PREVALENCE OF *BRUCELLA* IN MILK PRODUCED UNDER OPEN AND ZERO-GRAZING SYSTEMS IN RWANDA AND RISK OF EXPOSURE AMONG MILK CONSUMERS IN CATTLE-KEEPING HOUSEHOLDS

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A81/52133/2017

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD SCIENCE AND TECHNOLOGY

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY
FACULTY OF AGRICULTURE
UNIVERSITY OF NAIROBI

2022

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This thesis is my original work and has not been submitted for award of a degree in any other University.

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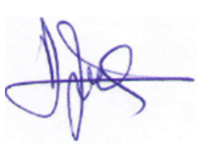
Signature  Date: April 25, 2022

Dedication

This work is dedicated to my late father who believed in education and always encouraged us to keep educating ourselves.

Acknowledgments

First and foremost, I would like to express my sincere gratitude to my supervisors, Dr. George Ooko Abong', Dr. Lucy Njue and Dr. Dasel Wambua Mulwa Kaindi for their support throughout my PhD study journey. I am grateful for your patience, your motivation and encouragement. Your guidance was crucial for the success of my PhD journey.

My sincere gratitude goes to BHEARD (Borlaug Higher Education for Agricultural Research and Development) for financially supporting my PhD study. Without your support my PhD journey would have not been possible.

I would like to thank the lab technicians, Jacinta W. Muchiri at the University of Nairobi, Faculty of Agriculture, Department of Food Science, Nutrition and Technology and Afsa Mukanyarwaya at the University of Rwanda, College of Agriculture, Animal Sciences and Veterinary Medicine, Department of Food Science and Technology. Thank you for your welcoming attitude and for your assistance in the laboratory analysis.

Finally, I would like to thank my mother Antoinette Mukazitoni, my brother, Jules Djangwani and my sisters, Julienne Djangwani, Jacqueline Djangwani, Janviere Djangwani and my in-laws, Eugenie Muhundwangeyo, Etienne Kanyamahanga and Sam Rugira for their moral support during my PhD study journey.

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List of Abbreviations

BAPA	: Buffered Acidified Plate Antigen Test
c-ELISA	: Competitive Enzyme-Linked Immuno-Sorbent Assay
CFT	: Complement Fixation Test
CI	: Confidence Interval
DAROs	: District Animal Resources Officers
EAC	: East African Community
FAO	: Food and Agriculture Organization
HH	: Household
HHH	: Household Head
HHs	: Households
i-ELISA	: Indirect Enzyme-Linked Immuno-Sorbent Assay
IgG-ELISA	: Immunoglobulin G Enzyme-Linked Immuno-Sorbent Assay
IgM-ELISA	: Immunoglobulin M Enzyme-Linked Immuno-Sorbent Assay
IgG/IgM-ELISA	: Immunoglobulins G and M Enzyme-Linked Immuno-Sorbent Assay
IgG/IgM-LFA	: Immunoglobulins G and M lateral flow assay
IPRC	: Integrated Polytechnic Regional College
MAT	: Microscopic Agglutination Test
MINAGRI	: Ministry of Agriculture and Animal Resources
MRT	: Milk Ring Test
N/A	: Not Applicable
NGOs	: Non-Governmental Organizations
OD	: Optical Density
ODK	: Open Data Kit

OIE : World Organization for Animal Health

OR : Odds Ratio

PAT : Plate Agglutination Test

PCR : Polymerase Chain Reaction

PP : Percent Positivity

PPE : Personal Protective Equipment

q-PCR : Quantitative Polymerase Chain Reaction

RAB : Rwanda Agriculture and Animal Resources Development Board

RBT : Rose Bengal Test

RBPT : Rose Bengal Plate Test

RDCP : Rwanda Dairy Competitiveness Program

Riv. T : Rivanol Precipitation Test

SAT : Serum Agglutination Test

SPSS : Statistical Package for the Social Sciences

SSA : Sub-Saharan Africa

TAT : Tube Agglutination Test

TVET : Technical and Vocational Education and Training

UK : United Kingdom

WHO : World Health Organization

General Abstract

Studies on *Brucella* prevalence in milk produced in Rwanda are limited. Brucellosis knowledge among cattle farmers and the risk of exposure to *Brucella* through milk consumption are, also, poorly documented. A study was, therefore, conducted to assess brucellosis knowledge among cattle farmers in Rwanda; to determine the prevalence of *Brucella* in milk produced under zero and open grazing cattle production systems in Rwanda and to assess the risk of exposure to *Brucella* through milk consumption in cattle keeping households. The study was cross-sectional and involved 198 zero grazing cattle keeping households and 132 open grazing cattle keeping households. Questionnaires and indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA) were used to collect data and data was analyzed using descriptive statistics and logistic regression in statistical package for social studies (SPSS) software.

More than half of all respondents (63.6 %; 210/330) had heard about brucellosis but only 3.8 %; 10.0 % and 4.3 % could correctly name at least two ways through which cattle contract brucellosis, at least two brucellosis clinical signs in cattle and at least two farm animals which can contract brucellosis, respectively. The overall knowledge score was very low with only 1.8 % (6/330) of all respondents having a knowledge score of ≥ 7.5 out of a possible maximum 15. Very few among farmers, 5.2 % (6.6 % in zero grazing study areas and 3.0 % in open grazing study areas) were using PPE while assisting cattle in parturition or handling aborted materials. Artificial insemination was practiced by 66.2 % (131/198) among zero grazing farms while no farm among open grazing farms reported using artificial insemination. Only a total of 8 farms, all of which were zero grazing, had vaccinated their cattle against brucellosis. Majority among respondents (63.9 %; 211/330) indicated they would seek veterinary help if their animal had or was suspected of having brucellosis.

