Metric ton
NISR	National institute of statistics of Rwanda
OR	Odds ratio
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic acid
spp.	Subspecies
VoiCE	Value of immunization compendium of evidence
WASH	Water, sanitation, and hygiene
WHO	World health organization

1. Introduction

Historically, livestock production in Rwanda, mainly cattle, has been an agricultural practice carried out by most of the population. Cows hold significant traditional and cultural value for Rwandans, particularly in contexts such as bride price negotiations in marriage arrangements (De Lame 1996). In rural areas, a cow's importance extends beyond its social, cultural, and economic value; it also provides manure, which is used as organic fertilizer for crop production (Verpoorten 2009).

The 1994 Genocide against the Tutsi, which killed more than 1 million people, also affected livestock particularly cattle wherein more than 80% of cows were decimated (Habiyaremye et al. 2021). In addition to cattle being killed during the genocide and the liberation war that began in 1990, animals were indirectly impacted by a lack of other aspects such as pasture, fodder, and veterinary care during the war (Verpoorten 2009). After the genocide, numerous efforts were made to repopulate livestock species, but progress has been slow. The country was extremely poor and had to prioritize other pressing issues, such as rebuilding the judiciary, health, and education sectors, among others. In 2006, the government of Rwanda (GoR) launched the "*Girinka*"—one cow per poor family—programme aimed at increasing cattle population and milk production (Habiyaremye et al. 2021). By June 2022, the programme had provided more than 400,000 heifer cows to the beneficiaries (MINAGRI 2023). In addition to animal genetic improvement projects, the Girinka programme has significantly boosted the cattle population in Rwanda. Prior to the programme's inception, the cattle population was under 1 million but it is now estimated to be 1.6 million (Figure 1). The programme has also had a significant impact on reducing poverty, enhancing food security, and fostering social cohesion among its beneficiaries (Nilsson et al. 2017). However, the nutritional benefits of the implemented programme remain unclear, given the high prevalence of stunting (38%) and low milk consumption (21%) among young children (National Institute of Statistics of Rwanda (NISR) [Rwanda] et al. 2015).

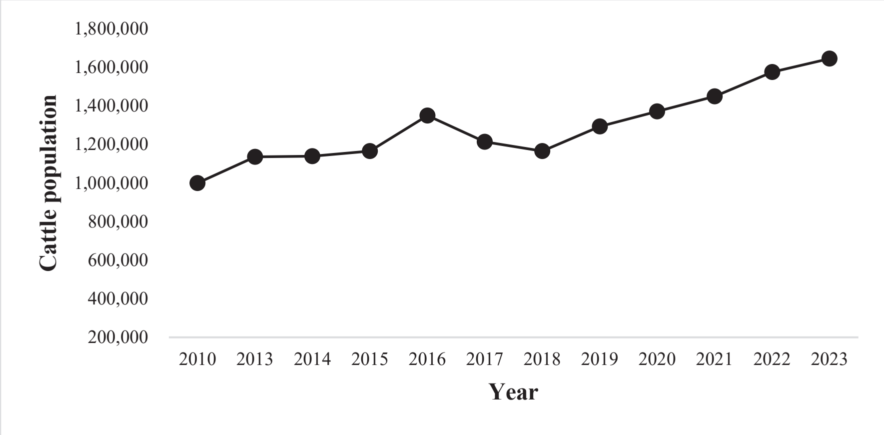

Figure 1. Rwanda cattle population growth for the last decade.

Source: MINAGRI, (2023)

Milk production increased by 160% from 2005 to 2010 (Figure 2), partly due to the implementation of the Girinka programme and the transition from traditional to modernized, business-oriented dairy farming (Ouma et al. 2023). In early 2006, the country began importing exotic bull semen and started artificial insemination to cross-breed and improve local breeds (Ankole cattle). This initiative led to an increase in total milk production from only 50,000 MT to nearly 1 million MT in 2022 (Figure 2). Despite the increase in milk production, milk processing capacity remains low, as less than 30% of the milk sold in Rwanda is processed (Ouma et al. 2023). The Rwandan milk market is also dominated by the unregulated informal channel, through which nearly 70% of raw milk is sold (Kamana et al. 2014). To address this issue, the government of Rwanda, through the Ministry of Agriculture and Animal Resources (MINAGRI), has issued guidelines on milk transport and handling to increase formal milk marketing and improve milk quality (MINAGRI 2016). Further, per capita milk consumption has increased from 68 liters in 2018 to 78.7 liters in 2023 (MINAGRI 2023). However, this is still below the World Health Organization (WHO) standard, which recommends 200 liters per person per year. The increased consumption is partly a result of government initiatives such as the ‘One Cup of Milk per Child' programme, which targets 80,000 children across 100 schools (Ouma et al. 2023).

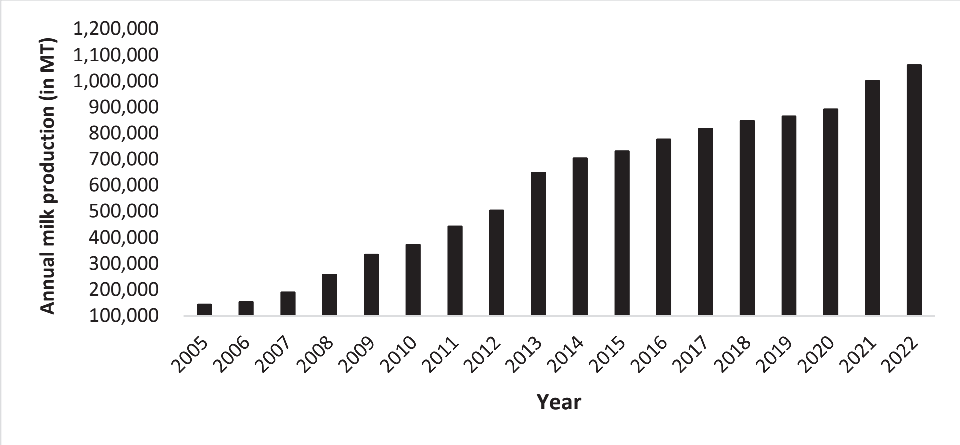

Figure 2. Rwandan annual milk production in thousands metric tons from 2000 to 2020.

Source**:** MINAGRI, (2023; 2015)

The Rwandan dairy sector is predominantly operated by smallholder farmers, with very little to no inputs. Consequently, these farmers face multiple challenges, such as low milk production due to poor feeding and management practices, diseases such as mastitis, issues with milk quality, and the misuse of antimicrobials (Iraguha et al. 2024; Ndahetuye et al., 2020a; Mpatswenumugabo et al., 2017).

A recent study highlighted that the prevalence of subclinical mastitis at the cow level was 62% (Ndahetuye et al., 2020a), which is higher than previous reports which found between 50% and 52% (Mpatswenumugabo et al., 2017; Iraguha et al., 2015). This increase in prevalence could be partly explained by the rise in improved exotic breeds, who are more prone to mastitis than local breeds (Biffa et al. 2005). Worldwide, mastitis is responsible for the highest costs incurred by dairy farmers (Huijps et al., 2008; Seegers et al., 2003). It causes direct losses such as reduced milk yield and treatment expenses, as well as indirect costs such as discarded milk, decreased future milk production and quality, increased culling risk (Tommasoni et al. 2023), and in some cases, death (Vaarst & Enevoldsen 1997). While mastitis pathogens in milk generally pose a lower public health risk if the milk is pasteurized, it is important to note that some zoonotic bacteria associated with mastitis, such as *E. coli* and *Staphylococcus aureus*, can cause illness. Therefore, consuming unpasteurized milk from mastitis cows could lead to milk-borne diseases (Hameed et al. 2007). Additionally, the improper use of antibiotics to treat mastitis poses a significant public health concern (Iraguha et al. 2024). Aside from mastitis, animal feeding is another considerable problem that hinders the productivity of dairy cows. In Rwanda, the majority of smallholder dairy farmers use zero-grazing systems and rely heavily on a "cut-and-carry" method with poor quality feeds and no supplementation, which negatively impacts production (Kamanzi & Mapiye 2012). Land scarcity is a major contributing factor to feed shortages, as the average land size per household is less than 0.5 hectares (MINAGRI 2023) which limits farmers' capacity to cultivate enough fodder for their animals (Kamanzi & Mapiye 2012).

Milk contaminated with pathogens and antibiotic residues is a significant public health concern. Bacterial contamination can occur at any stage of the dairy value chain, from the farm to the consumer's table. In Rwanda, bacteria such as *Salmonella*, *Escherichia coli*, and *Brucella* spp. antibodies have been found in milk at either the farm or collection centre (Ndahetuye et al. 2020b). In that study, the authors highlighted that these microorganisms may originate directly from the udder, be shed by infected animals, or come from the environment. A more holistic study that included all value chain segments, including dairy processing, concluded that unhygienic practices of dairy value chain actors are strongly associated with high levels of contaminated milk and dairy products (Kamana et al. 2017). Similar studies have found identical patterns in East Africa, suggesting serious public health risks to consumers (Mpatswenumugabo et al. 2023). Apart from poor handling practices, animal diseases (mastitis), personal hygiene, and a lack of adequate infrastructure (cooling facilities) and training for dairy farmers were identified as key factors associated with milk contamination at farm level (Kamana et al. 2017). Elevated levels of somatic cells and the detection of antibiotic residues in raw milk point to an additional public health issue in Rwanda (Ndahetuye et al., 2020b). The high somatic cell counts may be due to current or previous intramammary infections, such as mastitis. This could explain the presence of antibiotic residues in milk samples from certain farms (Ndahetuye et al. 2020b), likely due to the use of antibiotics without adhering to the required withholding periods (Iraguha et al. 2024).

2. Background

In recent decades, global milk production has greatly enhanced food security, nutrition, and the livelihoods of nearly 150 million households (FAO 2022a). Despite this, developing countries have a smaller share compared to developed nations. Rwanda's dairy sector has more than doubled its output from 2010 to 2018 (MINAGRI 2023) due to an increased cow population, and improved farming practices, and breeds, but still lags behind regional countries such as Kenya, Ethiopia, Tanzania, and Uganda (Habiyaremye et al. 2021). However, several constraints hinder the development of the Rwandan dairy sector, including inadequate veterinary and extension services, poor quality feeds (Habiyaremye et al. 2021), limited technological capacity, and organizational challenges (Bingi & Tondel 2015).

2.1 Milk production and quality

2.1.1 Management practices associated with milk yield and quality

The connection between milk quality and milk yield has been thoroughly studied in recent decades (Costa et al., 2020; Kaygisiz et al., 2015; Hagnestam-Nielsen et al., 2009). Overall, both milk quality and yield are influenced by a variety of factors, ranging from intrinsic factors—related to the animal to extrinsic factors—associated with management. Animal-related factors include breed, age, parity, lactation stage, and disease (Gustavsson et al., 2014; Hagnestam-Nielsen et al., 2009), while management factors encompass feeds and feeding strategies, weather conditions, farm hygiene, milking procedures, and milk handling practices (Hernández-Castellano et al., 2019; Ramírez-Rivera et al., 2019; Duguma and Janssens, 2016), among others.

The most critical management practice influencing milk production is reproduction, which includes planning, execution, and evaluation. In many cases, farmers are solely responsible for developing reproduction strategies (Henchion et al. 2022). However, the use of reproduction technologies involves a broader range of stakeholders, including veterinarians, industry professionals, AI technicians, and, to a certain extent, the government. Key decisions regarding when to breed, which breed to select, and which breeding technologies to use are crucial in determining milk production and yield. Further, poor reproduction management practices can significantly impact cow fertility, thereby reducing overall milk production (Ali et al. 2015; Walsh et al. 2011). Current research indicates that farm interventions targeted towards high milk quality and production have been implemented and provide promising results (Gülzari et al., 2020; De Vries et al., 2020; Gillah et al., 2014; Sraïri et al., 2009). For example, Båge & Magnusson (2024) proposed a more holistic approach to mitigating climate change and food insecurity through improvements in animal and reproductive health. According to the authors, by adopting sustainable and cost-effective farm management practices, farmers in low and middle-income countries could significantly reduce food insecurity and improve their livelihoods.

In Rwanda, similar to other developing countries, milk production is often low due to insufficient feed, suboptimal reproduction techniques and related diseases, and poor health statuses of dairy cows (De Vries et al. 2020). The enhancement of milk productivity and quality is sometimes hindered by a combination of farmers' knowledge in farm management and the lack of quality and sufficient resources, such as high-quality feeds and good infrastructure related to animal production and milk quality (De Vries et al. 2020). Feeding strategies in low and middle-income countries (LMIC) are directly influenced by the availability of feed resources (Thys et al. 2005). In these countries, the most widely practiced feeding strategy is the cut-and-carry system, characterized by low milk productivity and poor milk composition (Maleko et al. 2018). However, despite the majority of dairy farmers being smallholders, evidence shows that milk quality and yield could be improved by focusing on farm management practices (Vyas et al., 2020; McGuire et al., 2013; Sraïri et al., 2009; Hansson and Öhlmér, 2008).

2.1.2 Common milk quality issues in sub-Saharan Africa

It is projected that by 2050, the demand for milk in sub-Saharan Africa will increase threefold. This significant increase will be driven by rapid population growth and ongoing urbanization, which will create higher consumption needs and change dietary patterns across the region (Herrero et al., 2013; Thornton, 2010).

If productivity does not increase at the same rate or higher to meet the demand, a food crisis is likely. This may worsen the issue of poor human nutrition, which is already a major concern in sub-Saharan Africa (FAO 2017). The rise in demand for milk will also be accompanied by an emphasis on improving milk quality to meet consumer expectations and standards (Gülzari et al. 2020) to ensure safety. Milk quality is evaluated based on several parameters, including milk composition (Mwendia et al. 2018), microbiological properties (Opiyo et al. 2013), animal health (such as mastitis), environmental sustainability, and organoleptic characteristics (Castellini et al. 2023). In sub-Saharan African countries, milk quality issues are primarily linked to microbiological factors, such as animal diseases, poor milking and milk handling practices, milk composition, as well as adulterations (Gülzari et al. 2020).

Bacterial contamination of milk can originate from the milk itself, as it may be naturally contaminated, or from sick animals, humans, the environment, water, and the equipment used for milking and storing the milk. These sources of contamination include infected udders and/or teats, the animal's skin, udders that are muddy from faeces, contaminated milking and storage equipment, and water used during the cleaning process (Karimuribo et al. 2005). Other sources of bacteria include air, milkers, handlers, drugs (chemicals) used to treat sick animals, and water added for adulteration purposes by corrupt and unscrupulous sellers (Swai & Schoonman 2011). Occasionally, re-contamination can occur during and after processing, primarily due to unhygienic conditions and improper handling practices (Parekh & Subhash 2008).

Antibiotic residues are another milk quality concern. These are pharmacologically active substances and their metabolites that remain in animal-source foods after administering antibiotics to the animal (Menkem et al. 2019). Antimicrobials in livestock production are unavoidable because they are used in treating bacterial infections (Arsene et al. 2022). Previous reports indicate that in 2013, around 131,000 metric tons (MT) of antibiotics were used in livestock production globally, with a projected increase of 52% by 2030 (Van Boeckel et al. 2015). However, a recent report indicates a decline in antibiotic use in food-producing animals, particularly in China, the world's largest consumer, leading to an overall reduction in antibiotic usage (Tiseo et al. 2020). In a retrospective study conducted in Rwanda, Manishimwe et al. (2024) reported that the importation of antibiotics for food-producing animals is increasingly high, reaching nearly 12,000 MT annually. When adjusted for animal biomass, it was revealed that these importations were estimated to be approximately 30 mg/kg in 2021.

Milk and dairy products are sources of great nutritional, social, and economic importance all over the world (Virto et al. 2022). However, dairy production faces the most challenging disease, mastitis, which leads to an excessive use of antibiotics in the treatment. For instance, in the United States, over 75% of all dairy cows receive intramammary infusions of prophylactic doses of antibiotics, following each lactation (Landers et al. 2012). Other common infectious diseases in dairy cows include respiratory and uterine infections, as well as infectious foot diseases (Virto et al. 2022). Notably, when withdrawal periods are respected, fewer residues are found in milk and milk products. However, in LMICs, the levels of antibiotic residues are excessively high (Arsene et al. 2022) due to poor farming practices, non-compliance with withdrawal periods, and misuse of antimicrobials (Iraguha et al. 2024), among others. Health risks associated with antibiotic residues in milk include the development of antimicrobial resistance, hypersensitivity reactions, and cancer (Rahman et al. 2021). Developing countries are at greater risk than developed countries due to poor detection facilities, the lack of proper monitoring systems, and permissible thresholds for antimicrobial residues in foods (Pokharel et al. 2020).

2.2 Biosecurity measures and animal diseases prevention

Biosecurity measures are all steps involving segregation, hygiene, or management procedures designed to reduce the risk of introducing, establishing, or spreading potential pathogens in, within, or from a farm, operation, or geographical area (Huber et al. 2022). The importance of animal disease prevention goes beyond animal welfare; it also concerns financial implications, public health, and food safety (Nöremark et al. 2010).

In developed countries, biosecurity measures are well established and routine controls are conducted to reduce the risk of animal diseases spreading between farms or in the food chains (Léger et al., 2017; Marier et al., 2016; Noordhuizen et al., 2013).

Biosecurity measures include practices such as hygiene, sanitation, movement controls, vaccination, culling, feed management, and waste disposal to prevent disease spread (Msimang et al. 2022). Evidence shows that farmers' compliance with these practices varies across cultures, countries, types of livestock, farm classifications, and other factors. For example, a study on pig farms in Sweden found that larger-scale farmers were more likely to implement biosecurity measures compared to hobby farmers (Nöremark et al. 2010). However, in the United Kingdom, farmers faced challenges in implementing biosecurity measures during a foot and mouth disease outbreak (Gunn et al. 2008). There are several parameters to be included in the implementation of biosecurity measures in any setting. Through expert opinion, some studies have evaluated biosecurity measures based on their importance and effectiveness (Kuster et al. 2015). A more holistic approach entails assessing importance, effectiveness, feasibility, and cost, thus, the success of evaluating biosecurity measures relies on these parameters (Léger et al. 2017).

2.3 Animal-source foods and undernutrition

Malnutrition refers to a pathological condition caused by either insufficient nutrition (undernutrition) or excessive food intake (overweight) (Morales et al. 2024). While child malnutrition is endemic in developing countries, developed nations have fewer cases across all ages (Kramer & Allen 2015). Undernutrition is a broad term encompassing stunting (height-for-age), wasting (weight-for-height), and being underweight (weight-for-age) (Shetty 2006). It is the leading cause of child morbidity and mortality among children under five years of age (Crowe et al. 2014). In 2004, undernutrition alone was responsible for nearly 53% of all child deaths worldwide (Caulfield et al. 2004), showing a reduction of only 2% since 1995 (Pelletier et al. 1995). This slow reduction rate could be attributed to various factors, including environmental, socioeconomic, and political influences (Black et al. 2008).