Anti-*Brucella* antibodies were prevalent in 19.7 % (95 % CI, 15.5-24.4) of the 330 collected farm bulk milks with a significantly higher ($p < 0.05$) sero-prevalence among open grazing farms (37.9 % [50/132]) compared to zero grazing farms (7.6 % [15/198]). Practicing open grazing system (OR = 69.5; 95 % CI = 1.6 - 3033.6), history of abortion (OR = 19.5; 95 % CI = 8.1 -

46.8), and placenta retention (OR=4.2; 95 % CI = 1.7 - 10.3) were the significant risk factors for the presence of anti-*Brucella* antibodies in milk.

Overall, 14.2 % (47/330) of all surveyed households were exposed to *Brucella* by having at least one household member consuming raw milk while the farm bulk milk sample had turned *Brucella* seropositive. Notably and significantly ($p < 0.05$), raw milk was consumed in more open grazing households with a *Brucella* seropositive farm bulk milk sample (34.8 %; 46/132) than in zero grazing households with a *Brucella* seropositive farm bulk milk sample (0.5 %; 1/198). Compared to other household members, the cattle keeper was the household member most exposed (OR=19.9; 95 % CI, 5.9-66.2) to *Brucella* through milk consumption.

Brucellosis knowledge among surveyed respondents was generally poor. *Brucella* was prevalent in farm bulk milk especially milk from open grazing farms and the risk of exposure to *Brucella* through milk consumption was higher in households practicing open grazing cattle production in Rwanda. Therefore, educational campaigns are needed to raise awareness about brucellosis among cattle farmers in Rwanda. In addition to brucellosis educational campaigns, further research is needed to identify prevalent *Brucella* species and strains by cultural and molecular methods and implement more informed and appropriate brucellosis control programs in the country. With the observed scarcity and difficulty in accessing biosafety level three laboratories needed to culture *Brucella*, more efforts should be directed towards establishing more research infrastructure.

CHAPTER ONE: INTRODUCTION

1.1. Background

Cattle keeping in Rwanda is an important aspect of the Rwandan culture and economy (Karenzi *et al*., 2013). Cattle is kept under two main livestock rearing systems, the open grazing/extensive system and the zero grazing/intensive system, with 80%, 17% and 3% of the dairy farms practicing the zero grazing/intensive system, the open grazing and the semi-intensive system, respectively (RDCP, 2014). The cattle population in Rwanda is 1,449,888 heads while the annual milk production is estimated at 891,326 metric tons (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2021).

An important portion of the milk produced in Rwanda stays and is used on farms. According to the National Institute of Statistics, at least 36.7 % of the produced milk is consumed in producing dairy farms (National institute of statistics of Rwanda, 2018). Although detailed information on milk consumption patterns in Rwanda is limited, the portion of milk that stays on dairy farms is consumed in variety of locally prepared milk products including fresh raw milk, boiled milk, tea milk and traditionally fermented milk.

Different microorganisms, some of which are pathogenic bacteria, have been associated with milk and milk products. They include *Bacillus cereus*, *Brucella abortus* , *Cronobacter spp*., *E. coli*, *Campylobacter jejuni*, *Listeria monocytoenes*, *Mycobacterium bovis*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus spp*., *Yersinia enterolitica*, *Coxiella burnetii*, etc (Mcmahon, 2013). Among pathogenic bacteria associated with milk and milk products are the zoonotic bacteria which are especially a concern when milk is consumed raw or milk products are made from raw milk. Zoonotic bacteria that have been associated with milk and milk products include *Brucella abortus* , *Brucella melitensis, Campylobacter jejuni, Coxiella burnetti, enterohaemorragic Escherichia coli, Listeria monocytogenes, Mycobacterium bovis, Mycobacterium avium paratuberculosis, Salmonella* spp., *Staphylococcus aureus, Streptococcus agalactiae, Yersinia entrolitica* (Britz & Robinson, 2008; Dhanashekar et al., 2012; International Livestock Research Institute, 2012; Mcmahon, 2013; Mosalagae et al., 2011; Pelzer & Currin, 2009; WHO, 2015). The zoonotic bacteria, *Brucella*, which is of interest to this research work, contaminates milk from infected milk producing animals (Corbel, 2006; Godfroid *et al*., 2014).

One common way of *Brucella* transmission from livestock to humans is through the consumption of unpasteurized milk and milk products (Dadar *et al*., 2019). *Brucella* contaminates milk from brucellosis infected animal hosts such as cattle (Corbel, 2006; WHO, 2012; Thoen & De Kantor, 2006).

In Rwanda, brucellosis is among the main livestock diseases (Feed the Future Innovation Lab, 2016). Studies that have been conducted in the country indicate the presence of brucellosis in cattle with cow prevalence rates varying from 0.0 to 2.0 % in peri-urban areas of Kigali City (Manishimwe *et al*., 2015; Ntivuguruzwa *et al*., 2020), 8.3 % in districts bordering the national parks (Ntivuguruzwa *et al*., 2020) and 9.9 to 18.9 % in the district of Nyagatare (Chatikoba et al., 2008; Ndazigaruye *et al*., 2018).

Humans are exposed to brucellosis through different ways including milk consumption. Increased exposure of humans to brucellosis was associated with milk consumption and consumption of locally processed milk products in Rwanda and other countries including Uganda, Kenya and Bangladesh (Gafirita et al., 2017; Osoro et al., 2015; Rahman et al., 2012; Rujeni & Mbanzamihigo, 2014; Tumwine et al., 2015). While a lot of progress has been made to eradicate brucellosis in animals in developed countries, brucellosis is still among major zoonotic infections particularly in low income countries and the developing world including Rwanda (World Health Organization, 2012).

1.2. Statement of the problem

Bovine brucellosis that has been reported in Rwanda (Chatikoba *et al*., 2008; Manishimwe *et al*., 2015; Ndazigaruye *et al*., 2018; Ntivuguruzwa *et al*., 2020) presents a risk of bovine milk being contaminated with the zoonotic bacteria, *Brucella*, and a recent study investigating the general microbiological quality of milk from farms to milk collection centers in Rwanda detected anti-*Brucella* antibodies in milk from two milk collection centers (Ndahetuye *et al*., 2020). Fresh milk produced in Rwanda is, also, at risk of contamination with *Brucella* due to poor herd and farm management practices, milking techniques and hygiene (Kamana *et al*. 2014, Habarugira et al. 2014, Rujeni & Mbanzamihigo, 2014).

To date, however, the farmers' knowledge of brucellosis, the prevalence of *Brucella* in milk, the risk factors of milk contamination with *Brucella* and the risk of exposure to *Brucella* through milk consumption in Rwanda have not yet been established. While milk microbiological quality studies in Rwanda have been conducted for some zoonotic bacteria including *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* (Kamana, 2014; Kotto et al., unpublished), limited data exist on prevalence of the zoonotic *Brucella* in milk. Most previous brucellosis studies that have been conducted focused on the disease prevalence in animals and humans (Chatikoba *et al*., 2008; Manishimwe *et al*., 2015; Ndazigaruye *et al*., 2018; Ntivuguruzwa *et al*., 2020; Rujeni & Mbanzamihigo, 2014).

With the reported animal brucellosis and with human brucellosis studies in Rwanda having associated the infection in humans to the consumption of raw milk (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014), the prevalence of *Brucella* in milk and the risk of exposure to *Brucella* through milk consumption in Rwanda are lacking. Therefore, the current study aims at assessing the knowledge, attitudes and practices of Rwandan cattle farmers regarding brucellosis, determining the prevalence of *Brucella* in milk produced under the zero and open grazing systems in Rwanda and risk factors of milk contamination and assessing the risk of exposure to *Brucella* among milk consumers in cattle keeping households.

1.3. Justification of the study

Animals including cattle are known to be the natural hosts of *Brucella* with a possibility of transmitting the bacteria to humans through occupational exposure and animal sourced food consumption. While studies in Rwanda have already reported on cattle and human brucellosis, there is still need to assess the farmers' knowledge of brucellosis and investigate the food safety aspect to understand the milk food safety vis-à-vis *Brucella* and the role of milk consumption, if any, in transmitting the diseases to consumers.

It is anticipated that findings from this study will contribute new knowledge on the prevalence of *Brucella* in farm bulk milk in Rwanda as well as on the exposure to brucellosis for milk consumers in rural cattle keeping households. Findings from the study will, also, contribute information to brucellosis policy developers and results in better planned efforts to control brucellosis in the country and reduce the disease' associated burdens on animal and human health.

In addition to contributing new information and informing for policy development, findings from this study will provide more insights to the public, in general, and to dairy farmers, in particular, about cattle brucellosis and the public health concern it may pose through milk consumption. Among the Sustainable Development Goals (SDGs) that the United Nations (UN) and the government of Rwanda have set to achieve are no poverty, zero hunger and good health and well-being. Findings from this study will contribute information on brucellosis prevalence and help the government plan to prevent livestock diseases such as brucellosis which result in economic losses on the part of farmers and increased poverty. Findings from the study will, also, contribute information to review the existing brucellosis control strategies, control brucellosis in the country and ensure availability of milk in good quantity and quality to reduce hunger while preventing adverse health effect that may originate from consuming unsafe milk and milk products.

1.4. Objectives of the study

1.4.1. Overall objective

The overall objective of the current study was to evaluate the prevalence of *Brucella* in milk produced under open and zero grazing systems in Rwanda and the risk of exposure to *Brucella* among milk consumers in cattle keeping households.

1.4.2. Specific objectives

1. To assess knowledge, attitudes and practices on brucellosis among cattle farmers in the open and zero grazing systems in Rwanda.

2. To determine the prevalence and the risk factors of *Brucella* presence in farm bulk milk from open and zero grazing cattle production systems in Rwanda.

3. To assess the risk of exposure to *Brucella* among milk consumers in zero grazing and open grazing cattle keeping households.

1.5. Hypotheses

1. Knowledge, attitudes and practices on brucellosis among cattle farmers in both open and zero grazing systems in Rwanda do not significantly differ.

2. The prevalence and the risk factors of *Brucella* presence in farm bulk milk from open and zero grazing cattle production systems in Rwanda are not significantly different.

3. Milk consumers in cattle keeping households from both open and zero razing systems in Rwanda are not significantly exposed to *Brucella*.

CHAPTER TWO: LITERATURE REVIEW

2.1. Cattle production in Rwanda

Cattle keeping in Rwanda has been practiced for a very long time and has become an important aspect of the Rwandan culture and economy (Karenzi et al., 2013). Cattle are distributed and produced all over the country with some regions having more cattle than the others. The ministry of agriculture estimates the cattle population in Rwanda at 1,449,888 heads (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2021). The cows are owned and mainly kept on smallholder farms with an average land size of about 0.7 ha per farm (TechnoServe Rwanda, 2008; Mutimura, 2010) and two to three cows per farm (Bishop & Pfeiffer, 2008; Kamanzi & Mapiye, 2012; Miklyaev *et al*., 2017).