At the household and individual levels, child undernutrition is associated with many factors. For example, a recent study in Ethiopia found that child, maternal, and household characteristics were significantly associated with stunting and wasting among children under the age of five (Kassie & Workie 2020). A recent systematic literature review reported that, in addition to food intake-related factors, other determinants significantly associated with child malnutrition include household income, maternal education and nutrition status, child's age and birth weight, family size, and birth order (Katoch 2022). Programmes targeting poverty alleviation, child education, and food security have been crucial in reducing malnutrition and household food insecurity in many developing countries (Mozaffarian et al., 2018; Lavallee et al., 2010). Evidence indicates that agricultural practices can have a significant impact in reducing undernutrition and promoting community well-being (Kumar et al., 2019; Warinda and Nyariki, 2019; Mozaffarian et al., 2018; Hetherington et al., 2017).

Animal-source foods (ASF), including milk, and other dairy products, constitute a vital component of human diets (McKune et al. 2022). Research consistently demonstrates the significant benefits that are associated with ASF consumption, including a reduced risk of stunting and being underweight for children (Darapheak et al. 2015), as well as decreased morbidity and mortality among children under five (Dror & Allen 2011). Moreover, these advantages extend beyond childhood, as they benefit pregnant women and lactating mothers as well. For instance, a study focusing on pregnant mothers found that those who included fish in their diets during pregnancy and lactation typically birthed children with better weight-for-age measurements (Marques et al. 2008). Similar positive associations have been observed for other ASF, such as meat, milk, and eggs.

The significance of ASF consumption lies in its provision of essential nutrients which are crucial for human health and growth (Schönfeldt et al. 2013). Efforts to understand the relationship between livestock ownership, ASF consumption, and child nutritional outcomes are ongoing. Despite variations in research findings, evidence suggests that households with at least one livestock species generally have higher proportions of ASF consumption (Hetherington et al. 2017). Inadequate consumption of ASF, often exacerbated by poverty, correlates positively with malnutrition in developing countries (Leroy & Frongillo 2007). Recent evidence has shown that domestic food production is positively linked to the population's nutritional status, as an increase in food production enhances food supply and, consequently, food availability (Awad 2023).

Household food insecurity is defined as the inability of a household to access adequate and nutritious food (Bickel et al. 2000). The latest United Nations report on global food security and nutrition highlights a concerning situation despite ongoing efforts to combat hunger (FAO et al. 2023). Since 2019, over 122 million more people are experiencing hunger, and approximately 2.4 billion individuals lack access to sufficient, safe, and nutritious food (FAO et al. 2023). Women and children are the most affected by this. Additionally, the report reveals that in 2021, nearly 150 million children were stunted, 45 million were wasted, and 37 million were underweight (FAO et al. 2023).

While infants, young children, and women of childbearing age are typically identified as the most vulnerable to poor nutrition, studies have shown that inadequate nutrition affects adolescents, adults, and the elderly as well (McKune et al. 2022). This underscores the importance of promoting ASF consumption across all age groups to comprehensively address malnutrition. The consequences of undernutrition include various aspects, including but not restricted to the physical growth and development of children, their educational performance, and overall health (McKune et al. 2022).

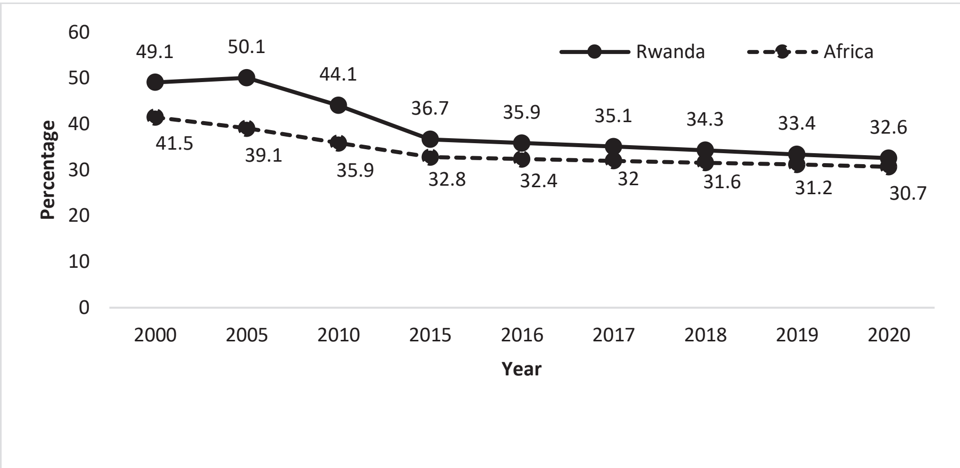

Figure 3. Stunting rates among Rwandan children under 5 years.

Source: FAO, (2022b)

2.4 Contribution of zoonotic infections to undernutrition

Zoonotic diseases, which arise from the interaction between animals and humans, are a major concern in LMICs, particularly at the household level (Matilla et al. 2018). Previously, the impact of zoonoses on human health was expressed in terms of disability-adjusted life years (DALYs) (Murray et al. 2000). However, a new concept that considers only the incidence and prevalence of zoonoses, was introduced—Zoonosis Disability Adjusted Life Years (zDALYs) (Torgerson et al. 2018). Despite advancements in estimating the impact of zoonotic diseases on human health, few reports have emphasized their contribution to undernutrition (Grace et al., 2012; Anantharam et al., 2021) and, to some extent, poverty (Grace 2014).

2.4.1 Household factors associated with zoonoses in children

On one hand, the consumption of milk and dairy products is undeniably linked to reductions in chronic undernutrition in early life, particularly stunting (Headey et al. 2024). However, evidence shows that most infants and young children in LMICs are fed monotonous diets with a low intake of nutrient-rich fruits, vegetables, and ASF (Gatica-domínguez et al. 2021). These poor diets are a root cause of undernutrition in early childhood, which can lead to compromised schooling, cognitive outcomes, and lower wages in adulthood (Black et al. 2013). On the other hand, increasing livestock production to meet growing demands has led to more interactions at the livestock-wildlife-human interface, thereby creating more opportunities for the spread of zoonotic diseases (Kelly et al. 2018). A recent review in East Africa highlighted that some pastoral communities continually engage in risky practices related to zoonotic diseases, such as drinking unboiled milk, assisting in animal births with bare hands, and living in close proximity with livestock (Mpatswenumugabo et al. 2023).

The impact of malnutrition on the immune system has been extensively studied, and findings indicate that malnourished individuals exhibit a weakened immune response (Liu et al. 2021; Stelmasiak et al. 2021). However, the role that the immune system plays in influencing nutritional outcomes is often misunderstood. A systematic review demonstrated that immune dysfunction is both a cause and an effect of malnutrition (Bourke et al. 2016). Recent research has also highlighted that the lack of secretory IgA and disrupted development of the gut immune system and microbiota are linked to environmental enteric dysfunction, a leading cause of nutrient malabsorption and diarrhea and thus, malnutrition (Perruzza et al. 2024). Young children's immune systems are weaker than adults, therefore, they are more vulnerable to many diseases, including milk-borne zoonoses. For instance, a study investigating the zoonotic risks of pathogens from sheep and their milk-borne transmission revealed that young children, pregnant women, and the elderly are at greater risk compared to others when consuming raw milk and raw milk products (van den Brom et al. 2020). Other household factors, such as a low socioeconomic status and poor access to clean water and sanitation, can also increase the risk of zoonotic infections in young children due to food contamination (Ngure et al. 2014).

The composition of milk makes it an ideal medium for the growth and multiplication of many pathogenic bacteria. To ensure milk safety, it is crucial to control temperature and maintain hygiene throughout the entire milk value chain (Porcellato et al. 2018). The gold standard for ensuring milk safety is pasteurization—a heat treatment method that, when properly applied, can effectively reduce the level of pathogenic bacteria in milk (van den Brom et al. 2020). However, in LMICs, rural communities often lack the infrastructure and resources to properly store and process milk and milk products (Grace et al. 2020). This deficiency predisposes household members, particularly children, to milk-borne zoonoses. Despite significant advancements in the medical and dairy sectors, infant mortality rates due to milk-borne zoonoses remain high (Currier & Widness 2018).

2.4.2 Common milk pathogens

As previously discussed, animal-source foods (ASF) provide a valuable array of high-quality nutrients to young children, including proteins, vitamins, and several minerals (Li et al. 2019). However, these benefits come with a risk, namely food-borne diseases, which can hinder the nutritional value of ASF and other nutritious foods (Havelaar et al. 2022). Common milk pathogens include bacteria, viruses, parasites, and prions (Almashhadany et al. 2022).

A recent review on milk contaminants in dairy products in sub-Saharan Africa has provided a detailed list of the potential pathogens that can be found in milk (Akinyemi et al. 2021). The most common bacteria present in milk are *Brucella abortus*, *Coxiella burnetti*, *Mycobacterium bovis*, *Bacillus, Campylobacter jejuni, Clostridium, Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella*, *Staphylococcus aureus*, and *Yersinia* *enterocolitica* (Akinyemi et al. 2021). These bacteria may originate from cows that are infected with mastitis, including pathogens such as *Staphylococcus aureus, Streptococcus uberis, Streptococcus agalactiae*, *Corynebacterium bovis*, coagulase-negative staphylococci, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Mycoplasma species* (Dalanezi et al. 2020; Klaas & Zadoks 2018; Kibebew 2017; Azevedo et al. 2016). Alternatively, they may come from other animal diseases or environmental sources, such as *Brucella abortus, Coxiella burnetii, Mycobacterium bovis, Bacillus, Campylobacter jejuni*, *Clostridium, Escherichia coli O157, Listeria monocytogenes, Salmonella* spp*.,* and *Yersinia enterocolitica* (Akinyemi et al. 2021; Cobirka et al. 2020).

On the other hand, viruses commonly isolated from milk include astrovirus, calicivirus, Central European encephalitis virus, coronavirus, enteroviruses, hepatitis A (HAV) and E viruses (HEV), norovirus, rotavirus, and tick-borne encephalitis virus (TBEV) (Long et al., 2017; van Doremalen et al., 2014). Since foot-and-mouth disease virus (FMDV) is endemic in some parts of Africa, it was isolated from cow milk in Tanzania (Armson et al. 2019). Additionally, rift valley fever virus was recently isolated from cow milk in Kenya (Grossi-soyster et al. 2019). Further, a few parasites have been found in cow milk, including *Toxoplasma gondii* (Boughattas 2017), and *Cryptosporidium* spp. (Ursini et al. 2020).

2.4.3 Relationship between diarrheal infections and undernutrition in young children

Malnutrition is a complex health condition which is primarily caused by an inadequate nutrient intake and diseases such as diarrhea (WHO & UNICEF 2009). Both acute and chronic malnutrition (stunting) are significant predisposing factors to death following moderate to severe diarrheal infections in children under 5 years of age (Kotloff et al., 2013; O'Reilly et al., 2012; Caulfield et al., 2004). A cross-sectional study investigating the impact of childhood nutritional status on pathogen prevalence and the severity of acute diarrhea among Kenyan children aged 6–59 months revealed that children with malnutrition exhibited more severe disease symptoms than those without malnutrition (Tickell et al. 2017). It is reported that these differences could be attributed to the reduced immune response to infection in undernourished children (Friis et al. 2014).

Besides nutrient loss, diarrheal infections have long-term effects on young children that go beyond dehydration and death, including gut dysbiosis (Chawla et al. 2022). The gut microbiome, an ecosystem of symbiotic microbes, is imperative for nutrient absorption in healthy individuals and influences other physiological processes, such as obesity and malnutrition in children of various age groups (Caesar et al., 2015; Chassaing et al., 2014; Ley, 2010). In undernourished children, the gut microbiome is characterized by increased Proteobacteria and decreased *Bifidobacterium* and *Lactobacillus* species (Iddrisu et al. 2021). This microbiome imbalance allows for intestinal colonization by potentially pathogenic bacteria such as *Fusobacterium mortiferum*, *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli,* and *Enterococcus faecalis*, which can subsequently cause diarrhea (Caesar et al., 2015; Ghosh et al., 2014). Significant research has highlighted the link between early childhood infectious diarrhea and its impact on health, development, and human capital (Brennhofer et al. 2017); Adair et al., 2013; Deboer et al., 2012). Studies estimate that national economic growth rates in developing countries decrease by 8% or more due to the reduced potential for education and productivity among their populations (Horton & Steckel 2014).

Various reports have highlighted the relationship between child deaths and malnutrition and infectious diseases (Caulfield et al., 2004; Rice et al., 2000; Pelletier et al., 1995). While nearly 53% of child deaths are associated with undernutrition, more than 60% of these deaths are caused by diarrheal infections (Caulfield et al. 2004). This phenomenon is described as “the vicious cycle” between undernutrition and infections (Figure 4). Previous predictions have estimated that 55% of child deaths worldwide are attributable to undernutrition (Pelletier et al. 1995), but recent data shows that the age-standardized proportion of deaths attributable to child and maternal undernutrition is 12.2% (Melaku et al. 2018). In 2009, the WHO reported that diarrheal infections were the leading cause of death in children under 5, accounting for 17% of all child deaths (WHO 2009). Undernutrition contributed to 73% of diarrhea cases, compared to 44% for pneumonia, which had a similar child death percentage (WHO 2009). Therefore, emphasizing adequate health and nutrition during pregnancy and early childhood is recommended, as higher birth weights and early growth correlate with greater adult height and educational outcomes (Scharf et al. 2014).

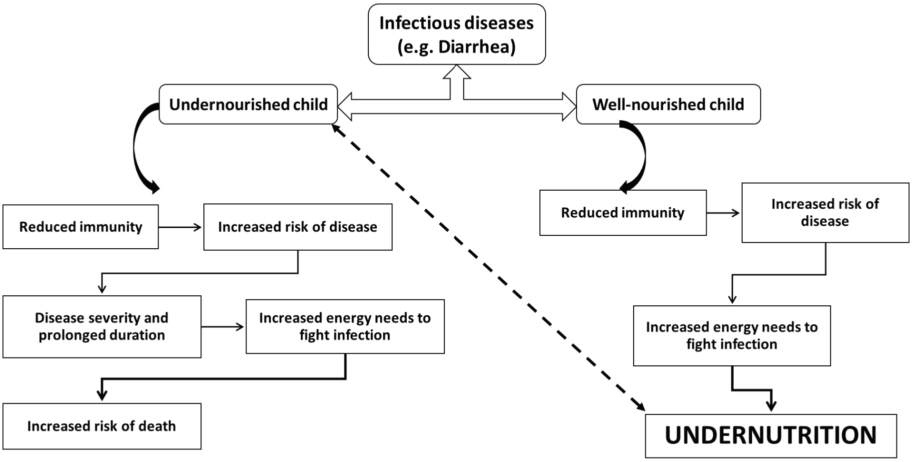

Figure 4. The vicious cycle between undernutrition and infections in children under 5 years.

Source: Adapted from VoiCE, (2022).

3. Aims

The overall aim of this thesis was to investigate management and household practices associated with milk production and quality in smallholder dairy farms, with the overarching goal of improving food security, food safety, nutrition, and livelihoods of poor smallholder households in northern Rwanda.

The specific aims were:

- To review the current knowledge on the relationship between milk consumption and the occurrence of selected bacterial zoonoses in East Africa.
- To evaluate the contribution of management factors to milk yield, composition, and quality in smallholder dairy farms.
- To characterize selected potential zoonotic pathogens present in milk and children's faecal samples collected from the same households.
- To investigate the risk factors associated with the prevalence of zoonotic pathogens in both milk and children’s faecal samples from the same households.
- To explore the sampling and analysis of child undernutrition determinants through a multidisciplinary approach.

4. Materials and methods

The studies included in this thesis used various study designs, data collection, and analytical methods. Table 1 summarizes the different methods and designs for each study.

Table 1. Description of study designs and data analysis.

Study	Study design	Data analysis
Study I	Systematic review	PRISMA guidelines and statistical analysis
Study II-III	Cross-sectional and observational	Laboratory and statistical analysis
Study VI	Population-based cross-sectional	Spatial and statistical analysis

4.1 Study population

This study is part of a multidisciplinary population-based research project targeted at households with children below 36 months of age, from the Northern Province of Rwanda, between 24th November and 30th December 2021. The study area was chosen because the Northern Province of Rwanda has the highest prevalence of undernutrition, with 41% of children under five being stunted (National Institute of Statistics of Rwanda (NISR) [Rwanda] et al. 2021).

4.2 Data collection and processing

4.2.1 Systematic review (Paper I)

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were used to select relevant articles for a systematic literature review (Moher et al. 2009). Articles published between 2010 and 2021 from the seven East African countries (Burundi, Ethiopia, Kenya, Rwanda, South Sudan, Tanzania, and Uganda) on milk quality and selected milk-borne zoonoses (brucellosis, campylobacteriosis, *Escherichia coli*, Q fever, salmonellosis, and tuberculosis) were retrieved from Web of Science, PubMed, and Scopus. The relevance of articles was assessed based on their titles and abstracts. Only cross-sectional, cohort, and longitudinal studies published in English were selected. .

4.2.2 Household selection and enrolment (Paper II-IV)

The overall sample size was determined using a formula for prevalence studies (Daniel & Cross 2018).

$$n = \frac{Z^2 p(1-p)}{d^2} DEFF \tag{1}$$

where n is the sample size, Z is the critical value at $\alpha/2$ (1.96 for 95% confidence level), p is the expected prevalence of stunting in the Northern Province (41%), and d is the degree of precision (0.05). $DEFF$ is the design effect of 1.5 (to account for the increased variability that might be due to intra-cluster correlation). The primary sample size was adjusted for a 10% non-response rate, resulting in a final sample size of 601 households.

Household selection was conducted in multiple stages. The first stage involved randomly selecting 137 villages (the smallest administrative unit in Rwanda) from the five districts of the Northern Province (Figure 5). The second stage involved randomly selecting 601 households with at least one child aged 1–36 months for the survey. Finally, out of these 601 households, 156 had lactating cows and were interviewed, and milk samples were subsequently collected.

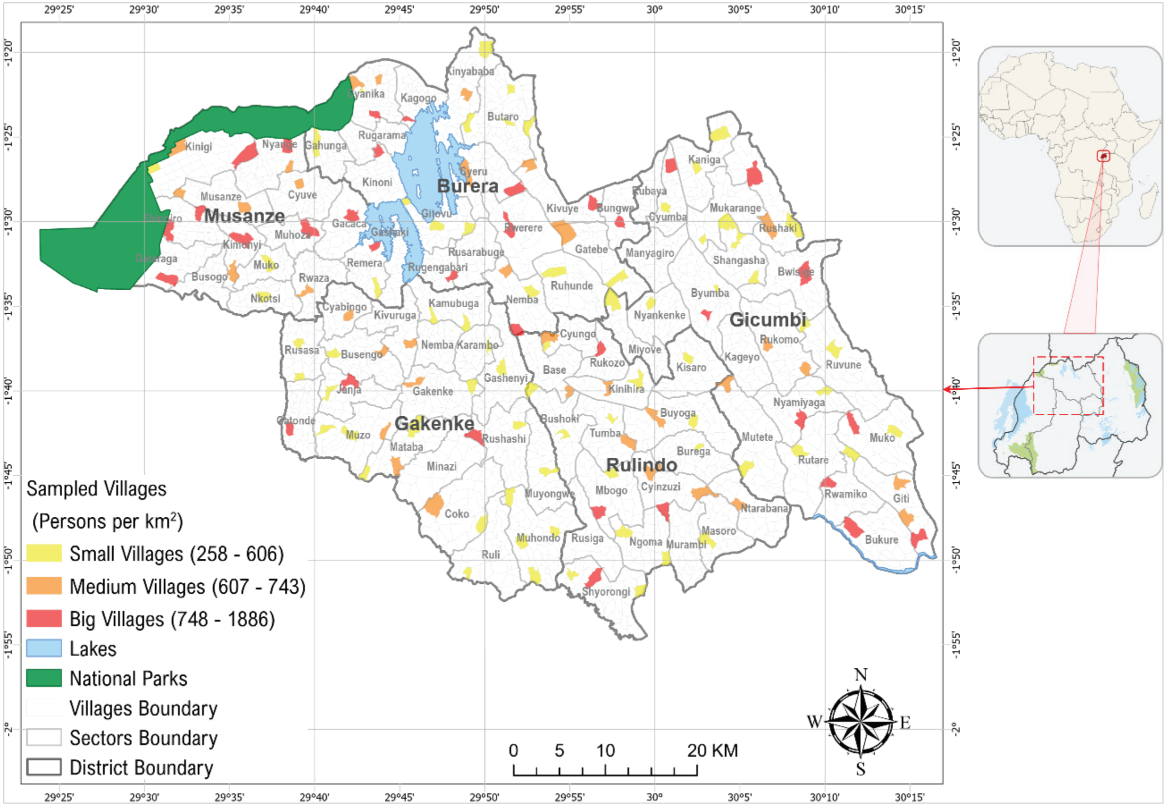

Figure 5. Study area in Northern Province, Rwanda.

Source: Kagoyire et al., 2024

4.2.3 Survey and questionnaire development

An android-based mobile GIS application called emGeo was created, leveraging the internet-based Information Management System for Environmental Protection and Disaster Risk Management (iMSEP) (Mansourian et al. 2023). This tool was designed to facilitate the collection, storage, analysis, management, and sharing of geographical data among researchers located in different places (Sweden and Rwanda) who were unable to be on site due to the Covid-19 pandemic.

A structured questionnaire was designed, adapted, uploaded on android tablets, and piloted. Before data collection began, two groups of enumerators were established. The first group, consisting of PhD students and enumerators from non-animal science disciplines, was responsible for collecting household and child data. The second group, made up of PhD students and data collectors with an animal science background, focused on gathering livestock-related data. To avoid confusion during data collection, a separate section was dedicated to collect data related to livestock production.

4.2.4 On farm observational data collection

A separate form was used to gather data on individual animals and farm management practices. Information at the animal level included breed, age, parity, lactation stage, body condition score, hygiene, and milk yield. At the farm level, data was collected on the grazing system, hygiene of animal housing, milking procedures, milking frequency, hygiene of the milkers, and the milking area.

4.2.5 Milk and faecal samples

Milk and child faecal samples were collected from households with lactating cows. Milk samples were aseptically collected from the milk storage container, which was kept at room temperature within the house. Along with milk samples, children's faecal samples were collected using a rectal swabbing technique. Rectal swabs were collected into screwcap tubes containing 1 ml transport media (E-swab, *FLOQSwabs*® with 1 ml liquid Amie’s medium, Copan, Italy) by a registered nurse from the first data collection group. Milk samples and rectal swabs were stored in separate cool boxes during the fieldwork period (2-4 hours) and kept at -20°C at the nearest health centre until Friday. To ensure a cold chain during the transportation of samples from the health centre to UR, a -20°C portable freezer (Labfreez, Beijing, China) was plugged into the car.

4.3 Laboratory analysis

4.3.1 Milk composition and quality (Paper II)

To determine milk composition and quality, a series of analyses were conducted at either the farm or in the Microbiology laboratory of the University of Rwanda. Manufacturers' instructions were followed to ensure reproducibility and accuracy of the results.

The Lactoscan SP (Ultrasonic Milk Analyzer, Milkotronic Ltd) was used to determine the milk composition. The analysis measured several components of the milk, including fat (%), protein (%), solids-non-fat (SNF) (%), density, and lactose (%). The results were printed using the analyzer's inbuilt printer and then manually recorded in Excel for statistical analysis.

Somatic cell counts analysis was completed using a portable DeLaval cell counter (DCC) (DeLaval International AB, Tumba, Sweden). The results, displayed in cells/µl, were subsequently converted to cells/ml (Kandeel et al. 2018). These results were then recorded into an Excel sheet containing the excerpt from the survey data to match them with the household and animal data.

Antibiotic residues in milk were assessed using the Delvotest SP-NT kit (DSM, Delft, the Netherlands). Results were interpreted visually: a negative result was indicated by yellow agar, while a positive result was indicated by purple agar.

4.3.2 Nucleic acids extraction (Paper III)

Due to logistical issues, nucleic acid extraction was conducted in both Rwanda and Sweden.

Nucleic acid extraction from milk samples was completed at the Michael Cranfield Regional One Health Laboratory at Gorilla Doctors in Musanze, Rwanda. Total nucleic acids were isolated using the AllPrep PowerViral DNA/RNA Kit (Qiagen, Helden, Germany) according to the manufacturer’s instructions. One hundred µl of the DNA/RNA products were stored at -20°C until they were transported frozen to Gothenburg University in Sweden for further analysis.

Nucleic acid extraction from children's faecal samples was completed at the Diagnostic Laboratory at the Department of Infectious Diseases, Institute of Biomedicine at Sahlgrenska Academy, University of Gothenburg, Sweden. Samples were thawed from -80°C and diluted; 250 µl of each sample was transferred to 2 ml of NUCLISENS® easyMAG® Lysis Buffer (Biomérieux, France). The samples were then placed in the eMAG® system (Biomérieux, France) for total nucleic acid extraction. After a 90-minute run, the eMAG system automatically eluted the nucleic acids into 100 µl of elution buffer, which were then transferred to a 2 ml storage tube for downstream applications.

4.3.3 Multiplex PCR (Paper III)

In this study, an in-house (Sahlgrenska Academy, University of Gothenburg, Sweden) multiplex quantitative real-time Polymerase Chain Reaction (qPCR) system was developed and used to detect selected pathogens in milk and faecal samples.

QuantStudio 6 Flex Real-Time PCR system (Life Technologies, USA) was used to run the PCR analysis. UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Thermo Fisher Scientific, UK) was used to dilute both primers and probes according to the manufacturers' instructions. Universal MasterMix (UMM ABI) (Applied Biosystems, USA) was used for bacteria, adenovirus, and parasites while Taqman Fast Virus 1-step Mastermix (Applied Biosystems, USA) was used for RNA viruses. Several reactions were constructed according to the target genes and pathogens, each of them containing 21 µl reaction volume. Reaction 1 contained 5 µl of template DNA, 10 µl of UMM ABI, 0.5 µl of forward primer, 0.5 µl of reverse primer, 0.5 µl of probe, and 4.5 µl of SuperQ PCR grade water for bacteria, parasite, and adenovirus. Reaction 2 contained 5 µl of template RNA, 5 µl of UMM ABI, 0.5 µl of forward primer, 0.5 µl of reverse primer, 0.5 µl of probe and 9.5 µl of SuperQ PCR grade water for RNA viruses.

4.4 Data management and statistical analysis

For Paper I, articles were individually recorded in an Excel sheet for further statistical analysis. Since we did not perform a meta-analysis, we computed basic statistics, including (1) the mean prevalence rates of each bacterial isolate, (2) the number of publications per country, and (3) the percentage of individual pathogens.

Survey responses, observational data, and laboratory results were recorded in an Excel sheet and exported to R software (R Core Team, Vienna, Austria) for descriptive and inferential statistical analyses. A multivariate analysis of variance (MANOVA) (Paper II) and multiple logistic regression (MLR) (Paper III) tests were conducted to identify any potential associations between outcome variables and independent variables, with statistical significance set at $p<0.05$. Multicollinearity was assessed among independent variables to ensure that the significant overall p-values represent the true significance of the statistical model. This was done by determining the Pearson correlation, where $p > 0.05$ was considered non-significant, indicating no relationship between independent variables in the equation.

4.5 Sampling and analysis of undernutrition among children

A multidisciplinary approach was employed to investigate the determinants of undernutrition among children aged 1 to 36 months in the study area. A team of 19 researchers, including six PhD students from fields such as health sciences, nutrition, animal sciences, and Geographic Information Science (GIS) and innovation sciences, along with eight nurses, four veterinary doctors, and one postdoctoral researcher, was formed. An android-based app on the emGeo platform was developed using a structured questionnaire to capture a wide range of factors associated with stunting. Additionally, haemoglobin levels were measured on a portable photometer (HemoCue, Ängelholm, Sweden) and anthropometric measurements were calculated as previously described (World Health Organization, 2006).

The preliminary data analysis involved calculating height-for-age z-scores (HAZ), weight-for-height z-scores (WHZ), and weight-for-age z-scores (WAZ). To explain the variability in stunting rates and associated risk factors, a classical binary logistic regression was applied based on the stunting data. Lastly, random machine learning and geographically weighted logistic regression analyses were carried out to identify the most important predictors of stunting.

4.6 Ethical considerations

An ethical approval application was submitted prior to data collection and was reviewed and granted by the Institutional Review Board (IRB) of the University of Rwanda, College of Medicine and Health Sciences (review approval notice no. 181/CMHS IRB/2021). Additional authorization to conduct data collection was obtained from designated government institutions, including the Ministry of Health, and the Ministry of Local Government. Informed consent was also obtained from participants before their voluntary participation in the study through consent forms. Participants were informed that refusing to participate or withdrawing from the research at any stage would not result in a penalty or loss, either currently or in the future. Due to the sensitive nature of the information, including geo-located data on surveyed households, a data anonymization protocol was implemented after data collection to reduce the risk of participant identification. This process ensured that the data remained untraceable throughout both the analysis and publication of the study results.

5. Main results

This section presents a summary of the key findings from the research. For more detailed information and an in-depth analysis, the individual Papers I-IV included in this thesis provide comprehensive insights and specific data on the topics covered.

5.1 Prevalence and risk factors associated with milk-borne zoonoses (Paper I)

A systematic literature review was carried out to evaluate the major risk factors associated with milk consumption and milk-borne bacterial zoonotic infections in East African communities. The review included 65 articles from seven East African countries—Burundi, Ethiopia, Kenya, Rwanda, South Sudan, Tanzania, and Uganda—published between 2010 and 2021. Several risk factors associated with zoonotic infections in humans have been identified from various studies (Table 2). Common factors included dietary habits, such as the consumption of raw milk and dairy products. Moreover, an increasing amount of research suggests that poor animal husbandry practices carry serious health risks to farmers in East African communities. These risks arise from living in close proximity to livestock, handling infected materials from aborted animals without proper protection, and assisting with the calving of cows without wearing gloves.

Table 2. Selected risk factors associated with incidences of milk-borne zoonotic infections in East Africa (2010-2021).

Category	Identified risk factors	Reference(s)
Food habits	Consuming unpasteurized milk and milk products	Ndeereh eta l., 2016; Njenga et al., 2020; Caudel et al., 2019; Onono et al., 2020; Onyango et al., 2021; Lado et al., 2012; Mburu et al., 2021; Sawi et al., 2010; Kansiime et al., 2014; Kazoora et al., 2015; Asiimwe et al., 2015
Animal husbandry practices	Attending to parturition	Onono et al., 2020; Ndeereh et al., 2018; Mburu et al., 2021; Njenga et al., 2020; Sawi et al., 2010; Asiimwe et al., 2015
	Living in close contact with animals	
	Handling animal products	
	Being agro-pastoralist	

Across all East African countries, *Brucella* spp. was the most frequently investigated pathogen, accounting for 54.5% of the research. Contrastingly, *Coxiella burnetii* and *Campylobacter* spp. were the least studied, each representing only 1.5% of investigations. Ethiopia contributed the most articles, with 17 out of 65 publications, while only one article was retrieved from Burundi. Culture-based microbiological techniques were the most commonly used method for identifying zoonotic pathogens in biological samples. Regardless of the sample type, *E. coli* had the highest prevalence rate at 41%, followed by *Mycobacterium* spp. at 28.1%, while *Salmonella* spp. had the lowest prevalence rate at 9.7% (Table 3).

Table 3. Distribution of isolated pathogens and their respective prevalence rates.

		Prevalence (%)				
Pathogen	**N**	**Mean**	**Median**	**Minimum**	**Maximum**	**SD**
Brucella spp.	22	17.6%	16.2%	0.6%	56.0%	12.7%
E. coli 0175:H	4	22.5%	17.6%	2.9%	52.0%	21.7%
Escherichia coli	10	41.0%	38.2%	12.0%	66.7%	21.2%
Salmonella spp.	9	9.7%	6.3%	3.3%	26.5%	7.2%
Mycobacterium spp.	2	28.1%	28.1%	18.8%	37.3%	13.1%

SD: standard deviation

The distribution of isolated pathogens and the samples from which they were obtained showed that more pathogens were isolated from milk samples than from human samples (Figure 6). Additionally, the mapping revealed that only *Brucella* and *Mycobacterium* spp. were isolated from human samples, highlighting why these two pathogens are of greater concern compared to the other studied pathogens.

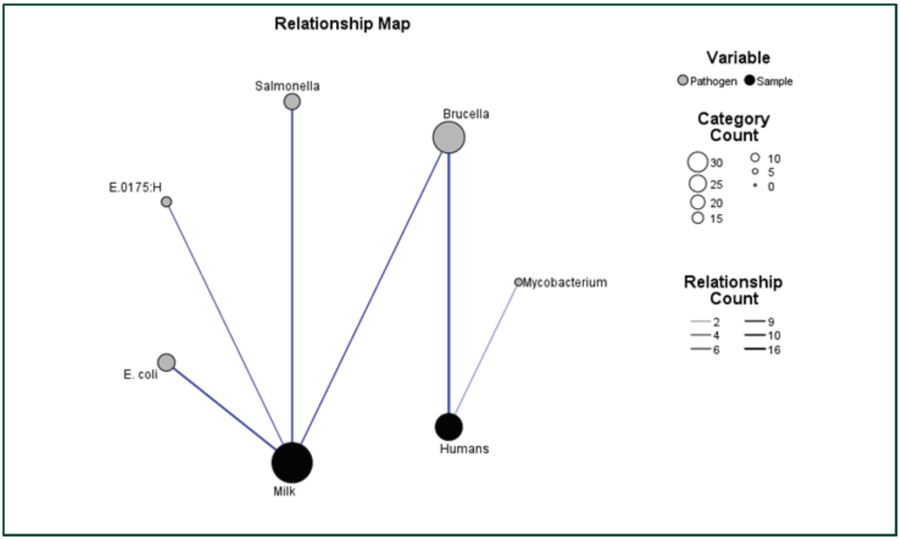

Figure 6. The relationship between isolated pathogens and sample types.

5.2 Socioeconomic characteristics of smallholder farmers in the study area (Paper II-IV)

The majority of households in the study area were male-headed (92.5%) and had an average of six people per household. Only 5% of male partners held skilled jobs, compared to just 1.3% of female partners, with the rest engaged in unskilled labor. This largely explains why 92% of households earned less than 100,000 Rwandan francs (approximately SEK 780) per month (Figure 7). Agriculture, including both crop and livestock production, was the primary source of income for most households. The findings show that each household owned at least one type of livestock. Thirty-eight percent of households had only cattle, and more than 60% had both cattle and other types of livestock, such as small ruminants, poultry, pigs, and rabbits.

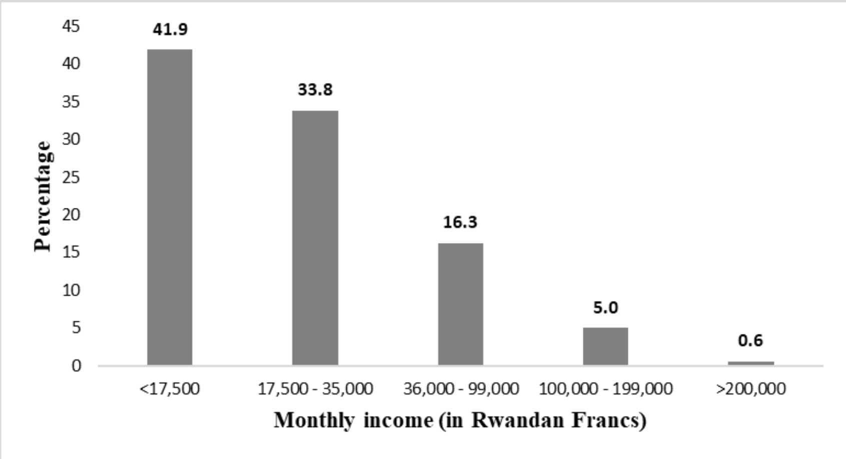

Figure 7. The monthly income per household included in Papers II-III.

5.3 Farm management practices and biosecurity measures (Paper II-III)

Most farmers, over 67%, did not own land, and more than 95% of them used the cut-and-carry technique and crop residues to feed their animals. The most common dairy breed, found in 87.5% of cases, was a cross between local Ankole cattle and exotic breeds such as Friesian or Jersey. The average daily milk production per cow was around 4 liters, with households typically owning between 1 and 2 cows. The predominant feeding system in the study area was zero grazing, practiced by 84.2% of farmers. Record keeping was uncommon, with only 59% of farmers keeping fertility and reproduction records. Interestingly, nearly 93% of farmers reported washing their hands and the cow's udder before milking.

Regarding reproduction techniques, artificial insemination was not widely used among dairy farmers, with only about 15% reporting its use, while 80% relied on natural mating. Mastitis was the most prevalent dairy disease, reported in 76.3% of cases by farmers. The most common reproductive disorders were the absence of heat and repeated breeding, accounting for 46.8% and 28.8% of cases, respectively. Only 15% of farmers were aware that artificial insemination can help reduce the spread of diseases among animals, whereas nearly 80% believed that vaccinating young calves was the most effective method for preventing animal diseases.

5.4 Factors associated with milk yield and quality (Paper II)

The findings demonstrated that 12.9% of milk samples contained antibiotic residues, and 34.2% had a somatic cell count exceeding 300,000 cells/ml. However, no statistically significant association was found between these outcomes and the management practices included in the model. Contrastingly, milk yield was significantly influenced by factors such as milking frequency, breed, parity, and body condition score (BCS). It was revealed that milking twice resulted in a higher mean yield ($p < 0.001$) compared to milking once. The Ankole breed showed a lower yield ($p < 0.001$), compared to other breeds. Similarly, a good BCS was associated with a higher milk yield ($p <0.001$) compared to a moderate and poor BCS ($p >0.05$), while multiparous cows produced more milk ($p = 0.002$) than primiparous cows (Table 4).