In Rwanda, the cows are raised following different livestock production systems which have been reviewed and classified in three categories: extensive production/open grazing, intensive production/zero grazing and semi-intensive production (Mazimpaka, 2017). In the eastern and north-western parts of Rwanda with higher cattle populations and relatively more land for grazing, cattle are raised mainly under the open grazing systems in which cattle are left to graze on fenced farms, while in regions with lower cattle populations and smaller holder farms, cattle are raised under the zero grazing system in which cows are kept in-doors and farmers cut and carry forage, crop residues and water to feed the cows (Feed the Future Innovation Lab, 2016; Mazimpaka, 2017). Countrywide, the zero-grazing system is the most common and practiced by up to 80 % of farms while open grazing and semi-grazing are practiced by 17 % and 3 % of farms, respectively (Land O' lakes, 2014).

2.2. Milk production and milk consumption in Rwanda

There are five main milk sheds in Rwanda: Kigali milk shed, Eastern milk shed, Southern milk shed, Northern milk shed and North-Western milk shed (Miklyaev *et al*., 2017). Milk production is concentrated in the milk shed and has been estimated differently by different institutions, all of which show an increase in milk production in Rwanda. According to the Ministry of Agriculture and Animal Resources, the annual total milk production has more than doubled from 442,337 metric tons in 2011 to 891,326 metric tons in 2020 (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2021). A report on investment opportunities in the Rwanda Dairy sector, also, reported that the annual milk production in Rwanda had increased from 50,000 metric tons in 2000 to 816,000 metric tons in 2019 (Steeg & Bonnier, 2019). In 2018, the National Institute of Statistics estimated that about 2 million liters of milk were produced every day in Rwanda (National institute of statistics of Rwanda, 2018) which was equivalent 730,000 metric tons of annual milk production.

Most of the produced milk is consumed on producing farms and channel through the informal milk market. From a daily milk production in Rwanda it was estimated that 36.7 % of the production is own-consumed by producing farmers, 32.7 % sold to others, 18.4 % sold at milk collection center, 7.9 % given to others , 3.2 % lost due to spoilage while 1.1 % are used in other ways (National institute of statistics of Rwanda, 2018). Similarly to milk production, the milk consumption in Rwanda has also increased. The reported figures show that the per capita annual milk consumption has increased from 20 liters in the 1990s to 68 liters and 80 liters in 2019 and 2020 respectively (Steeg & Bonnier, 2019).

The milk products that are consumed in Rwanda include raw milk, pasteurized milk, fermented milk ("Ikivuguto"), yoghurt, cheese, cream and butter (Steeg & Bonnier, 2019). While it is not yet easy to determine in what proportions the milk products are consumed, a portion of milk that is consumed on dairy farms and in rural households is consumed fermented into the popular traditional sour milk locally known as "ikivuguto" (Karenzi *et al*., 2013). "Ikivuguto" is very popular among Rwandan consumers who prefer the product over related dairy products such as yoghurt and the industrialized form of "ikivuguto" due to its consistency and flavor (Karenzi *et al*., 2013). "Ikivuguto" is also produced in local milk shops and sold both in rural and urban areas (Karenzi *et al*., 2013).

Few reports exist on the exact figures of the consumption of raw milk in Rwanda. For urban consumers, a survey conducted in Musanze town, the second largest city in Rwanda, indicated that 66.7 % of milk buyers boiled it before consumption (Kamana *et al., 2014)*. When asked randomly, the majority of consumers in rural areas would respond that they never consume or use milk without boiling it. However, it was reported that constraints related to time, cost of charcoal or wood used to boil milk in rural areas are hindrances to boiling or not properly boiling milk in rural households in Rwanda (Miklyaev *et al*., 2017).

2.3. Brucella sp. and brucellosis infection

2.3.1. *Brucella* spp.

Brucella spp, the causative agents of brucellosis, are short, non-spore forming, non-capsulated, facultative intracellular Gram negative coccobacilli of 0.5 to 0.7µm in diameter and 0.5 to 1.5µm in length. (Food and Drug Administration, 2012). There are 12 species of *Brucella* spp. that have been identified, each species having a preferential animal host (Table 1). *Brucella* species that are most important to livestock animal health and public health are *B. abortus* from cattle, *B. melitensis* from goats and sheep, *B. suis* from swine and *B. canis* from dogs (Corbel, 2006; Food and Drug Administration, 2012).

Table 1: Brucella species and their animal hosts

***Brucella* species**	**Animal host**	**References**
B. abortus	Cattle	(Bang, 1897)
B. melitensis	Goats, sheep	(Bruce, 1887)
B. suis	Swine	(Traum, 1914)
B. canis	Dogs	(Carmichael & Bruner, 1968)
B. ovis	Sheep	(Buddle & Boyes, 2008)
B. neotomae	Rodents	(Stoenner & Lackman, 1957)
B. microti	Voles	(Scholz *et a*l., 2008)
B. pinnipedialis	Seals	(Kroese *et al*., 2018)
B. ceti	Dolphins and whales	(Foster *et al*., 2007)
B. papionis	Baboons	(Whatmore *et al*., 2014)
B. vulpis	Foxes	(Scholz *et al*., 2016)
B. inopinata	*	(Scholz *et al*., 2010)

*Isolated from a breast implant wound of a female patient

Although different *Brucella* species have different preferential animal hosts, cross-species infections can occur. *B. abortus* which has cattle as its natural preferential host has been isolated from goats and sheep raised together with cattle (Wareth *et al*., 2015); *B. melitensis* which preferentially infects goats and sheep has been isolated from cattle (Kolo *et al*., 2018) and *B. suis* which has swine as its natural host has been isolated in cattle (Fretin *et al*., 2013).

2.3.2. Brucellosis infection

2.3.2.1. Brucellosis infection in animals

Brucellosis has been reported in many different animals. In addition to livestock animals such as cattle, sheep, goats and pigs, brucellosis infections have been reported in camels (Musa *et al*., 2008), buffaloes (Holt *et al*., 2011) and wildlife including bisons, hares, reindeers (Whatmore, 2009), elks, deers, feral swine, wild boars, hares, caribou, chamois (Godfroid *et al*., 2013; Whatmore, 2009). Among the animals affected by brucellosis, livestock animals, especially cattle, goats and sheep are a concern from public health, animal health and economic point of views. Livestock animals contract brucellosis when they are infected with the bacteria *Brucella* spp. Worldwide, the most important *Brucella* spp. responsible for the infections in livestock are *B. abortus*, *B. melitensis B. ovis* and *B. suis* (Godfroid *et al*., 2014).

The sources of infection for livestock animals are infected animals which transfer the bacteria to healthy animals. Aborted materials, fetal membranes, vaginal discharges, milk, manure from infected animals are infected with *Brucellae* (Hamdy & Amin, 2002; Kaur *et al*., 2018; Langoni *et al*., 2000; Mugizi *et al*., 2015; Tekle *et al*., 2019) and are important means of transmission between animals (Corbel, 2006; Poester *et al*., 2013). Apart from directly infecting healthy animals, the highly infected materials end up infecting the environment and remain active environmental sources of contamination. *B. abortus* was for example reported to survive for 28 days in water and to remain viable for up to 132 days in milk (Kaden *et al*., 2018). It was also reported that *Brucellae* can survive on fetal tissue, soil and vegetation for 21 to 81 days depending time of the year, temperature and exposure to sunlight (Aune *et al*., 2012). Following infection in the animals, *Brucellae* invade a variety of cells including epithelial cells, placental trophoblasts, dendric cells and macrophages (Gorvel, 2008). *Brucellae* survive and multiply within host's cell thanks to their capacity to modify the cell intracellular environment favoring their own replication (Kim, 2015; Sangari & Agüero, 1996).

In livestock animals such as cattle, goats, sheep and pigs, the most frequent clinical sign of brucellosis is abortion in female animals (Schmutz *et al*., 1996; Boukary *et al*., 2013; McDermott *et al*., 2013). In sexually mature animals, *Brucellae* spread in different areas and especially in the reproductive system where they cause placentitis and metritis (Poester *et al*., 2013) which in turn results in abortions (Ul-Islam *et al*., 2013). Abortion usually happens once and during the second half of gestation (Lampel *et al*., 2012) but animals that aborted only once due to brucellosis may stay infected the rest of their lives (Godfroid *et al*., 2010). Other signs of brucellosis in livestock animals are also reproductive disorders and include stillbirths, weak calves, retained placenta and longer calving intervals (Acha *et al*., 2001). In male animals such as bulls, brucellosis symptoms are epididymitis, seminal vesiculitis, orchitis and testicular abscesses which may result in infertility (Parkinson & McGowan, 2019)

In addition to these rather reproduction related symptoms, brucellosis in animals exhibit other external clinical signs, mainly hygromas whichappear especially when brucellosis infection in the animal has progressed from an acute or sub-acute form to a chronic form and can occur on the jaw, bursa, thigh, flank, hip, shoulder, neck and leg joints (Musa *et al*., 1990). Hygromas were strongly associated with brucellosis in tropical countries and were commonly considered as a manifestation of brucellosis (Thienpont *et al*., 1961; OIE, 2009). In some animals, brucellosis infection may not exhibit any signs or symptoms (Food and Drug Administration, 2012). Actual testing remains therefore the best means of determining or confirming brucellosis in animals.

Whether brucellosis symptoms are exhibited, disappear or are completely absent, infected animals can remain carriers of *Brucella* and continue to shed the bacteria in fetus materials, vaginal discharges, milk and manure (Hamdy & Amin, 2002; Kaur *et al*., 2018; Langoni *et al*., 2000; Mugizi *et al*., 2015; Tekle *et al*., 2019). When the infection has become chronic, *Brucellae* also localize in the supra-mammary lymph nodes and mammary glands and can then be secreted into milk for the whole life of an infected lactating cow (Hamdy & Amin, 2002).

From an economical point of view, brucellosis infections in animals such as cattle and other livestock small ruminants result into tremendous losses (Ullah *et al*., 2014). Brucellosis affects productivity by causing abortions (meaning loss of offspring) which are associated with temporary or definitive infertility and reduced or total absence of milk production (Mangen *et al*., 2002; Corbel, 2006; Ul-Islam *et al*., 2013). In cases where animals are infected with brucellosis but do not abort or abort only once, there is still reduction in milk production which has been estimated to be up to 25% (Acha *et al*., 2003).

2.3.2.2. Brucellosis infection in humans

Human brucellosis is one of the most common zoonotic diseases. It is estimated that every year about 500,000 cases of human brucellosis are reported (Godfroid *et al*., 2013) and this may be an underestimation considering how brucellosis is underreported especially in the developing world (Corbel, 2006; Halliday *et al*., 2015; WHO, 2012). Humans contract brucellosis when *Brucella*'s animal hosts transmit the bacteria directly or indirectly to humans. Therefore, efforts to eradicate human brucellosis should focus on eradicating it in primary animal hosts and reservoirs.