Table 4. Statistical significance of management practices associated with milk yield.

Management practice	Mean	SD	p-value
Milking frequency			***<0.001***
Once	3.5	2.0	
Twice	5.0	2.4	
Body condition score			***<0.001***
Good	5.4	2.6	
Moderate	3.7	1.9	
Poor	2.3	1.3	
Parity			***0.002***
Primiparous	3.9	2.1	
Multiparous	5.0	2.6	
Breeds			***<0.001***
Ankole	2.0	1.1	
Crossbreed	4.1	2.1	
Friesian	8.0	2.8	
Jersey	6.0	2.3	

SD: standard deviation

5.5 Characterization and prevalence of milk and faecal pathogens (Paper III)

The study found that 35.9% of milk samples contained pathogens, with *Salmonella* spp. being the most common (25.6%). However, 84.6% of faecal samples tested positive for various pathogens, with *E. coli* strains being the most prevalent. Specifically, virulence factor genes (*eae*) associated with enteropathogenic *E. coli* (EPEC) were detected in 38.5% of faecal samples, while toxin genes (*eltB*) related to enterotoxigenic *E. coli* (ETEC) were present in 31.4%. Other *E. coli* toxin genes were found in less than 10% of faecal samples. Adenovirus was the most prevalent virus, found in 37.8% of faecal samples (Table 5).

Table 5. Characterization of isolated pathogens and their respective prevalence rates.

	Milk samples		Faecal samples	
Pathogens	**N**	**Prevalence (%)**	**N**	**Prevalence (%)**
Adenovirus	0	0.0%	59	37.8%
Astrovirus	0	0.0%	7	4.5%
EPEC-*bfpa* gene	0	0.0%	7	4.5%
Campylobacter spp	0	0.0%	13	8.3%
Cryptosporidium parvum/hominis	0	0.0%	3	1.9%
ETEC-*eltB* gene	1	0.6%	49	31.4%
ETEC-*estA* gene	0	0.0%	9	5.8%
EHEC *vtx1* gene	0	0.0%	14	9.0%
EHEC *vtx2* gene	1	0.6%	9	5.8%
EPEC *eae* gene	0	0.0%	60	38.5%
Hepatitis E	6	3.8%	0	0.0%
Norovirus GI	0	0.0%	11	7.1%
Norovirus GII	0	0.0%	8	5.1%
EHEC O157	0	0.0%	22	14.1%
Rotavirus	12	7.7%	12	7.7%
Salmonella spp	40	25.6%	9	5.8%
Sapovirus	1	0.6%	12	7.7%

Shigella spp	2	1.3%	29	18.6%
Overall prevalence		**35.9%**		**84.6%**

Multiple logistic regression (MLR) analysis showed no significant association between faecal pathogens and independent variables, but handwashing and grazing systems were associated with milk pathogens (Table 6).

Table 6. MLR analysis of selected practices and the prevalence of milk pathogens.

Variable	p-value	OR	95%CI
Washing hands			
Always	Reference		
Sometimes	**0.003***	0.27	[0.12 0.64]
Rarely	0.090	0.26	[0.05 1.24]
Grazing system			
Grazing	Reference		
Zero grazing	0.187	11.41	[0.31 426.32]
Semi-zero grazing	**0.048***	51.08	[1.04 2499.91]
CI – confidence interval, OR – odds ratio			

Although some risk factors were not linked to the prevalence of pathogens in either milk or faecal samples, certain household and farm practices were common across the study area (Table 7). For example, the majority of farmers (84.6%) practiced a zero-grazing system, and 87.2% of farmers milked their cows in animal sheds, most of which had soil floors. Moreover, 70% of households had access to an improved source of drinking water, only 53.8% reported boiling drinking water before use.

Table 7. The distribution of household hygiene and milking practices in the study area (N=156).

Practice	Category	Proportion (%)
Source of drinking water	Improved	105 (67.3)
	Unimproved	51 (32.7)
Drinking water treatment	Yes	84 (53.8)
	No	72 (46.2)
Washing hands	Always	69 (44.2)

	Sometimes	76 (48.7)
	Rarely	11 (7.1)
Grazing system	Grazing	8 (5.1)
	Zero grazing	132 (84.6)
	Semi-zero grazing	16 (10.3)
Type of animal house floor	Concrete	10 (6.4)
	Soil	140 (89.7)
	Wooden	3 (1.9)
	No animal house	3 (1.9)
Milking place	Cowshed	136 (87.2)
	Milking parlour	6 (3.8)
	Open area	14 (9.0)
Animal hygiene	Clean	7 (4.5)
	Moderately dirty	59 (37.8)
	Slightly dirty	68 (43.6)
	Very dirty	22 (14.1)

5.6 Nutritional status of children and influencing factors (Paper IV)

According to the height-for-age z-scores calculated in this study, 27% of all children were found to be stunted, with boys showing a higher rate of stunting (33.8%) compared to girls (20.9%). To explain the variability in stunting rates and associated risk factors, a classical binary logistic regression was applied based on the stunting data.

It was found that household economic factors, such as not having access to electricity ($p<0.001$) and food insecurity ($p<0.001$), were strongly associated with child stunting. Other associated factors included underweight status, child age, gender, birth weight, and breastfeeding status, all of them with $p<0.001$. The distance to the nearest health facility ($p= 0.02$), availability of handwashing facilities at home ($p<0.001$), whether the child received deworming tablets ($p<0.001$) and vitamin A supplements ($p= 0.001$), the type of milk products consumed (fresh or fermented), and not owning a home garden were also significant contributors. Multivariate logistic regression identified additional risk factors for stunting. For instance, a mother's decision-making autonomy in major household purchases was associated with an increased likelihood of stunting (OR = 1.2935).

To explore the underlying causes and spatial distribution of stunting in this study, GIS and spatial analyses were conducted. The findings identified 26 variables as the most relevant predictors of stunting, which explains 30% of the variability in stunting. Additionally, the geographically weighted logistic regression showed that 35% of the variation was accounted for by a combination of independent variables and geographic location (Figure 8).

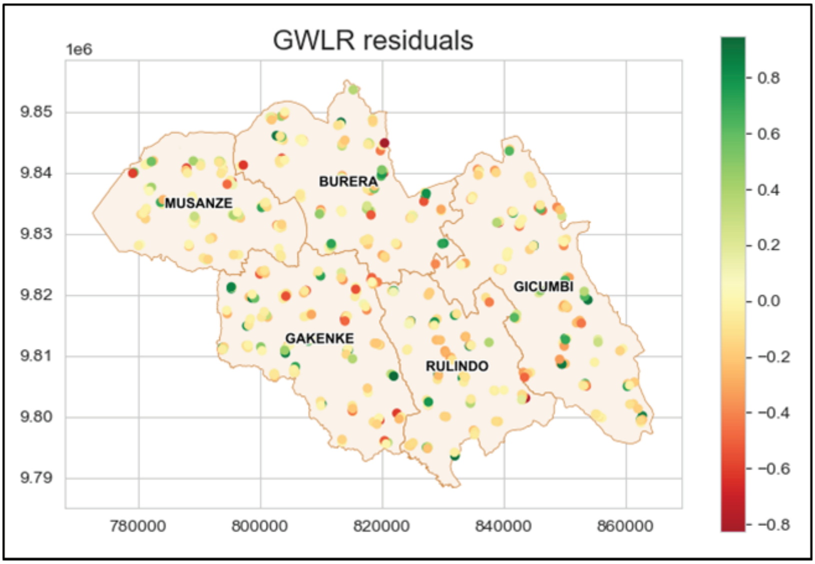

Figure 8. Spatial distribution of the GWLR model residuals.

Source: Kagoyire et al., 2024

6. General discussion

The overall objective of this thesis was to investigate management and household practices related to milk production and quality in smallholder dairy farms, with the aim of enhancing food security, food safety, and nutrition for those poor smallholder households in northern Rwanda. This section highlights the key findings on milk consumption and the prevalence of zoonotic infections, as well as the risk factors linked to milk production and quality. It also outlines specific household hygiene practices that are associated with the presence of zoonotic pathogens in milk and children's faecal samples and discusses their impact on children's nutritional status. Additionally, it highlights the advantages of a multidisciplinary approach in recognizing the necessity of the collaborative methods employed in this project. Lastly, it identifies research gaps and offers personal reflections on key topics to deepen the scientific insights discussed in the earlier sections.

6.1 Milk consumption patterns and occurrence of zoonotic infections

The relationship between milk consumption and the occurrence of five milk-borne bacterial zoonoses—brucellosis, salmonellosis, campylobacteriosis, *Escherichia coli* infections, and tuberculosis—based on published literature from East Africa between 2010 and 2021 was assessed. Current findings describing the distribution of the most studied milk-borne pathogens revealed that *Brucella* spp. (54.5%) was the most prevalent, followed by *Escherichia coli* (18.2%), *Salmonella* spp. (12.1%), *Mycobacterium* spp. (6.1%), and *Escherichia coli* O157:H7 (6.1%). Despite its irrefutable nutritional benefits for many adults and young children, consuming unpasteurized milk poses significant health consequences. For example, another review reported that raw milk consumption was directly associated with food-borne disease outbreaks involving *Campylobacter spp., Salmonella spp.,* shiga toxin-producing *Escherichia coli, Brucella melitensis, Mycobacterium bovis*, and the tick-borne encephalitis virus (Zastempowska et al. 2016).

Moreover, several factors associated with reported incidences of milk-borne infections have been identified across all the studies included in the review. Consumption of unpasteurized milk, low levels of education, living in close contact with livestock, and unprotected handling of infected aborted material were the most commonly reported risk practices associated with the occurrence of bacterial zoonoses among East African communities. These risks are especially concerning for vulnerable populations, including immunosuppressed individuals, young children, pregnant women, and the elderly (Lund & O'Brien 2011). Cultural practices and inadequate infrastructure in low and middle-income countries, such as a lack of cooling facilities and electricity, contribute to the high consumption of raw milk (Headey et al. 2024). In these settings, household pasteurization (boiling) is the most commonly used method to make milk safe for consumption (Awasthi et al. 2012).

Although boiling milk significantly reduces bacterial contamination, evidence shows that poor storage and handling practices can lead to re-contamination (Tremonte et al. 2014). In resource-poor settings, households face multiple challenges that increase their vulnerability to food-borne infections. Firstly, they often lack the essential infrastructure for producing and preparing safe food, such as access to clean water and electricity (Langiano et al. 2012). A recent study found that children from households with coliform bacteria in their drinking water were 10 times more likely to suffer from nutritional deficiencies than those from households with uncontaminated water sources (Shrestha et al. 2020). In addition to poor hygiene practices, farmers frequently do not comply with food safety standards, such as discarding milk from infected animals and adhering to antibiotic withdrawal periods (Iraguha et al. 2024), which leads to the consumption of contaminated milk and dairy products. Moreover, limited knowledge regarding the zoonotic risks of animal-source foods, often due to low levels of education (Yemane & Tamene 2022), further exacerbates the problem. It is important to note that while heat treatment can eliminate most heat-labile pathogens, their toxins remain unaffected in the milk, which poses a serious risk of food-borne infections in humans, particularly among vulnerable groups such as young children (Zastempowska et al. 2016). Additionally, the health risks associated with consuming contaminated milk extend beyond food-borne diseases, as it can also be a source of antibiotic-resistant bacteria (Serwecinska 2020), which can potentially compromise treatment outcomes in human infections (Huemer et al. 2020).

Whether food is produced in a factory, prepared in a restaurant, or cooked at home, evidence indicates that poor handling practices during food preparation are linked to food-borne outbreaks (Yemane & Tamene 2022). According to the WHO, any strategic interventions to reduce food-borne infections should focus on households (Yemane & Tamene 2022), as outbreaks of these infections within the home often go unreported (Byrd-Bredbenner et al. 2013). However, the effectiveness of these interventions must be carefully evaluated as there are numerous barriers within the food value chain in low and middle-income countries. One significant barrier is the predominance of informal markets. For example, in Rwanda over 70% of milk is traded through informal channels (Kamana et al. 2014). Along with informal marketing, challenges include a limited consumer awareness and ability to pay for safer food, as well as a lack of incentives for investing in food safety along the supply chain (Leahy et al. 2022; Hoffmann et al. 2019). These issues are further exacerbated by the weaknesses of public institutions which are responsible for regulatory enforcement (Hoffmann et al. 2019; Mayett-Moreno & Oglesby 2018).

To address these challenges, new strategies must be implemented. For example, Johnson et al. (2015) suggested that adopting a more holistic theory of change, which includes all key players in the food chain from farm to fork, could lead to measurable outcomes that enhance food safety throughout the animal-source food market chain. In developing countries, where informal channels dominate the food market chain, it is argued that improving the quality of products could lead to an increased consumption of animal-source foods, which in turn could help reduce the prevalence of undernutrition (Johnson et al. 2015). Regardless of the differences in milk and dairy product marketing, it is crucial for the dairy industry to apply standard principles of good hygiene and good manufacturing practices to address the challenges related to the quality and safety of dairy products (Ntuli et al. 2023). At every segment of the food chain, identifying and managing sources of contamination should be an integral part of the production and processing processes (Ntuli et al. 2023).

6.2 Farm management practices associated with high-quality milk yield

From a production perspective, several factors influence milk yield including breed, reproduction technologies, feeding systems, and animal health issues (Kashongwe et al., 2017; Mayberry et al., 2017; Gustavsson et al., 2014).

The results showed that milk yield was positively correlated with cow breed, with improved breeds producing more milk than local breeds, which aligns with findings from existing literature (Duguma 2020; Manzi et al. 2020; Galukande et al. 2013). These crossbreeds have a higher genetic potential for milk production and longer lactation periods compared to local breeds (Abraha et al. 2009). A recent meta-analysis study investigating the relationship between farm management practices and milk yield in East Africa reported similar findings (Bateki et al. 2020). The study found that milk yield was positively associated with improved breeds and better feeding systems, while heat stress and calf suckling reduced daily milk production on most farms (Bateki et al. 2020).

It is likely that farm management practices such as insufficient and unbalanced diets, inadequate disease control, poor housing, improper milking routines, and limited water availability were the primary factors contributing to the recorded low milk yield. This suggests that farm management strategies targeted at these smallholder farmers may not achieve the desired outcomes, as these farmers often lack the necessary resources to implement such strategies (Ritter et al. 2017). Nonetheless, increasing milk yield should remain a priority to help address food insecurity and malnutrition in these resource-poor households. Genetic improvement of local breeds by crossbreeding them with high-yielding cows through artificial insemination (AI) is a widely recommended reproductive technique (Sharma et al. 2024), and is particularly important for smallholder farmers in the tropics (Baruselli et al. 2018). However, the current findings revealed that only 15% of farmers use AI, which contributes to the recorded low milk yield at the farm level.

Although statistical analysis did not find any association between milk quality and the investigated risk factors, it is important to recognize that various factors can affect milk quality at different stages of the value chain. Evidence indicates that factors such as intramammary infections, stage of lactation (early and late), parity, body condition, milking frequency, and stress are commonly linked to an increased somatic cell count (SCC) in milk (Lianou et al. 2021; Paape et al. 2007). The current findings also showed that 13% of the milk samples tested positive for antibiotic residues. An explanation for this could be that animals were undergoing antibiotic treatment, coupled with a poor compliance with withdrawal periods (Rahman et al. 2021). As discussed earlier, consuming microbiologically contaminated milk and dairy products poses significant health risks to consumers. Similarly, chemical contamination—primarily from antibiotic and pesticide residues, milk preservatives, and environmental pollutants—raises serious public health concerns (Prache et al. 2022). To meet the growing demand for animal-source foods, driven by population growth and shifting dietary habits, it is essential to focus on increasing production and improving quality (Fróna et al. 2019; Hernández-Castellano et al. 2019). Primary responsibility lies with producers, who ensure the availability and quality of these foods, while affordability and accessibility depend on the combined efforts of producers, industry, and governments (Yee et al. 2005). Government institutions should establish milk quality standards, while the industry should support producers through capacity building and ensuring compliance with these standards (Vyas et al. 2020).

Increasing individual cow milk productivity is essential to boost total milk production at the farm level. Efforts should focus on selecting appropriate breeds that are adapted to local conditions, adopting technologies that enhance reproductive performance (Vyas et al. 2020), and implementing effective feeding systems and disease control strategies. The use of artificial insemination is imperative for bridging the gap between milk yield and quality, as it enhances the genetic potential of dairy cows and helps reduce the transmission of animal diseases such as brucellosis (Baruselli et al. 2018). Additionally, veterinary extension services play a vital role in improving milk production, therefore, customized extension service programmes should be established to provide farmers with affordable and high-quality support. These programmes should prioritize different farmer categories, particularly smallholders, who make up the majority of farmers in developing countries and often lack access to quality extension services (Aker 2011).

6.3 Household factors influencing child health and nutritional status

In recent years, the stunting rate among children under the age of five in Rwanda's Northern Province has significantly decreased, dropping from 41% in 2019 to 27% in 2023 (Utumatwishima et al. 2024). Despite this remarkable progress, several key factors continue to hinder the eradication of malnutrition, particularly among poor families in the region. The studies outlined in this section aimed to investigate the risk factors contributing to child undernutrition, and to characterize zoonotic pathogens and the risk factors associated with their presence in both milk and children's faecal samples from the same households, through a multidisciplinary approach.

Various socio-economic factors were associated with stunting, including household food insecurity, low education level of parents, limited access to basic infrastructure (such as electricity, water, and healthcare), and the female spouse's decision-making power in household purchases. Additionally, child-related factors such as age, gender, breastfeeding, and health status were also associated with an increased likelihood of stunting. These findings are consistent with several studies from other developing countries. For example, a recent review article examining the relationship between risk factors and the nutritional status of South African children under five reported similar results (Mkhize & Sibanda 2020). Further, the review identified additional contributing factors such as low household income, unemployment, and poor caregiver nutritional knowledge (Mkhize & Sibanda 2020). Likewise, a study involving data from 35 developing countries revealed that rural children are more likely to experience poor nutrition compared to their urban counterparts (Fox & Heaton 2012). These disparities are largely due to the lower socio-economic status of rural households in developing countries, compounded by cultural norms (Fox & Heaton 2012). In the present study, boys were found to experience higher rates of stunting compared to girls, which aligns with findings from previous research in Rwanda (Binagwaho et al. 2020). The increased risk of stunting among male children is linked to their typically higher birth weight, which results in greater energy demands during the early stages of growth and development (Bork & Diallo 2017) and the period when they begin solid foods (Abeway et al. 2018). This biological difference is more pronounced in low and middle-income countries, where many households face food insecurity and inadequate dietary intake (Wamani et al. 2007). A recent study exploring the link between household practices and children's nutritional status in Nepal found that children from households without agricultural production were more likely to be undernourished compared to their peers (Shrestha et al. 2020), emphasizing the critical role of access to animal-source foods to combat malnutrition. Recent studies have explored the relationship between livestock ownership and patterns of animal-source food consumption in Rwanda (Flax et al. 2023; Flax et al. 2021). Findings indicate that in households with lactating cows, nearly 50% of children did not consume milk due to low milk production or because the milk was sold to meet other family needs (Flax et al. 2021). Indeed, an insufficient intake of animal-source protein can partially explain why the stunting rate among male children is higher than that of female children in the present study. However, interventions aimed at increasing milk production could potentially shift this consumption pattern through social and behavioral changes among smallholder dairy farmers (Flax et al. 2023).

Food-insecure households are often characterized by inadequate food intake, driven by factors such as low purchasing power, large household size, or insufficient family (farm) production (Babatunde et al. 2007). These characteristics partly explain why malnutrition rates among children in these families are consistently higher compared to those in food-secure households. In these households, parents are often less educated, leading to limited job opportunities and lower wage rates, which further contributes to food insecurity (Coleman-Jensen et al. 2013). However, in developing countries, and even in families where parents are educated and have better jobs, gender dynamics often significantly influence decision-making. Female spouses generally have less authority over major purchases compared to their husbands, highlighting the persistent disparities in household power and control (Christian et al. 2023). Therefore, intervention programmes designed to alleviate malnutrition should always incorporate strategies for empowering women to achieve their intended outcomes (Christian et al. 2023).

The relationship between milking practices and household hygiene factors and the prevalence of zoonotic pathogens in cow's milk and children's faecal samples from the same households was assessed. The results showed that zoonotic pathogens were more prevalent in faecal samples (84.6%) than in milk samples (35.9%). These findings are consistent with previous studies. For example, research conducted in Mozambique reported that 86% of children's stool samples contained enteric pathogens (Knee et al. 2018). Similarly, a study in Rwanda found that 92% of acute gastroenteritis cases had at least one enteric pathogen present (Kabayiza et al. 2014). Although no association was found between the prevalence of zoonotic pathogens in faecal samples and the risk factors included in the current study, several studies have identified key factors linked to gastroenteritis in young children. These include handwashing after defecation, the presence of coliforms in main water sources, and the separate storage of drinking water and water for other purposes (Njuguna et al. 2016). This research also highlighted several household hygiene malpractices that could contribute to the spread of pathogens, such as not treating drinking water before use, and failing to wash hands before food preparation, after defecation, or after assisting a child with defecation.

Salmonella spp. was the most prevalent bacteria in milk samples, detected in 25.6% of cases. This prevalence is significantly higher than previous reports from Rwanda (Kamana et al. 2014) and the region (Reta et al., 2016; Schoder et al., 2013), where prevalence rates ranged from 3.3% to 10.1%. The higher prevalence in the current study could be attributed to the use of advanced diagnostic techniques, such as PCR, with higher sensitivity and specificity (Van Lint et al. 2016), compared to conventional microbiological methods used in earlier studies (Reta et al., 2016; Schoder et al., 2013). Studies using similar diagnostic techniques support these findings. For instance, a study in Ethiopia found *Salmonella* spp. in 21.3% of milk samples at collection points (Bedassa et al., 2023). Additionally, the higher isolation rate of *Salmonella* spp. in the current study may be due to contamination from environmental sources during milking or milk handling, as suggested by previous research (Ruzante et al. 2010). Furthermore, existing literature indicates that when milk is collected directly from the cow, there could be a high prevalence of *Salmonella* spp., which suggests there is an ongoing infection (Castañeda-Salazar et al. 2021). Over two decades ago, it was reported that milk contamination can arise from three primary sources: infected cows (e.g., mastitis), the surface of the udder, and environmental factors such as milking equipment and the milker’s hands (Murphy & Boor 2000). The authors concluded that the level and type of microbial contamination is partly or even entirely dependent on the cow's health and hygiene, the farm environment, the procedures used for cleaning and sanitizing milking equipment, and the temperature and duration of milk storage. These findings have been consistently confirmed in later studies (Claeys et al. 2013; Gleeson et al. 2013). However, the situation is more concerning in developing countries, wherein smallholder farmers and informal market channels predominate, exacerbating the risks (Grace et al. 2020).

The second most prevalent pathogens in milk samples were rotavirus and hepatitis E virus. Their presence could be attributed to environmental contamination, as bovine rotavirus typically affects young calves rather than adult cows, thus it is unlikely to be shed into milk by lactating cows (Geletu et al. 2021). This is further supported by the fact that most farmers in the study area reported milking within the cowshed, which is a potential source of contamination. Moreover, statistical analysis revealed a strong correlation between hand-washing practices and the prevalence of pathogens in milk, indicating that poor hygiene among milk handlers contributes to the presence of these pathogens (El-Senousy et al., 2020; Cho and Yoon, 2014).

In faecal samples, adenovirus was the most prevalent virus, detected in 37.8% of samples. This prevalence aligns with a previous study in Rwanda, which found adenovirus in 39.7% of stool samples from children with acute gastroenteritis (Kabayiza et al. 2014). In contrast, rotavirus was only detected in 7.7% of samples, much lower than the 36.9% reported by Kabayiza et al. (2014). This discrepancy is possibly because the previous study analyzed clinical samples, while in the current study, only 24.4% of children had diarrhea within two weeks of data collection. Regarding bacterial pathogens, the most common in faecal samples was *E. coli*, with two major strains: enteropathogenic *E. coli* (EPEC) found in 38.5% and enterotoxigenic *E. coli* (ETEC) in 31.4% of samples. These findings are lower than a previous study in East Africa, which reported that EPEC-*eae* genes were found in 58% and ETEC-*eltB* genes in 60% of stool samples (Andersson et al. 2018). Differences in population, sample selection, and health status of children could explain these variations in pathogen prevalence.

This study also aimed to determine if a connection existed between pathogens found in milk and those present in faecal samples, but no direct link was identified. This suggests that pathogens present in raw milk may have been eliminated through milk boiling, a common practice of home pasteurization in the study area. These findings are consistent with a previous study conducted in the same area, which found that boiling milk at the household level reduced the risk of infection by 4.9% for children and 4.6%

for adults per year (Kamana et al. 2016). These results highlight the importance of milk boiling as an effective method to ensure food safety and prevent the transmission of zoonotic diseases through milk consumption. However, this hypothesis should be interpreted with caution. A recent review on the health and economic implications of milk-borne pathogens in developing countries recognized that the detection of pathogens in milk indicates a potential risk to consumers (Grace et al. 2020). Nevertheless, the review also suggested that other factors, such as co-infection and host factors, could play a role in mitigating these health risks. The absence of identical zoonotic pathogens in both milk and children's faecal samples from the same household could also suggest that the children had not consumed milk prior to data collection. This aligns with findings from another study in Rwanda, involving a larger sample size (n=458), where over 50% of mothers reported that their children had not consumed milk in the week before the interviews (Flax et al. 2023). Regardless of the reasons for the lack of a direct link between pathogens in these two sample types, ensuring the safety of animal-source foods should be a priority. This is vital to ensure that their consumption provides nutritional benefits without risk, particularly for young children, as this could result in severe consequences, including death, from zoonotic infections (Currier & Widness 2018).

6.4 The advantages of applying a multidisciplinary approach in the human undernutrition programme

The findings from the study on sampling and analysing spatial determinants of undernutrition highlights the complexity and multifactorial nature of child undernutrition. Twenty-six key predictor variables of child stunting in Rwanda were identified (Paper IV). Although several studies have attempted to understand and identify the factors associated with malnutrition in young children in Rwanda (Binagwaho et al. 2020; Uwiringiyimana et al. 2019), none have adopted a multidisciplinary approach. This fragmentation limits the effective implementation of recommendations due to a lack of integrated evidence and coordination across sectors (Morris et al. 2008).

Arguably, one of the most effective ways to understand and address malnutrition is through a multidisciplinary approach that applies efficient sampling methods to accurately estimate malnutrition rates (Morris et al. 2008). The current findings demonstrate that by integrating various statistical and spatial analytical tools, variability in child stunting rates was accounted for, and each analysis revealed its contribution to these variations. Such studies are likely to produce evidence-based, targeted interventions that align with the dynamic changes in nutritional status, agricultural technologies, and climate change (Morris et al. 2008). A recent study on the impact of an integrated intervention package on malnutrition among pregnant women in Rwanda acknowledges the lack of evidence on the effectiveness of these interventions in reducing maternal undernutrition (Habtu et al. 2022). Nevertheless, studies from other countries have recommended a multidisciplinary approach to investigate malnutrition and develop comprehensive and inclusive intervention programmes to address malnutrition among vulnerable populations (Bischoff et al. 2017; Beck et al. 2016). Moreover, a deeper understanding of malnutrition's disaggregated patterns is necessary to effectively guide policymakers (Pomati & Nandy 2020).

Collaboration among different research teams working on the same issue in the same area could significantly reduce costs and improve data sharing and management. While the benefits of multidisciplinary research may not be immediately apparent, the long-term benefits of collaboration and enhanced data sharing justify the investment (Brown et al. 2023). Furthermore, strengthening human and institutional resources beyond the national level would enhance the effectiveness of multidisciplinary research, with international universities offering quality education to researchers from developing countries who are involved in malnutrition programmes (Morris et al. 2008).

6.5 Personal reflections on milk production and quality in Rwanda

This section reflects my personal perspective of milk production and quality in Rwanda, incorporating key insights from studies I contributed to during my PhD journey (Iraguha et al. 2024; Garcia et al. 2023) that are not included in this thesis.

As previously highlighted, dairy cattle farming is deeply embedded within Rwandan culture due to its social, economic, and nutritional significance. These values have laid the groundwork for pro-dairy government policies aimed at reducing poverty and food insecurity while enhancing the livelihoods and nutrition of rural farmers. Since 2006, Rwanda's dairy sector has made substantial progress, driven by government initiatives aimed at boosting milk production and addressing poverty and malnutrition. Despite these advancements, dairy farmers continue to face significant challenges, including low productivity per cow, poor milk quality, and price volatility, among others.

In Garcia's study, we introduced the Dairy Dynamic Management (DDM) programme in the Northern and Eastern provinces of Rwanda, a novel approach that integrates education, research, and extension services to enhance milk production and quality through a One Health perspective. As part of the DDM programme, extension service providers and university scientists received training on milk quality and safety, enabling them to assist veterinary students and dairy farmers in implementing DDM principles. These principles include the clinical diagnosis of mastitis and surveillance of food-borne pathogens in milk. Over a 16-week period, DDM specialists trained and assessed 30 dairy farmers on milk production and quality, with a focus on screening and preventing mastitis. Each week, 100 cows were screened for clinical and subclinical mastitis, and milk samples were collected for microbiological analysis. The results indicated a significant reduction ($p \leq 0.001$) in total bacterial count (TBC) and the prevalence of bacterial species such as *Staphylococcus* spp., *Staphylococcus aureus*, non-aureus staphylococci (NAS), and coliform counts at cow level. Furthermore, smallholder farmers showed substantial improvement in following hygienic milking protocols, with over 90% successfully performing practices such as udder cleaning, pre-dipping, removal of pre-dip, hygienic milking, and post-dipping. These findings demonstrate that well-implemented and community-based interventions can greatly enhance milk production and quality among smallholder farmers, who often lack the resources to afford quality extension services. Achieving this goal requires the development of tailored educational and training programmes that focus on the specific needs and challenges faced by smallholder farmers. These initiatives can enhance food production, improve animal health, and ultimately promote economic well-being.

It is widely recognized that cows with higher milk production are more prone to intramammary infections (Koivula et al. 2005). Even when a cow is not reaching its full production potential, possibly due to inadequate feeding, a genetically high-producing cow is still likely to develop intramammary infections. In such cases, the use of antimicrobials, particularly antibiotics, is often the preferred choice for many farmers and veterinarians worldwide. In certain situations, particularly in developing countries where farmers have limited financial resources and a lack of knowledge about the potential consequences, the misuse of antibiotics becomes widespread (Hosain et al. 2021). This misuse often stems from an absence of proper veterinary guidance, leading to self-medication of livestock without considering appropriate dosages or withdrawal periods (Dione et al. 2021). This can result in the development of antibiotic-resistant bacteria, threatening both animal and human health.

In Iraguha's work, we conducted a longitudinal study to evaluate practices and factors related to antibiotic use, investigate the presence of antibiotic residues in cow's milk, and implement a comprehensive training programme aimed at improving the quality of milk production. We enrolled a total of 42 dairy farmers from the same geographical area, the Nyabihu district in the Western Province of Rwanda, all of whom supply milk to the same milk collection centre. Structured questionnaires were used and interviews with farmers were conducted, along with the analysis of milk samples to detect the presence of antibiotic residues. The results revealed several concerning malpractices, including administering antibiotics without laboratory results (100%), failure to comply with withdrawal periods (88.1%), and administering antibiotics without a veterinary prescription (85.7%). Factors contributing to these practices included farmers' limited knowledge about antibiotics, easy access to antibiotics in local veterinary shops, and inadequate veterinary services. Additionally, out of 451 bulk tank milk samples tested, 27 samples (6%) contained antibiotic residues, predominantly tetracyclines (55.3%) and beta-lactams (44.7%). Adopting better farm practices, such as regular animal health monitoring, implementing biosecurity measures to prevent disease, and consulting veterinarians, could significantly reduce the need for antibiotics. Collaborative efforts among stakeholders are highly recommended to improve dairy farmers' capacity and support research initiatives. Additionally, strengthening regulations on the responsible use of antibiotics within Rwanda's food production system is strongly advised to help curb antimicrobial resistance in both animal and human populations.

Both milk production and quality can be enhanced through the implementation of systematic and targeted intervention programmes that address all players in the dairy value chain using a One Health approach. This method is essential to ensure that safe, nutritious, and wholesome food is available to meet local nutritional needs while also being produced sustainably to meet multiple UN Sustainable Development Goals (SDGs). Further research is needed to identify and overcome barriers to the effective implementation of existing programmes aimed at boosting milk production and food safety.

7. Conclusions

The overall aim of this thesis was to investigate management and household practices related to milk production and quality in smallholder dairy farms, with the ultimate goal of enhancing food security, food safety, nutrition, and the livelihoods of poor smallholder households in the Northern Province of Rwanda. The current results demonstrate that:

- A systematic review on the relationship between milk consumption and selected zoonoses in East Africa concludes that brucellosis is the most studied zoonotic disease, as more than half of the reviewed studies focused on *Brucella* spp. Various factors were linked to the risk of zoonotic infections in humans, with the most significant being the consumption of unpasteurized milk and dairy products.
- Management factors contribute to milk yield, composition and quality in smallholder dairy farms. Low milk productivity per cow was related to inadequate feeding practices. Additionally, factors such as breed types, milking frequency, body condition scores, and parity show the potential for increasing milk yield.
- The presence of antibiotic residues and high levels of somatic cells in milk suggests animal health issues that are known to lower milk yield and impair milk quality.
- The detection of zoonotic pathogens in raw milk highlights a potential health risk to consumers, with the high prevalence of *Salmonella* spp. indicating poor milking and milk-handling practices.
- The stunting rate among young children has decreased, from 41% (under five years) in 2020 to 27% (under three years) in the current study, but multiple factors are still contributing to the stunting rate.

- The multidisciplinary approach used to investigate malnutrition offers evidence-based recommendations that address the complexities of the issue. This also indicates that adopting a multidisciplinary approach to design comprehensive and integrated intervention programmes could lead to increased milk production and quality, thereby enhancing the nutrition and livelihoods of smallholder farmers in Rwanda and other low-income regions worldwide.

8. Practical recommendations

Based on the conclusions outlined above, the following recommendations are proposed to address the key issues identified:

- A tailored training program for all participants in the milk value chain should be implemented to improve the safety of milk that is sold through informal channels, with an emphasis on adopting a One Health approach.
- Smallholder farmers should be educated on the best practices in farming and milking to enhance both milk production and quality.
- Strengthening household hygiene practices and farm biosecurity measures is crucial for ensuring safe food production, proper handling, and the prevention of zoonotic diseases.
- Comprehensive and holistic interventions should be developed to address the current high rates of malnutrition.

9. Future perspectives

The current findings have highlighted several key areas for future research that can help increase milk production, improve milk quality, and reduce malnutrition among young children. Below are some examples of actions that should be considered for future intervention programmes or research projects:

1. **Investigating factors associated with knowledge, attitude, and practicesthat influence reproductive performance and milk productivity, including the low adoption of artificial insemination (AI).** Despite farmers' awareness of the benefits of AI in enhancing genetic progress, livestock production, and animal health, the majority still rely on natural mating, as observed in Paper II. The underlying factors contributing to this low adoption rate need to be better understood. Future research could explore a cost-benefit analysis of AI technology and other barriers to its use. Identifying these factors will help in designing targeted interventions, such as education, subsidies, or improved service delivery to increase AI uptake and improve herd genetics, productivity, and farmer livelihoods.
2. **Investigating additional household water, sanitation and hygiene (WASH) practices.** Further research is needed to identify the sources of zoonotic pathogens at the household level by examining additional household WASH practices.
3. **Culturing samples and sequencing pathogens.** Since milk and faecal samples were not cultured in **Paper** III, it is possible that PCR detected dead cells. Culturing samples could increase the likelihood of recovering live bacteria, which might raise the prevalence rates of certain pathogens. Furthermore, sequencing the PCR products could help identify the **true**

source of pathogens in milk samples, whether from environmental contamination or infected cows. Future research should focus on culturing and sequencing technologies to gain a better understanding of zoonotic pathogens in both human and milk samples.

4. **Investigating the source of *Salmonella* spp. in milk samples.** In Paper III, a high prevalence of *Salmonella* spp. in milk samples was reported. Since the health status of cows at the time of milk collection was not assessed; therefore, it is not possible to confirm the source of these pathogens. Future research should include individual animal health data to estimate the contribution of environmental sources and inform the design of preventive strategies based on research findings.
5. **Adopting an interdisciplinary approach to combat malnutrition.** Findings from Paper IV identified multiple factors contributing to stunting rate among young children. Future research and intervention programmes targeting malnutrition among young children in Rwanda should take an interdisciplinary approach to address the root causes within each community. It is believed that this approach will help in designing targeted interventions that address both the root and underlying causes of malnutrition, rather than merely focusing on its immediate causes.

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Popular science summary

Recent advancements in milk production have significantly contributed to alleviating food insecurity, improving nutrition, and enhancing the livelihoods of millions of households worldwide. Key factors in this increase include the improved genetic potential of local dairy cattle, driven by biotechnologies such as artificial insemination and embryo transfer. Despite these advancements, many developing countries face persistent challenges that hinder optimal milk yields. These challenges include feed shortages, inadequate infrastructure, climate change, insufficient veterinary services, limited technology, and the prevalence of animal diseases. Additionally, factors intrinsic to the animals—such as breed, body condition, age, and lactation stage—also play a crucial role in milk production. Implementing biosecurity measures can mitigate disease risks on farms, thereby improving cow productivity.