The animal that are known to be involved in the transfer of *Brucallae* to humans are domestic animals mainly cattle, goats, sheep and pigs (Corbel, 2006; Godfroid *et al*., 2014). Humans are contaminated when their skin (especially skin with cuts), their digestive, conjunctival or nasopharyngeal mucous tissues/membrane come in contact with materials from infected animals such as abortion materials, fetuses, placental materials, vaginal discharges, urine and manure (Corbel, 2006; Pappas *et al*., 2006; Estradaa *et al*., 2016).

Brucellosis is an occupational disease and some occupational groups such as farm workers, veterinarians, butchers are therefore at high risk of getting brucellosis (Lytras *et al*., 2016; Pappas *et al*., 2006; Tabak *et al*., 2008; Tsegay *et al*., 2017). Just as farm workers and veterinarians, laboratory workers manipulating and culturing *Brucella* spp. are also at high risk of getting infected (Sam *et al*., 2012; Traxler *et al*., 2013). The other commonly way of transmission of brucellosis from infected animals to humans is through animal sourced foods and human brucellosis caused by the consumption of unpasteurized milk or milk products from unpasteurized milk from infected animals has been reported in many publications (Dadar *et al*., 2019). Transmission through the consumption of improperly cooked meat from infected animals may also occur (Casalinuovo *et al*., 2016).

Brucellosis is rarely transmitted from one person to the other. The few brucellosis cases of human-to-human transmission that have been reported include breast feeding from an infected mother to the infant (Carrera *et al*., 2006; Tikare *et al*., 2008), intra-uterine transmission from an infected mother to her infant (Tian *et al*., 2019), sexual activity between an infected and non-infected person (Vigeant *et al*., 1995) and blood transfusion from an infected person to a no-infected person (Wang *et al*., 2015).

Among *Brucella* species, 4 species from domesticated animals are known and reported to be responsible for most of infections in humans. They differ in their pathogenicity and, in a descending order of their pathogenicity, the 4 species are *B. melitensis, B. suis, B. abortus* and *B. canis* (Corbel, 2006; WHO, 2012; Moreno, 2014). The most virulent, *B. melitensis*, results in a rather acute infection in humans compared to other human infecting species which results in sub-acute and prolonged infections (Mantur *et al*., 2007). In addition to its highest virulence, *B. melitensis* is also responsible for most reported human brucellosis cases worldwide (Godfroid *et al., 2014; Pappas et al., 2006; Moreno, 2014)*.

Human brucellosis results in illness symptoms and the signs of brucellosis in humans appear within 3 weeks following the infection (Mantur *et al*., 2007). Patients with brucellosis experience symptoms which include intermittent fevers with body temperature varying between 37 °C in the morning and 40 °C in the afternoon, sweats with peculiar odor, chills, weakness, malaise, headache, insomnia, anorexia, joint and muscle pain, constipation, sexual impotence, nervousness and depression (Corbel, 2006; Pappas *et al*., 2006; Acha & Szyfres, 2003). In human females, brucellosis may also be accompanied by abortion and cases of abortion following infections with *Brucella* have been reported especially in the first and second trimesters of their pregnancy (Ali *et al*., 2016; Khan *et al*., 2001; Yang *et al*., 2018). In human males, brucellosis can lead to orchitis and epididymitis (Al-Tawfig, 2006; Gonzalez *et al*., 1997; Ron-Román *et al*., 2012).

Brucella infections in humans are often misdiagnosed and underreported due to the non-specific symptoms (McDermott & Arimi, 2002). Brucellosis symptoms can easily be confused with those of other diseases including enteric fever, malaria, rheumatic fever, fungal infections, tuberculosis and thrombophlebitis (Mantur *et al*., 2007). Brucellosis symptoms are also particularly confusing in areas where typhoid fever and malaria are common (Corbel, 2006; Halliday *et al*., 2015; WHO, 2012). Human brucellosis should be diagnosed and treated early. Delays in diagnosis and treatment during the first acute phase of the infection results in the transition of the infection into a localized and sub-acute or chronic brucellosis which is hard to treat (Roushan & Ebrahimpour, 2015; Young, 1995).

2.4. Brucellosis prevalence with reference to East African community countries

2.4.1. Brucellosis prevalence in Burundi

Burundi is the second smallest EAC country located at the West of the regional block (Figure 1) and sharing borders with Rwanda at the North, Tanzania at the East and South, Rwanda and Democratic republic of Congo at the West. Burundi has a total area of 27,834 square kilometres and a population estimated at 11.5 million (World Bank, 2019). Burundi's most popular livestock are small ruminants (Desiere et al., 2015) with 40 to 60 % of rural households owning goats and/or sheep compared to 10 to 20 % of the rural households owning cattle (Jeníček & Grofová, 2015). According to 2018 estimations by FAO, there were 3,249,827 goats, 1,110,936 cattle, 774,689 pigs and 548,608 sheep in Burundi (FAO Corporate Statistical Database, 2020).

Cattle rearing is especially observed in the Imbo plain at the most western part of the country and in the provinces of Bururi, Mwaro and Muramvya (Manirakiza et al., 2017; United States Agency for International Development, 2009). In Burundi, the traditional extensive agro-pastoral cattle production system is gradually being replaced by the mixed crops-livestock system in which cattle is kept in closed spaces and fed with cut and carried forage and crop residues. Around cities like Bujumbura where the demand in fresh milk is high, the intensive cattle production system is the most commonly practiced (Manirakiza et al., 2020).