In Rwanda, milk production is particularly vital for rural communities, as it provides smallholder farmers with income through the sale of milk, manure, and livestock. Household consumption of milk has a profound impact on nutrition, particularly for vulnerable groups such as young children, pregnant women, and the elderly. Research has shown that milk and dairy products are essential for the growth and development of children, contributing to reduced stunting and underweight prevalence and lowering child morbidity and mortality rates. However, the health risks associated with contaminated milk cannot be overlooked. Contamination can result from pathogens or harmful chemicals, including antibiotic residues and aflatoxins, which stem from sick animals, environmental sources, or poor practices during milking, storage, or preparation. Food-borne diseases linked to contaminated food are responsible for 420,000 deaths annually, with children under five accounting for 40% of these fatalities, mainly in developing countries.

To understand the key issues related to milk quality and safety in the region, a systematic literature review was conducted. This study focused on identifying risk factors associated with zoonotic diseases across seven East African countries. The systematic review highlighted brucellosis as the most extensively studied zoonotic disease, primarily associated with the consumption of unpasteurized milk.

A multidisciplinary team comprised of 19 researchers, including PhD students and data collectors, implemented field research involving 601 households with children aged between 1 and 36 months. Out of these, 156 were smallholder farmers with lactating cow. This study evaluated household management practices concerning milk yield, quality, and safety, and estimated stunting rates in young children, while also analyzing cow's milk and children's faecal samples for zoonotic pathogens. Findings indicated that milk production in the study area is significantly below the genetic potential of the predominantly crossbred cattle. The primary cause of this low productivity is inadequate feed quality. Importantly, factors such as improved breeds, regular milking, good body condition, and higher parity contribute to increased daily milk yields per cow. However, milk quality remains a challenge; laboratory results showed that almost 13% of milk samples contained antibiotic residues, while 34% had somatic cell counts indicative of mastitis, a disease known to reduce milk yield and impair milk quality. Body measurements of the children revealed that approximately 27% were stunted, with boys experiencing higher stunting rates than girls. The study identified multiple factors associated with stunting.

In conclusion, the low milk yield indicates a critical need for farmer education on feeding, herd health management, and proper milking techniques. Improving these practices can enhance both milk production and quality, benefiting the local dairy industry and public health. Furthermore, addressing food insecurity should include water, sanitation, and hygiene (WASH) practices to ensure safe food production and handling. Given the unique agroecological conditions across provinces, the multidisciplinary approach employed in this project can be adapted and scaled across the country as well as in similar low-income settings in other parts of the world.

Populärvetenskaplig sammanfattning

Utvecklingen inom mjölkproduktionen har bidragit till att förbättra livsmedelsförsörjningen, näringstillgången, och levnadsförhållandena för miljoner hushåll världen över. Nyckelfaktorer för dessa framsteg har varit den förbättrade genetiska potentialen hos korna, vilken har fått stor spridning med hjälp av reproduktionstekniker som artificiell insemination och embryo transfer. Trots dessa framsteg kvarstår utmaningar i många låginkomstländer, vilka hindrar att optimala mjölkmängder nås per ko. Dessa utmaningar utgörs av brist på foder och vatten, otillräcklig infrastruktur, klimatförändringar, brist på veterinära tjänster och annan djurhälsopersonal, begränsad teknologi, samt förekomst av djursjukdomar. Utöver detta spelar även djurens egenskaper som ras, hull, ålder och laktationsstadium en viktig roll för mjölkproduktionen. Dessutom är goda rutiner för hygien och smittkontroll viktiga för att minska risken för sjukdomar på gårdarna och därmed öka kornas produktivitet.

I Rwanda är mjölkproduktionen extra viktig för samhällen på landsbygden genom att den ger inkomst från försäljning av mjölk, gödsel och boskap. Hushållens konsumtion av mjölk har en viktig roll för nutritionen, speciellt för sårbara grupper som små barn, gravida kvinnor och äldre. Forskning har visat att mjölk och mjölkprodukter är livsviktiga för små barns tillväxt och utveckling, och att de bidrar till minskning av hämmad tillväxt, undervikt, sjuklighet och dödlighet hos barn. Däremot bör hälsorisker som hör samman med kontaminerad mjölk inte underskattas. Sjukdomsalstrande bakterier, antibiotikarester och aflatoxiner kan finnas i mjölk. De kan härröra från sjuka djur, omgivningen eller dåliga rutiner vid mjölkning, förvaring och tillredning av mjölk. Livsmedelsburna sjukdomar kopplade till kontaminerad mjölk orsakar, framförallt i låginkomstländer, årligen 420 000 dödsfall, varav 40 procent hos barn under fem år.

För att förstå risker inom livsmedelshygien och livsmedelssäkerhet kopplat till konsumtion av mjölk gjordes en systematisk litteraturöversikt av vetenskapliga artiklar från sju östafrikanska länder. Studien fokuserade på att identifiera riskfaktorer som kan associeras till zoonotiska sjukdomar, det vill säga sjukdomar som kan överföras mellan djur och människa och vice versa. Litteraturöversikten visade att brucellos var den mest studerade zoonotiska sjukdomen, primärt kopplad till konsumtion av opastöriserad mjölk.

En flerdisciplinär forskargrupp med 19 forskare, inklusive doktorander och datainsamlare, gjorde en fältstudie i norra Rwanda hos 601 fattiga hushåll med barn i åldrarna 1-36 månader, och 156 av dessa hushåll var också småbönder med mjölkande kor. Studien utvärderade hushållens uppgifter om kornas mjölkproduktion och rutiner som påverkar mjölkkvalitet och livsmedelssäkerhet, samt bedömde tillväxten hos de små barnen. I de hushåll som hade mjölkkor gjordes dessutom gjordes parallella analyser av komjölk och avföringsprover från barn avseende bakterier som kan ge zoonotiska sjukdomar.

Resultaten visade att mjölkproduktionen i norra Rwanda är betydligt lägre än vad man kan förvänta sig hos kor av den genetiska potential som de vanligaste, lokala korsningsraserna har. Den primära orsaken till denna låga produktion är otillräcklig foderkvalitet. Viktigt att poängtera är att faktorer som förbättrade raser, regelbunden mjölkning, gott hull och äldre djur bidrar till ökad mjölkmängd per ko. Emellertid fortsätter mjölkkvaliteten att vara en utmaning; resultaten från laboratorieanalyserna visade att nästan 13 procent av mjölkproverna innehöll antibiotikarester, och att drygt 34 procent hade celltal som indikerade att kon hade juverinflammation, en sjukdom känd för att sänka mjölkmängden och försämra mjölkkvaliteten. Mätningar av barnens kroppsmått visade att 27 procent av barnen hade hämmad tillväxt, och att pojkarna var mer hämmade i tillväxten än flickorna. Studien identifierade flera faktorer som kunde kopplas till hämmad tillväxt, bland annat att familjen inte ägde boskap och att hushållen hade låg inkomst.

Avhandlingens slutsats är att den låga mjölkproduktionen hos småbönder i norra Rwanda visar på ett stort behov av utbildning av lantbrukare inom utfodring, besättningshälsa och mjölkningsrutiner. Genom att förbättra dessa rutiner kan både mjölkproduktionen och mjölkkvaliteten ökas, vilket är positivt både för den lokala mjölkbranschen samt för folkhälsan. För att hantera osäker livsmedelsförsörjning bör dessutom vatten-, hygien- och sanitetsrutiner tas i beaktande för att garantera säker livsmedelsproduktion och livsmedelshantering. Det flerdisciplinära arbetssätt som använts i detta projekt kan anpassas efter de lokala förutsättningar som råder för lantbruk i andra provinser i Rwanda, och skalas upp och användas i hela landet liksom i andra delar av världen med liknande förutsättningar.

Incamake (Popular science in *Kinyarwanda*)

Iterambere ry'ubworozi bw'inka zitanga umukamo ryashoboye kugabanya ikigero cy'ubukene, ryatumye imirire iba myiza kurushaho, ndetse ryazamuye iterambere ry'imiryango myinshi cyane mu gihugu cyacu no ku isi muri rusange. Ibi byagezweho kubera ko ikoranabuhanga mu bworozi ryateye imbere, cyane cyane mu kuvugurura icyororo cy'inka zitanga umukamo mwishi hakoreshejwe gutera intanga ndetse n'insoro mu nka za gakondo hagamijwe kongera umukamo uzikomokaho.

Ariko n'ubwo ibyo byabashije kugerwaho, mu bihugu byinshi bikiri mu nzira y'amajyambere, harimo n'u Rwanda, haracyagaragara umusaruro w'amata ukiri hasi. Ibyo bigaterwa no kubura ubwatsi buhagije, n'ububonetse bukaba budafite intungamubiri zituma amata yiyongera. Hari kandi n'ubumenyi buke bw'aborozi mu bijyane n'ikoranabuhanga mu bworozi, kutabona serivisi n'amahugurwa bihagije, imihindagurikire y'ikirere n'ibindi. Gusa ntitwakwirengagiza n'impamvu zishingiye ku nka ubwazo. Nko kuba hari ubwoko bw'inka zidatanga umukamo uhagije, indwara z'amatungo (twavuga nk'ifumbi y'amabere), n'izindi nyinshi. Gusa ubushakashatsi bwagaragaje ko kubahiriza ingamba zikumira indwara mu bworozi nabyo bigira uruhare mu kongera umusaruro ukomoka ku mata kuko birinda amatungo kurwara.

Mu Rwanda, ubworozi bw'inka bufite akamaro kanini cyane, kandi buri mu muco w'Abanyarwanda. Bufasha cyane cyane abatuye mu byaro kubona ifumbire, amata yo kunywa, ndetse bakabasha no gukirigita ifaranga biturutse mu kugurisha amata, ifumbire, inyana cyangwa ibimasa byazikomotseho. Ikindi kandi, kunywa amata bifasha imiryango kurwanya imirire mibi cyane cyane ku bana bato, agabore batwite n'abonsa ndetse n'abageze mu zabukuru. Ubushakashatsi bwagaragajeko amata n'ibiyakomokaho bifite intungamubiri z'ingenzi ku mikurire y'abana kandi ko zibarinda kugwingira no kurwaragurika. Ariko kandi, ntitugomba kwirengagiza ingaruka zo kunywa amata yanduye cyangwa ibiyakomokaho mugihe byakozwe mu mata atujuje ubuziranenge. Amata ashobora kwanduzwa no gukama inka irwaye, imyanda y'aho inka zororerwa, umukamyi cyangwa ibikoresho bidafite isuku ihagije bikoreshwa bakama cyangwa bayatereka. Ubushakashatsi bwagaragaje ko buri mwaka, abantu basaga miliyoni 600 barwara bazize ibiryo byanduye, harimo n'amata, abagera ku bihumbi magana ane na makumyabiri (420,000) muri bo bakicwa n'izo ndwara. Ikibabaje kandi kurushaho, ni uko muribo hafi ijanisha rya 40 (40%) baba ari abana bafite mu nsi y'imyaka itanu y'amavuko, kandi bakaba biganje mu bihugu bikiri mu nzira y'amajyambere.

Kugirango dusobanukirwe n'uburemere bw'ikibazo cy'ubuziranenge bw'amata mu karere duherereyemo, twasomye ibyavuye mu bushakashatsi bwakozwe mu bihugu birindwi bigize Afurika y'Iburasirazuba. Ibyavuyemo byagaragaje ko indwara y'Amakore ariyo abo bashakashatsi benshi bakozeho igaragara mu bantu, kandi ko kunywa amata adatetse ari impamvu nyamukuru yatumye abantu bandura izo ndwara.

Mu bushakashatsi bwanjye buri muri iki gitabo, mfatanije n'abandi bashakashatsi bo mu Rwanda, twagerageje kureba impamvu zitandukanye zituma umukamo n'ubwiza bw'amata mu Ntara y'Amajyaruguru bikiri hasi. Twashakiye hamwe kandi impamvu zituma umubare w'abana bagwingiye ukomeza kuba hejuru muri iyi Ntara. Nanone kandi twafashe amata y'inka ndetse n'umusarani w'abana batararenza imyaka itatu, kugirango turebe udukoko turi muri ayo mata ndetse no mu musarani w'abana. Twari tugamije kureba ubuziranenge bw'amata, ariko tunareba ingaruka zigera k'umwana unyoye amata arimo udukoko. Twafashe kandi ibipimo by'imikurire ku bana 601 kugirango tumenye ikigero cy'ubugwingire bariho. Ibyavuye mu bushakashatsi bwacu byagaragajeko mu bana twapimye, abagera ku ijanisha rya 27, nukuvuga abana 163 bose bagwingiye. Twabonye kandi ko umukamo muke watewe n'uko aborozi batagaburiye neza inka zabo. Twasanze mu mata harimo udukoko twinshi dushobora kuba twaratewe n'umwanda w'abakamyi, ibikoresho bakamiyemo, ndetse n'inka zimwe na zimwe zari zirwaye ifumbi. Ikindi ni uko mu mata twakuye mu ngo 156, hafi ijanisha rya 13 (ingo 19) harimo ibisigazwa by'imiti y'amatungo.

Dushingiye ku byavuye muri ubu bushakashatsi, biragaragako aborozi bo mu Ntara y'Amajyaruguru bakeneye amahugurwa ahoraho ku bijyanye no kugaburira neza inka, kubugangabunga ubuzima bwazo, kugira isuku mu gukama, gutereka amata, ndetse no kuyategura. Ibi biramutse byubahirijwe, twizeyeko umusaruro ukomoka ku nka zitanga umukamo n'ubuziranenge bw'amata byakwiyongera. Kandi bigafasha mu kurwanya indwara zikomoka ku mwanda, bikagabanya ikigero cy'igwingira ry'abana, ndetse bikanazamura ubukungu bw'aborozi muri rusange. Turemeza kandi ko ibi byagerwaho habayeho ubufatanye bw'inzego zitanyuranye harimo abafatanyabikorwa, abashakashatsi, abanyabuzima, n'abandi bose bafite aho bahurira n'abaturage umunsi ku munsi. Turifuza kandi ko ubu bushakashatsi bwakorwa no mu zindi Ntara z'u Rwanda kuko bwagaragaje ibisubizo ku bibazo byinshi Abanyarwanda bahuriyeho, birimo ko igwingira ry'abana rikiri ku gipimo cyo hejuru, indwara z'amatungo, umukamo udahagije, n'ibindi.

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I

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Review Article

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A systematic literature review of milk consumption and associated bacterial zoonoses in East Africa

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Abstract

Consumption of unsafe animal-source foods is the major cause of foodborne disease outbreaks in low-income countries. Despite current knowledge of the threat posed by raw milk consumption to human health, people in many countries in East Africa still consume unboiled milk. This literature review explored the association between milk consumption and the occurrence of five milk-borne bacterial zoonoses: brucellosis, salmonellosis, campylobacteriosis, *Escherichia coli* infections, and tuberculosis. A search for literature published up to 1 October 2021 was conducted through the Web of Science, PubMed, and Scopus databases, using Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines. The selection process yielded 65 articles describing studies conducted in East Africa 2010–2021, which were carefully scrutinized. The most investigated pathogen was *Brucella* spp. (54.5%), followed by *E. coli* (18.2%), *Salmonella* spp. (12.1%), *Mycobacterium* spp. (6.1%), and *E. coli* O157: H7 (6.1%). The most common predisposing factors for potential milk-borne disease outbreaks were consumption of contaminated raw milk, inadequate cold storage along the milk value chain, poor milk handling practices, and lack of awareness of the health risks of consuming unpasteurized milk. Thus, a tailor-made training program is needed for all milk value chain actors to enhance the safety of milk sold in informal markets, and a One Health approach should be applied. Future studies should employ more advanced diagnostic techniques and countries in East Africa should invest in modern diagnostic tools and equipment, both in hospitals and in local rural settings where most cases occur.

Keywords: dairy, East Africa, raw milk, foodborne disease, risk factors, food safety

Introduction

Zoonoses are infectious diseases caused by pathogens that spread between humans and animals, with transmission through either direct or indirect contact. The main routes by which humans are infected include animal handling and husbandry, close habitation with livestock or animals, and consumption of contaminated animal products. Consuming undercooked animal-source foods such as meat, milk, and dairy products are a major risk factor for foodborne diseases. Additionally, consuming unboiled milk increases the risk of spread of multidrug-resistant pathogens to humans (Caudell et al. 2018), thus posing a public health concern. Unboiled milk is preferred by certain pastoral communities in East Africa and, this preference is positively linked to increased human infections (Nato et al. 2019, Makala et al. 2020). Raw milk consumption is reported to be the leading factor in transmission of foodborne pathogens through milk and milk products. For example, of 16 reported cases of human campylobacteriosis screened in the USA, 15 were found to have consumed raw milk (Oliver et al. 2009). This high incidence was local, however, as it occurred in one of the few federal states that permit sale of raw milk (Oliver et al. 2009). Otherwise, milk-borne diseases are rarely observed in high-income countries, due to hygienic milk production, pasteurization, low consumption of unprocessed milk, and hygienic food preparation (Nyokabi et al. 2021b).

The past decade has seen rapid development of dairy farming in East African countries. The top six milk-producing countries in Africa now include two from East Africa, namely Kenya, and Ethiopia, with annual milk production of 5 528 900 and 3 644 000 metric tonnes, respectively (FAO 2021). However, this increase in milk production has not been accompanied by changes in cultural behaviors and traditions relating to milk consumption (Prakashbabu et al. 2020), which remain strongly anchored in African communities. Milk is regarded as having important social, cultural, and economic value in many African countries, and its consumption is often governed by traditions and cultures (Ndambi et al. 2008). Some communities are still engaging in risky practices in terms of zoonotic diseases, such as drinking unboiled milk, assisting animal births with bare hands, and living near livestock (Amenu et al. 2019a). Milk processing rates are very low in East Africa, e.g. in Kenya only 15% of milk is processed and the remaining 85% is consumed raw, of which 40% is consumed by farmers and their families (Dolecheck and Bewley 2018).

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Scientific evidence has shown that pasteurization of raw milk kills pathogenic bacteria but does not affect its nutritional values (Macdonald et al. 2011). Pasteurization involves heating food to a specific temperature for a set period of time to inactivate non-spore-forming pathogenic bacteria, to destroy most heat-sensitive bacteria, and extend the shelf life of foods (LeJeune and Rajala-Schultz 2009). The most common method of pasteurization in developing countries is cooking or boiling in the household, which lacks standardization (Murchie 2016). It is thus difficult to effectively eliminate pathogenic bacteria from pasteurized foods, while in developed countries like the USA, high-temperature short-time pasteurization is the most commonly used method (Ahmed and Ramaswamy 2007).

Some factors associated with zoonosis outbreaks have been identified. For example, in Tanzania, assisting an aborting animal, proximity of cattle to residential neighborhoods, and religious beliefs have been found to be associated with brucellosis infection in humans (John et al. 2010). These contributing factors vary from country to country, but most are very common in East Africa. Moreover, several studies have demonstrated that knowledge of some zoonotic diseases is low among communities in low-income countries (Morse et al. 2012, Worsley-Tonks et al. 2022). Additionally, some zoonotic diseases are neglected or confused with febrile illnesses, most of which are confused with endemic malaria, hindering effective diagnosis and treatment, and also prevention (Chipwaza et al. 2014, Carugati et al. 2019). Q fever; an infectious disease caused by *Coxiella burnetii*, remains a neglected zoonosis in many developing countries and this has implications for its management and resurgence (Njeru et al. 2016). Although evidence suggests that Q fever prevalence is relatively low in some African countries, its endemicity requires good human awareness for appropriate control measures (Wardrop et al. 2016). Therefore, it is very important to raise awareness among communities and health service providers about the burden and management of zoonotic diseases (Zhang et al. 2016).

Studies conducted specifically in East Africa have shown a trend for brucellosis, tuberculosis, and Q fever outbreaks in pastoralist communities (Cavalerie et al. 2021). In knowledge, attitude, and practices (KAP) studies on dairy farmers regarding zoonotic diseases, a correlation has been found between farmers' behavior and an increase in zoonotic disease incidence (Amenu et al. 2019b, Prakash-babu et al. 2020, Nyokabi et al. 2021b). Several recommendations for control programs for major zoonotic diseases in East Africa have been formulated (Cavalerie et al. 2021), including enhancing national research agendas and promoting the One Health approach. Prevention of bacterial zoonoses is a key component of future agricultural policies that could be implemented to reduce the health and economic burden of these diseases on the local community members in East Africa. Therefore, this review focused on the relationship between milk consumption and occurrence of bacterial zoonoses in East Africa and particularly on risk factors associated with the occurrence of important milk-borne bacterial zoonoses [brucellosis, salmonellosis, campylobacteriosis, *Escherichia coli* infections, tuberculosis, and some neglected zoonoses (Q fever)] in humans consuming unsafe raw cow milk and other predisposing factors. The analysis addressed the following research question: "What are the most prevalent zoonoses, and what is the relationship between milk consumption and occurrence of these bacterial zoonoses in East African countries?"

Materials and methods

Study design

A systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Fig. 1).

Search strategy

An initial review of the literature was conducted through a search in databases such as Web of Science, PubMed, and Scopus for all relevant and recent articles published from 2010 to 2021 on milk quality and bacterial zoonoses (brucellosis, campylobacteriosis, *E. coli*, Q fever, salmonellosis, and tuberculosis) in seven East African countries (Burundi, Ethiopia, Kenya, Rwanda, South Sudan, Tanzania, and Uganda). Since the search strategies involved complex combinations, milk-related terms (e.g. cow milk, milk quality, and milk contamination), disease-specific names, and finally country, search strings were systematically constructed. Disease-specific search strings were formed using a combination of scientific disease name (e.g. tuberculosis), and agent name (e.g. *Mycobacterium*), combined using the Boolean operator, 'OR'. Additionally, the Boolean operator 'AND' was used to combine milk-related terms to zoonotic diseases and specific countries. The following search terms, combined with Boolean operators, were used to conduct the literature review: cow milk, milk product, milk consumption, milk contamination, milk quality, bacterial zoonosis, zoonos∗, tuberculosis, *Mycobacterium*, *Brucell*∗, *Salmonell*∗, *Campylobact*∗, *E. coli*, *E. coli*, and Country. When a relevant publication was identified, the reference list was examined to identify additional relevant publications. Search hits were exported to Endnote, combined into one library, and scanned for duplicates using methods described previously (Bramer et al. 2016). A manual check was performed to confirm the relevance of the final set of papers.

Selection of studies and data extraction

For this review, only papers published in English were considered. Apart from that, cross-sectional, longitudinal, and cohort studies were considered eligible. Articles involving milk consumption and prevalence of zoonotic infections in humans were considered eligible. Additionally, KAP studies were included to obtain relevant information on risk factors associated with zoonotic disease outbreaks in the study area, but meta-analysis papers were not included (Fig. 1). Papers with titles and/or abstracts that were not pertinent to the current research interest were excluded. The last search was made on 1 October 2021. All searches were conducted independently by two authors (J.P.M. and M.A.M.), and disagreements were resolved by consensus or by consultation with a third author (J.B.N.). The following data were extracted from the original articles: (1) country of publication; (2) year of publication; (3) zoonose occurrence; (4) type of study (cross-sectional, KAP); (5) source of pathogens/data; and (6) name of authors.

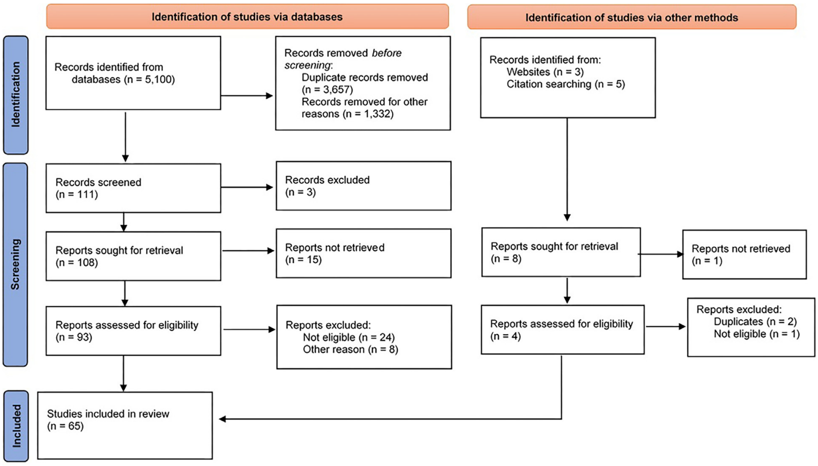

Figure 1. PRISMA flow diagram showing the number of papers retained in different stages of the literature search. The final selection of papers to be included in the review was done by considering both country and type of databases.

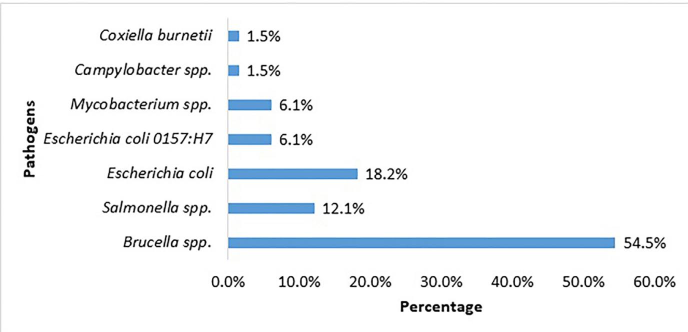

Figure 2. Distribution of different pathogens studied in published papers for countries in East Africa (2010–2021).

Results

A review of the selected papers revealed that the most investigated pathogen was *Brucella* spp. (54.5%), followed by *E. coli* (18.2%), *Salmonella* spp. (12.1%), *Mycobacterium* spp. (6.1%), and *E. coli* O157: H7 (6.1%) (Fig. 2). The least studied pathogens were *C. burnetii* and *Campylobacter* spp. (both 1.5%). Regardless of the country, brucellosis was the most studied disease in East Africa. Among the selected countries, Uganda and Tanzania had the highest number of publications on brucellosis [27.8% and 25.0% ($n = 36$), respectively], while Burundi had the lowest number (2.8%) (Table 1).

In terms of study type, cross-sectional studies were predominant (72.2%), while KAP studies only represented 11% of all studies included in the review. Only five case controls and one cohort study were considered eligible due to their target population and descriptive findings. Overall, studies on humans, raw milk, livestock-humans, and livestock represented 41.6%, 19.4%, 19.4%, and 16.7% of the total, respectively (Table 2).

Studies on both pathogenic and commensal *E. coli* were included in the dataset (Table 3). Out of 15 articles retrieved, 26.6% (4/15) described pathogenic *E. coli* O157: H7 serotype, while 66.6% (10/15) reported on other *E. coli* strains in general. Most of the studies on *E. coli* O157: H7 were carried out in Ethiopia (3/4), with only one in Tanzania. All these studies were cross-sectional (100%) in nature, and the majority of bacteria were isolated from raw cow milk (80%). Irrespective of the *E. coli* serotype studied, Ethiopia accounted for the majority (46.6%) of the papers retrieved, followed by Kenya (26.6%), Tanzania (13.3%), Rwanda (6.6%), and Uganda (6.6%), while no articles were found for Burundi and South Sudan.

In total, seven articles on *Salmonella* spp. were included in the dataset, all from four countries (Table 4). The majority of these studies were cross-sectional (85.7%), with only one longitudinal study (14.3%). Most were carried out in Ethiopia (42.8%), followed by Rwanda (28.6%), while only one

Table 1. Number of publications per pathogen and country.

Country	*Brucella* spp.	*Salm.* spp.	*E. coli*	*E. coli* 0157:H7	*Mycob.* spp.	*Camp.* spp.	*C. burnetii*	Total
Burundi	1	0	0	0	0	0	0	1
Ethiopia	5	4	4	3	1	0	0	17
Kenya	5	0	4	0	0	0	1	10
Rwanda	2	2	1	0	0	0	0	5
South. Sudan	4	0	0	0	1	0	0	5
Tanzania	9	1	2	1	0	1	0	14
Uganda	10	1	1	0	2	0	0	14
Total	36	8	12	4	4	1	1	66

Salm., Salmonella; *E. coli, Escherichia coli*; *Mycob., Mycobacterium*; *Camp., Campylobacter*.

article was retrieved for Tanzania (14.3%) and one for Uganda (14.3%).

Analysis of the dataset revealed that KAP studies accounted for 58.8% (10/17) and studies on risk factors 35.2% (6/17) (Table 5). Regardless of the type of zoonotic disease investigated, most studies were carried out in Kenya (35.2%), followed by Uganda (23.5%), while Ethiopia and Tanzania each accounted for 17.6%, and only one study was carried out in South Sudan (5.8%). No studies were retrieved for the remaining two East African countries considered (Burundi and Rwanda). Different laboratory techniques were used to isolate, identify, and characterize selected bacteria, ranging from standard microbiological methods to molecular diagnostic techniques (Table 6).

Discussion

In the selected dataset, brucellosis was the most frequently investigated of prevalent zoonotic diseases in East Africa. The analysis revealed some discrepancies between the diagnostic methods used, which suggests underestimation of the true prevalence rates of the zoonotic pathogens investigated and their relationship to the milk consumption patterns. It also revealed that consumption of unpasteurized milk, lack of knowledge about zoonotic diseases, and poor milk handling were associated with the occurrence of zoonotic bacterial infections. In low- and middle-income countries, the burden of foodborne disease is disproportionately high and originates from many food sources (Havelaar et al. 2022), and their effects may be acute or chronic in nature. Evidence from various studies indicated a positive association between milk consumption and the risk of contracting bacterial zoonoses in Africa (Kazoora et al. 2016, Dadar et al. 2019, Nyokabi et al. 2021). Conventionally, raw milk is boiled before consumption (Fusco et al. 2020), but some African communities do not boil milk owing to traditional and cultural beliefs, including that “if you boil milk you will provoke udder inflammation in the cows that produced the milk” (Prakashbabu et al. 2020). While boiling milk greatly reduces the presence of most bacteria (Tremonte et al. 2014), it is possible for milk to be re-contaminated due to poor storage and handling practices. In fact, one study found no statistically significant difference between the levels of bacterial contamination in raw and boiled milk (Häsler et al. 2014). Locally processed milk and milk products could potentially pose a risk to consumers, possibly due to inadequate cooling chain, poor processing, and/or handling practices when serving customers. This risk was confirmed by findings in a study where human brucellosis seroprevalence was higher among people consuming locally processed milk products (OR 2.54, 95% CI 1.12–5.78) (Tumwine et al. 2015).

Brucellosis

Although both animals and humans are at high risk of contracting brucellosis, a comparative study of humans and livestock in Kenya revealed that the seroprevalence was two-fold higher in animals than in humans living in the same pastoral community (Osoro et al. 2015). Reported seroprevalence rates of *Brucella* spp. isolated from raw milk varied by country, with the highest rate recorded in Tanzania (Swai and Schoonman 2011) and the lowest in Uganda (Makita et al. 2010). Interestingly, similar findings on human brucellosis were reported in studies on pastoral communities in Tanzania (Asakura et al. 2018a) and on pregnant women living close to wildlife and livestock areas who suffered abortions in association with some predisposing factors to brucellosis (Assenga et al. 2015, Bodenham et al. 2020, Makala et al. 2020, Ntirandekura et al. 2020). Other studies have found that consuming unboiled raw milk (Nasinyama et al. 2014) and living in proximity to livestock (Asiimwe et al. 2015, Tumwine et al. 2015) are strongly associated with occurrence of human brucellosis. A study in Kenya concluded that consumption of unpasteurized milk, handling infected aborted materials without protection, and consuming raw meat and raw blood were potential routes of exposure to brucellosis and other zoonoses (Onono et al. 2019). Another study reported that 79.5% of nomadic pastoralists participate in risky practices for human brucellosis, e.g. drinking unboiled milk showed a positive correlation with *Brucella* spp. seroprevalence (68.2%) (Njenga et al. 2020). Majalija et al. (2018) found that 14% of respondents reported drinking raw milk, of which 46.4% were seropositive to *Brucella abortus*, compared with 1.2% seropositive among those who did not drink raw milk. They also found a significant association between consuming raw milk and seroprevalence of *B. abortus* (OR 2.162, 95% CI 0.021–1.379), while other risk factors did not show any significant correlation (Majalija et al. 2018). Other studies observed a similar pattern where seroprevalence of human brucellosis was positively associated with raw milk consumption (OR 406.15, 95% CI 47.67–3461.69) (Migisha et al. 2018) or consumption of unpasteurized milk ($P = .023$; OR 2.57; 95% CI 1.14–5.80) (Muloki et al. 2018).

Low education level coupled with lack of other sources of income were reported to leave the communities concerned with no choice but to engage in risky milk consumption practices (Njenga et al. 2020). Many studies showed that economic

Table 2. Studies on livestock and human brucellosis in East Africa (2010–2021).

Country	Prevalence rate	Type of study	Source of pathogens/data	Reference(s)
Burundi				
	14.7% (95% CI 9.4%–20.8%)	Cross-sectional	Dairy cattle	Musallam et al. (2019)
Ethiopia				
	2.4% (95% CI 1.4%–3.7%) in cattle 2.6% (95% CI 1.2%–5%) in humans	Cross-sectional	Ruminants and pastoralists	Edao et al. (2020)
	3.0% in cows and 2.4% in humans	Cross-sectional	Livestock and humans	Lakew et al. (2019)
	31.5% (95% CI 27.4%–36.0%)	Cross-sectional	Humans	Mehari et al. (2021)
	NA	KAP study	Farmers	Legesse et al. (2018)
	3.5% (95% CI 2.4%–4.5%)	Cross-sectional	Cattle	Megersa et al. (2011)
Kenya				
	15.4% (95% CI 12.0%–19.5%)	Cross-sectional	Humans	Kiambi et al. (2020)
	OR 7.7 (95% CI 1.5–40.1)	Case-control	Humans/unboiled milk	Muturi et al. (2018)
	NA	KAP Study	Livestock and humans	Njenga et al. (2020)
	NA	Cross-sectional and case-control	Raw milk	Onyango et al. (2021)
	16% in animals and 8% in humans	Cross-sectional	Livestock and humans	Osoro et al. (2015)
Rwanda				
	19.7% (95% CI 15.5%–24.4%)	Cross-sectional	Raw milk	Djangwani et al. (2021)
	25%	Cross-sectional	Humans	Rujeni and Mbanzamihigo (2014)
South Sudan				
	NA	Case-control	Humans	Lado et al. (2012)
	31% (95% CI 28.0%–34.2%) in cattle and 33.3% (23.9%–44.3%) in herders	Cross-sectional	Cattle and herders	Madut et al. (2018a)
	23.3% (97/416)	Cross-sectional	Humans	Madut et al. (2018b)
	27.2% (95% CI 23.9%–30.6%)	Cross-sectional	Humans	Madut et al. (2019)
Tanzania				
	44.4% (55/124, 95% CI 35.5%–53.5%)	Cross-sectional	Cattle	Asakura et al. (2018a)
	7.0% (28/673, 95% CI 5.7%–8.4%)	Cross-sectional	Cattle	Asakura et al. (2018b)
	0.6% (95% CI 0.1–2.1%) in humans and 6.8% (95%CI: 5.4%–8.5%) in cattle	Cross-sectional	Livestock, wildlife, and humans	Assenga et al. (2015)
	NA	Case-control	Livestock	Assenga et al. (2016)
	6.10%	Cross-sectional	Humans	Bodenham et al. (2020)
	10.9% (34/313)	Cross-sectional	Humans	Makala et al. (2020)
	NA	KAP study	Local community	Mburu et al. (2021)
	21% (95% CI 12.5%–32%) in women and 5% (95% CI 3.1%–8%) in ruminants	Cohort	Livestock and humans	Ntirandekura et al. (2020)
	56%	Cross-sectional	Raw cow milk	Swai and Schoonman (2011)
Uganda				
	NA	Case-control	Pastoralists	Asiimwe et al. (2015)
	26.5% (49/185)	Cross-sectional	Raw cow milk	Asiimwe et al. (2015)
	NA	KAP study	Pastoralists	Kansiime et al. (2014)
	7.5% ($n = 200$)	Cross-sectional	Humans	Majalija et al. (2018)
	12.6% (90% CI 6.8%–18.9%)	Risk assessment	Raw cow milk	Makita et al. (2011)
	14.9% (95% CI 10.6%–20.1%)	Cross-sectional	Humans	Migisha et al. (2018)
	18.70%	Cross-sectional	Humans	Muloki et al. (2018)
	5.8% (95% CI 3.3%–8.3%) in farmers and 9% (95% CI 13.3%, 4.7%) in milk consumers	Cross-sectional	Cattle farmers and consumers	Nasinyama et al. (2014)
	25.50%	Cross-sectional	Raw cow milk	Rock et al. (2016)
	17.0% ($n = 235$)	Cross-sectional	Humans	Tumwine et al. (2015)

NA, not available/applicable.

Table 3. Studies on *E. coli* spp., including *E. coli* O157: H7, in East Africa (2010–2021).

Country	Prevalence rate (%)	Type of study	Source of pathogens/data	Reference(s)
Ethiopia				
	5.2% (*E. coli* O157:H7)	Cross-sectional	Raw cow milk	Ababu et al. (2020)
	2.5% (*E. coli* O157:H7) 51.7% (*E. coli*)	Cross-sectional	Ready to consume milk	Amenu et al. (2019a)
	2.9% (*E. coli* O157:H7)	Cross-sectional	Raw cow milk	Disassa et al. (2017)
	33.9% (*E. coli*)	Cross-sectional	Raw cow milk	
	58% (*E. coli*)	Cross-sectional	Raw cow milk	Reta et al. (2016)
	29.6% (*E. coli*)	Cross-sectional	Raw and pasteurized cow milk	Garedew et al. (2012)
	NA (*E. coli*)	Cross-sectional	Raw cow milk	Berhe et al. (2020)
Kenya				
	66.7% (*E. coli*)	Cross-sectional	Unpasteurized milk	Brown et al. (2020)
	25% in cow milk and 32% in camel milk (*E. coli*)	Cross-sectional	Cow milk and camel milk	Nato et al. (2019)
	13.8% (*E. coli*)	Cross-sectional	Raw cow milk	Ngaywa et al. (2019)
	42.4% (*E. coli*)	Cross-sectional	Raw cow milk	Nyokabi et al. (2021a)
Rwanda				
	8.5% (*E. coli*) at farm level 62.5% (*E. coli*) at MCC level	Cross-sectional	Raw cow milk	Ndahetuye et al. (2020)
Tanzania				
	66% (*E. coli*)	Cross-sectional	Raw cow milk	Ngasala et al. (2015)
	10.1% (*E. coli* O157:H7)	Cross-sectional	Raw cow milk and milk products	Schoder et al. (2013)
Uganda				
	12% (*E. coli*)	Cross-sectional	Raw cow milk	Majalija et al. (2020)

NA, not available/applicable.