Very few studies on brucellosis prevalence in Burundi exist in literature. Although the detection of the first cases of human brucellosis were reported in what used to be Rwanda-Urundi in 1930s (Pergher & Noel, 1936), a few further studies on brucellosis in Burundi were reported, one in the 1960s and the other in the 1980s (Merker & Schlichting, 1984; Thienpont *et a*l., 1961). Recently and in the last decade, one study on brucellosis prevalence in Burundi was found in searched literature. The study, which was conducted on dairy herds in West and Central Africa by testing farm bulk milks with milk Enzyme Linked Immuno-Sorbent Assay, reported a sero-prevalence of 14.7 % (95 % CI: 9.4 – 20.8) among dairy cattle herds in peri-urban Bujumbura (Musallam *et al.*, 2019).

For the purpose of this review, recent (2010 to 2019) brucellosis studies on other livestock and on humans in Burundi were not found or were not reported in searched literature.

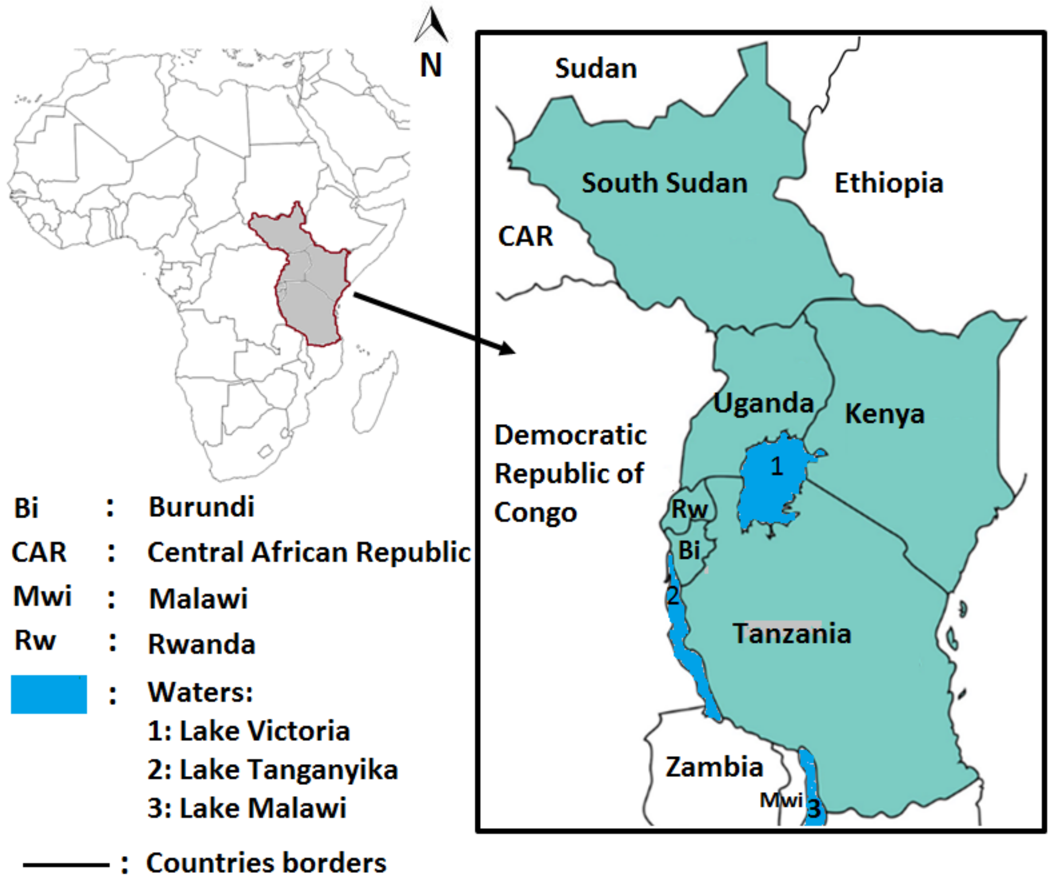

Figure 1: Map showing the location of the six EAC member countries (in grey)

2.4.2. Brucellosis prevalence in Kenya

Kenya is the biggest economy in EAC. Kenya is located at the East of the regional block (Figure 1) and sharing borders with Ethiopia and South Sudan at the North, Somalia at the East, and Tanzania at the South and Uganda at the West. Kenya is 580,370 square kilometres and has a population of 47.6 million (Kenya National Bureau of Statistics, 2019). Kenya's livestock census of 2009 indicated that cattle population was estimated at 17,467,774 heads and goats were 27,740,153 heads while the sheep population was 17,129,606 (Kenya National Bureau of Statistics, 2019). According to the same census, high populations of livestock were observed in the Rift Valley region.

In Kenya, livestock is raised under different livestock production systems including intensive, semi-intensive and extensive production systems. Dairy cattle, which are an important part of livestock in Kenya, are raised under different production systems. It was estimated that 45 % of dairy cattle farms practice the semi-intensive/semi-grazing system while 35 % of the farms practice the intensive system but on a small scale. The rest of dairy cattle farms practice controlled extensive system (10 % of farms), uncontrolled extensive system (5 % of farms) and intensive system on a large scale (5 % of farms) (FAO, 2018). Livestock, in general, depends on rangelands resources which support 70 % of the livestock in the country (Kenya Ministry of Agriculture, Livestock, 2019).

Brucellosis has been studied in Kenya for a long time as shown by a recent review on the disease frequency in humans and animals and risk factors for human infection from studies conducted as early as 1916 (Njeru *et al*., 2016). In the last decade (2010-2019), a number of studies have also reported on brucellosis prevalence in Kenya (Table 2). Using a variety of diagnostic tests most of which were serological (Table 2), prevalence in cattle varied between 0.2 % and 21.9 % (Chota *et al*., 2016; Enström *et al*., 2017; Fèvre *et al*., 2017; Kairu-wanyoike *et al*., 2019; Kosgei *et al*., 2014; Gicheru *et al*., 2015; Nakkel *et al*.,2016; Okumu *et al*., 2019; Osoro *et al*., 2015). For the small ruminants, brucellosis prevalence ranged from 0.0 to 20.0 % in goats and 0.0 to 13.8 % in sheep (Chota *et al*., 2016; Kairu-wanyoike *et al*., 2019; Kosgei *et al*., 2014; Nakkel *et al*., 2016).