Table 4. Studies on *Salmonella* spp. in East Africa (2010–2021).

Country	Prevalence rate (%)	Type of study	Source of pathogens/data	Reference(s)
Ethiopia				
	6%	Cross-sectional	Raw milk	Ejo et al. (2016)
	6.3%	Longitudinal	Raw milk	Geresu et al. (2021)
	3.3%	Cross-sectional	Raw milk	Reta et al. (2016)
Rwanda				
	5.2%	Cross-sectional	Raw milk	Kamana et al. (2014)
	14%	Cross-sectional	Raw milk	Ndahetuye et al. (2020)
Tanzania				
	10.1%	Cross-sectional	Raw milk and milk products	Schoder et al. (2013)
Uganda				
	5%	Cross-sectional	Raw cow milk	Majalija et al. (2020)

insecurity and culture are positively associated with engagement in risky food practices (James et al. 2014, Kopetz et al. 2014, Cheng et al. 2016). Education is important to prevent the spread of disease in such communities, e.g. 33.3% seroprevalence to *B. abortus* was found among those with no formal education in Uganda (Majalija et al. 2018) and poor community knowledge of brucellosis was shown to be significantly associated with human brucellosis seroprevalence and risky practices such as drinking raw milk (17.6%, $P < .01$) and blood (35.3%, $P < .01$) (Asakura et al. 2018b). In some low-income settings, cultural beliefs are a constraint to education. For example, Maasai communities in Tanzania were found to rate education less highly than other communities in that country, and are thus more likely to employ more risky practices for human brucellosis infection, such as drinking raw milk ($P = .06$) or blood ($P < .01$) and helping calf delivery with bare hands ($P = .03$) than other tribes (Asakura et al. 2018a). Other studies suggested that community knowledge about the zoonotic nature of brucellosis is not positively associated with a decrease in use of risky practices by community members. Legesse et al. (2018) conducted a KAP study on human brucellosis and found that the majority (89.3%) of interviewed farmers had good knowledge of brucellosis but still consumed unpasteurized milk. Lack of knowledge about brucellosis among community members is a predisposing factor to human brucellosis (Mburu et al. 2021). It is possible that there may be perpetual transmission of brucellosis to humans from raw milk consumption in these communities, due to increased malpractices. These findings emphasize the need for awareness campaigns and training programs for smallholder dairy farmers in order to foster behavioral change and improve milk quality along the whole value chain.

Table 5. KAP studies and risk factors associated with milk-borne bacterial zoonoses identified in East Africa (2010–2021).

Country	Milk-borne diseases of interest/pathogen	Type of study	Target population	Reference(s)
Ethiopia				
	Brucellosis	KAP study	Farmers	Legesse et al. (2018)
	Salmonella spp.	Quantitative risk assessment	Raw milk	Weldeabezgi et al. (2020)
	Bovine tuberculosis	Cross-sectional	Cattle	Kemal et al. (2019)
Kenya				
	C. burnetii (Q fever)	KAP study	Residents	Ndeereh et al. (2016)
	Brucella spp.	KAP study	Nomadic pastoralists and non-pastoralists	Njenga et al. (2020)
	Zoonotic diseases	KAP study	Informal value chain actors	Nyokabi et al. (2018)
	N/A	KAP study	Farmers	Nyokabi et al. (2021b)
	Brucellosis	KAP study	Farmers	Onono et al. (2019)
	Brucellosis	Cross-sectional and case-control	Pastoral communities	Onyango et al. (2021)
South Sudan				
	Brucellosis	Case-control	Humans	Lado et al. (2012)
Tanzania				
	E. coli	Cross-sectional	Humans	Caudell et al. (2018)
	Brucellosis	KAP study	Local community	Mburu et al. (2021)
	Zoonoses	KAP study	Local community	Swai et al. (2010)
Uganda				
	Brucellosis	Case-control	Pastoralists	Asiimwe et al. (2015)
	Human brucellosis	KAP study	Local community	Majalija et al. (2018)
	Brucellosis	KAP study	Pastoralists	Kansiime et al. (2014)
	Human tuberculosis	Cross-sectional	Livestock and humans	Meisner et al. (2019)
	Mycobacterium spp.	KAP study	Cattle farmers	Kazoora et al. (2016)

Campylobacter spp.

Isolation and identification of *Campylobacter* spp. are difficult due to its long incubation period and special culture requirements, such as microaerobic conditions (Brandl et al. 2004), which makes it difficult to study. Only one study on *Campylobacter* spp., in raw milk from East Africa (Kashoma et al. 2016), was included in this review. That study found that 13% of the sampled raw milk contained different *Campylobacter* species. In contrast, studies in developed countries show that *Campylobacter* species are highly frequently isolated from raw milk and are associated with campylobacteriosis in humans. For example, Del Collo et al. (2017) detected *Campylobacter* spp. at 46% of bulk tank milk samples from US dairies, while a follow-up study of a milk-borne campylobacteriosis outbreak in Finland revealed persistent *C. jejuni* contamination of bulk tank milk for several months (Jaakkonen et al. 2020). Further, a retrospective cohort study conducted in England revealed a positive correlation between campylobacteriosis outbreaks in humans and consumption of unpasteurized milk (Kenyon et al. 2020). These findings suggest that raw milk is likely to be contaminated with pathogenic *Campylobacter* spp. and, if consumed, could pose a potential human health risk.

Escherichia coli

Escherichia coli is a gram-negative, rod-shaped, facultative anaerobic bacterium. Most *E. coli* strains are non-pathogenic and colonize the gastrointestinal tract of both animals and humans, but some strains have become very pathogenic through genetic evolution (Kaper et al. 2004). The most frequently isolated pathogenic *E. coli* is the enterohemorrhagic *E. coli* (EHEC) serotype O157: H7 (Shridhar et al. 2017). Since cattle are the natural reservoir of *E. coli* O157: H7 and between 1% and 50% of healthy cattle carry and shed *E. coli* O157: H7 in their feces (Cho et al. 2006), raw milk contamination can occur during milk handling. Some of the studies reviewed suggested that the presence of *E. coli* bacteria in raw and pasteurized milk is associated with poor and unhygienic milk handling practices, fecal contamination, higher environmental contamination, and poor storage conditions (Ngasala et al. 2015; Reta et al. 2016). Other studies reported that *E. coli* is frequently detected at milk collection center (MCC) level, possibly due to bacterial contamination by workers and equipment used in milk storage (Ndahetuye et al. 2020). The presence of *E. coli* O157: H7 bacteria in raw milk is a global public health concern. In Africa, the risk of *E. coli* O157: H7 outbreaks is highly associated with raw milk consumption. Moreover, a study in Tanzania found that consumption of raw milk was associated with an increased probability of carrying multidrug-resistant *E. coli* strains, which was most likely connected to scarcity of potable water and to the informal sales channels for raw milk that prevail in low-income countries operating outside national quality control standards and regulations (Caudell et al. 2018). In the USA, researchers have found a positive correlation between *E. coli* O157: H7 outbreaks and consumption of raw cow milk (Denny et al. 2008). Similar findings have been reported in Brazil (Cerva et al. 2014).

Salmonella spp.

In the set of papers reviewed, presence of *Salmonella* spp. in raw milk was significantly associated with poor hygiene practices at farm level (Ndahetuye et al. 2020) or poor milk handling practices by traders due to lack of adequate training (Schoder et al. 2013). Similarly, findings in Uganda suggest that the main sources of *Salmonella* spp. in raw milk can be grouped into poor hygiene, handling, and transporta-

Table 6. Diagnostic techniques used in isolating and identifying zoonotic pathogens.

Pathogens	Laboratory method	Number of references	Reference(s)
Brucella spp.	Milk ring test (*only*)	1	Swai and Schoonman (2011)
	Milk ring test and ELISA	1	Kamwine et al. (2017)
	ELISA (*only*)	9	Makita et al. (2010), Muloki et al. (2018), Muturi et al. (2018), Asakura et al. (2018a), Madut et al. (2019), Musallam et al. (2019), Djangwani et al. (2021), Osoro et al. (2015), and Rock et al. (2016)
	Rose Bengal (*only*)	1	Rujeni and Mbanzamihigo (2014)
	Rose Bengal and ELISA	5	Asakura et al. (2018b), Assenga et al. (2015), Edao et al. (2020), Madut et al. (2018a), and Makala et al. (2020)
	Rose Bengal and any other test (complement fixation, culture, agglutination, fluorescence polarization)	5	Megersa et al. (2011), Tumwine et al. (2015), Migisha et al. (2018), Makala et al. (2020), and Ntirandekura et al. (2020)
	Rose Bengal, agglutination, and ELISA	1	Madut et al. (2018b)
	Agglutination (*Only*)	2	Asiimwe et al. (2015) and Majalija et al. (2018)
	Agglutination and ELISA	1	Nasinyama et al. (2014)
	PCR (*only*)	1	Kiambi et al. (2020)
	Culture and PCR	1	Bodenham et al. (2020)
Campylobacter spp.	Multiplex PCR	1	Kashoma et al. (2016)
E. coli	Standard microbiological techniques (culture, biochemical tests, and microscopic examination)	12	Caudell et al. (2018), Amenu et al. (2019b), Ababu et al. (2020), Berhe et al. (2020), Brown et al. (2020), Garedew et al. (2012), Nato et al. (2019), Majalija et al. (2020), Ndahetuye et al. (2020), Nyokabi et al. (2021b), Reta et al. (2016), and Gwandu et al. (2018)
	Standard microbiological techniques confirmed by PCR and sequencing	1	Ngaywa et al. (2019)
	Viable counts (TBC, TCC)	2	Schoder et al. (2013) and Ngasala et al. (2015)
Salmonella spp.	Standard microbiological techniques (culture, biochemical tests, and microscopic examination)	6	Kamana et al. (2014), Ejo et al. (2016), Majalija et al. (2020), Ndahetuye et al. (2020), Geresu et al. (2021), and Reta et al. (2016)
	Viable counts (TBC, TCC)	1	Schoder et al. (2013)
Mycobacterium spp.	Ziehl–Neelsen (ZN) and fluorescence microscopy (FM)	1	Boyong et al. (2018)
	Tuberculosis skin test (TST)	1	Meisner et al. (2019)

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; TBC, total bacteria count; and TCC, total coliform count.

tion practices (Majalija et al. 2020). However, Reta et al. (2016) observed a more complex contamination process that included all critical control points along the raw milk value chain, resulting in an increase in total bacterial counts from the start (12% on-farm) to the end (42.9% at the point of sale) of the value chain. Similarly, a study in Rwanda found that *Salmonella* spp. isolation rate was much lower (5.2%) at farm level than in milk shops (21.4%) (Kamana et al. 2014). In that study, the high isolation rate at milk shops was tentatively attributed to poor handling and unhygienic practices by the personnel (Kamana et al. 2014). However, contradictory findings were made by Ndahetuye et al. (2020), who recovered no *Salmonella* spp. in milk samples from MCC, whereas a significant proportion (14.0%) of milk samples from farms showed presence of *Salmonella* spp. This difference could have been due to the dilution effect resulting in undetectable levels of *Salmonella* spp. in milk samples from MCC (Ndahetuye et al. 2020). This is supported by the fact that MCCs have cold chain infrastructure that reduces growth of microorganisms and improves milk quality (Demirbaş et al. 2009, O'Connell et al. 2017), in contrast to direct sales from farms.

Tuberculosis

Tuberculosis (TB) is a leading zoonotic infectious cause of human death worldwide, which is transmitted to humans through consumption of raw, unpasteurized or contaminated milk, dairy products and other animal products (Kazoora et al. 2016). Two studies dealing with perceptions of the local communities and risk factors associated with the occurrence of TB in humans were included in this review (Kazoora et al. 2016, Meisner et al. 2019). The two studies revealed that local communities engage in predisposing risk practices such as consuming raw milk, consuming dairy products from raw milk, living in proximity with livestock among others (Kazoora et al. 2016, Meisner et al. 2019). It was also reported that the majority of study respondents have very low knowledge and poor practices regarding zoonotic aspect of TB (Meisner et al. 2019). It is important to note that despite the knowledge about TB, many people still consume raw milk as a necessity due to lack of time or resources to properly pasteurize milk before consumption (Kazoora et al. 2016). This poor practice is also associated with low education level and living in low-resource settings (Njenga et al. 2020).

Q fever

Q fever is an emerging zoonotic disease caused by an intracellular bacterium *C. burnetii* (Porter et al. 2011). *Coxiella burnetii* is transmitted to humans through direct contact, inhalation of contaminated aerosols, or by consuming contaminated animal products, mainly unboiled milk (Celina and Cerný 2022). Only one article investigating the knowledge, attitudes, and practices about Q fever and rickettsioses were included in this review (Ndeereh et al. 2016). The study revealed that the local communities have no knowledge about Q fever, while only 9.1% of health providers know about the disease (Ndeereh et al. 2016). This low knowledge among health providers could be justified by the fact that Q fever is poorly diagnosed in low and middle-income countries, and the disease is worldwide considered a neglected and re-emerging zoonotic disease (Ullah et al. 2022). It is currently known that ingestion of contaminated raw milk poses a low risk of contracting Q fever since the oral route is reported to be a less efficient way of transmission (Arricau-Bouvery and Rodolakis 2005). Despite this low probability of transmission, we recommend special attention to reducing exposure to raw milk for at risk people (young children, old people, pregnant women, patients with cardiac pathology, or immunosuppressed) and promoting consumption of pasteurized milk and its products to decrease the prevalence of Q fever.

Investigation methods

Scrutiny of the investigation methods used in the articles included in this review revealed that cross-sectional study was the most common method used. This method has scientific limitations when assessing temporal variations and the causal effect of independent variables on the response variable (Wang and Cheng 2020). However, the trends in the dataset revealed a common pattern of prevalence of the investigated diseases or pathogens in all selected countries, confirming the validity of the results. Laboratory diagnostic methods used in the papers included rapid tests, conventional microbiological techniques, and enzymatic techniques coupled with molecular diagnosis. However, the laboratory methods employed in many studies resulted in some data gaps. For example, Kamwine et al. (2017) found that the milk ring test for brucellosis identification had lower sensitivity (85%) and specificity (95.5%) than the indirect enzyme-linked immunosorbent assay (sensitivity 98.5%, specificity 99.5%). They concluded that the presence of *Brucella* spp. antibodies in milk could not be associated with a current infection in lactating cows, and recommended a field study to confirm the source of the antibodies in raw milk (Kamwine et al. 2017). Most ELISA methods do not report the current infection and sometimes detect some immunoglobulins (Ig) and not others (Osoro et al. 2015). Similarly, a study on patients presenting with febrile illnesses and seeking healthcare services in Kenya found that the febrile brucella plate agglutination test, which is commonly used in hospitals, had low diagnostic performance compared with real-time polymerase chain reaction, with estimated sensitivity of only 36.6% (95% CI 24.6%–50.1%) and specificity of 69.3% (95% CI 64.0%–74.3%) (Kiambi et al. 2020). Additionally, identification and differentiation of mycobacteria belonging to the *Mycobacterium tuberculosis* complex could have provided evidence of their zoonotic nature and their transmission in humans. These discrepancies in diagnostic methods result in underreporting of many zoonotic diseases in low-income countries, due to lack of adequate diagnostic capability (Ndeereh et al. 2016). Finally, access to improved diagnostic tools and equipment in low- and middle-income countries is very difficult due to insufficient funding allocated to disease diagnosis, lengthy procurement processes, to cite a few (Yadav et al. 2021).

Conclusions

Overall, this review revealed potential health risks to raw milk consumers in East Africa. There is sufficient evidence to show that milk-borne bacterial infections in East African countries are associated with consumption of contaminated raw milk, which is common practice in many East African counties. Inadequate cold chain conditions along the milk value chain, poor milking practices, and lack of awareness of the health risks of consuming unpasteurized milk further predispose milk consumers to the risk of contracting milk-borne diseases, as do lack of infrastructure, inadequate boiling, and recontamination of boiled milk due to poor storage conditions and handling practices. However, the findings should be interpreted with caution because most of the articles reviewed described cross-sectional studies that could not provide clear evidence on the persistent exposure of consumers to contaminated raw milk. Therefore, further studies involving isolation and identification of pathogenic bacteria in milk are needed to identify zoonotic risk factors associated with raw milk consumption.

Only papers published in English were included in the review, which may explain why there were few articles from Rwanda (a bilingual country) and Burundi (a French-speaking country). In addition, Rwanda and Burundi are smaller than other East African countries from which many publications were retrieved. However, the findings are still generalizable to those two countries. In fact, since similar findings were found for different countries at different times, the conclusions on associations between risk factors and the occurrence of zoonotic infections are likely to be valid. Considering the limitations in diagnostic methods used in some of the studies reviewed, it is obvious that the true prevalence rates of these zoonotic diseases might be higher than reported. Therefore, further livestock and human studies using advanced investigation methods are needed. Additionally, East African countries need to invest in modern diagnostic tools and equipment, in hospitals and especially in local rural settings. Greater access to these tools should be supported to improve surveillance and control programs for both humans and animals. Finally, a tailor-made training program for all milk value chain actors should be implemented to improve the safety of milk sold via informal channels and a One Health approach should be applied.

Conflict of interest

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

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Jean Pierre Mpatswenumugabo (Investigation, Methodology, Writing – original draft), Marie Anne Mukasafari (Investigation, Methodology, Writing – original draft), Jean Baptiste Ndahetuye (Conceptualization, Supervision, Writing – review & editing), Ewa Wredle (Conceptualization, Supervision, Writing – review & editing), and Renée Båge (Conceptualization, Project administration, Supervision, Writing – review & editing)

Data availability

The authors declare that all data supporting the findings of this study are available within the article (Tables 1–6).

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Milk is essential for children's growth, but Rwanda struggles with low milk yield and quality, affecting food security and public health. This thesis assessed factors influencing milk yield, quality, zoonoses and child malnutrition, linking low yield to feeding practices and breeds. *Salmonella* was detected in 25.6% of milk samples and 27% of children were stunted. A multidisciplinary approach has demonstrated potential when investigating child malnutrition, and coordinated interventions will contribute to its eradication.

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