Human brucellosis was studied especially for small livestock keeping communities and for patients attending hospitals with brucellosis symptoms and the reported prevalence ranged from 0.6 % to 35.8 % in humans (Chota *et al*., 2016; de Glanville *et al*., 2017; Fèvre *et al*., 2017; Kairu-wanyoike *et al*., 2019; Maiyo & Obey, 2016; Nakkel *et al*., 2016; Njeru *et al*., 2016; Osoro *et al*., 2015). However, a higher county level prevalence of 46.5 % among humans was reported in the county of Marsabit in a study conducted for a group of three counties (including Marsabit) to determine sero-prevalence and risk factors for brucellosis among humans and their livestock (Osoro et al., 2015).

Of the two recent studies which covered brucellosis in camels, one reported a prevalence of 11.1 % in camels in Marsabit county (Osoro *et al*., 2015) while the other reported a prevalence of 0 % in 5 camels tested in West Pokot county (Chota *et al*., 2016).

In the last decade, a few studies also covered or extended their investigations on the prevalence of *Brucella* antibodies in milk in Kenya and one recent study, which investigated the incidence and knowledge of brucellosis in Kahuro district, Murang’a county, used the Milk Ring Test

(MRT) and reported that 22 % of the analyzed 150 pooled milk samples were positive to brucellosis (Njuguna *et al*., 2017). In the same study, 230 individual farm bulk milks were collected from farmers and analyzed and 24 % were positive to brucellosis. Such prevalence in raw milks, although not directly indicating the individual animal prevalence in cattle, are an indication of brucellosis prevalence in cattle in Kenya and the risk of transmission to consumers when milk is not pasteurized prior to using.

Table 2: Studies on livestock and human brucellosis prevalence in Kenya in the last decade (2010-2019) (a)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Cattle</u>				
Subnational	356	CFT	0.9-4.6[a]	(Chota et al., 2016)
Subnational	225	i-ELISA	12.4 (7.7–15.4)	(Enström et al., 2017)
Subnational	983	Rapid immuno-chromatographic flow assay	0.2 (0.0-0.5)[c]	(Fèvre et al., 2017)
Subnational	441	c-ELISA	6.3 (4.0–8.6)	(Kairu-wanyoike et al., 2019)
Subnational	149	RBT	10.7[a]	(Kosgei et al., 2014)
Subnational	208	MRT	7.7[a]	(Gicheru et al., 2015)
Subnational	250	RBT & c-ELISA	21.9[a]	(Nakkel et al., 2016)
Subnational	398	c-ELISA	16.8 (13.2–20.4)	(Okumu et al., 2019)
Subnational	2,978	i-ELISA	4.1 (3.4–4.8)	(Osoro et al., 2015)

Table 3: Studies on livestock and human brucellosis prevalence in Kenya in the last decade (2010-2019) (b)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Goats</u>				
Subnational	123	CFT	0.0-20.0[a]	(Chota et al., 2016)
Subnational	961	c-ELISA	3.3 (2.1–4.4)	(Kairu-wanyoike et al., 2019)
Subnational	92	RBT	13.0[a]	(Kosgei et al., 2014)
Subnational	167	RBT & c-ELISA	7.3[a]	(Nakkel et al., 2016)
Subnational	4,080	c-ELISA	10.7 (9.3–12.3)	(Osoro et al., 2015)
<u>Sheep</u>				
Subnational	30	CFT	0.0 to 13.8[a]	(Chota et al., 2016)
Subnational	623	c-ELISA	1.4 (0.5– 2.3)	(Kairu-wanyoike et al., 2019)
Subnational	73	RBT	8.2[a]	(Kosgei et al., 2014)
Subnational	167	RBPT & c-ELISA	8.6[a]	(Nakkel et al., 2016)
Subnational	3,088	c-ELISA	7.3 (6.1–8.8)	(Osoro et al., 2015)

Table 4: Studies on livestock and human brucellosis prevalence in Kenya in the last decade (2010-2019) (C)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Humans</u>				
Subnational	562	[b]	17.1[a]	(Chota et al., 2016)
Subnational	2,113	Rapid immuno-chromatographic flow assay	0.6 (0.2-0.9)	(Fèvre et al., 2017)
Subnational	1,022	IgG-ELISA	35.8 (32.8–38.8)	(Kairu-wanyoike et al., 2019)
Subnational	317	RBT & c-ELISA	1.3[a]	(Nakkel et al., 2016)
Subnational	2,811	IgG-ELISA	16.4 (13.5–19.6)	(Osoro et al., 2015)
Subnational	825	Rapid immuno chromatography flow assay	3.4[a]	(de Glanville et al., 2017)
Subnational	1,043	[b]	32.3[a]	(Maiyo & Obey, 2016)
Subnational	1,067	RBT, IgM/IgG-ELISA & q-PCR	13.7 (11.7–15.9)	(John Njeru et al., 2016)

[a]: Prevalence confidence interval not provided in the original article
[b]: Diagnostic test not specified (Study based on health center results' records)
[c]: The computed confidence interval value was < 0.0
CFT: Complement Fixation Test; i-ELISA: Indirect Enzyme-Linked Immuno-Sorbent Assay; c-ELISA: Competitive Enzyme-Linked Immuno-Sorbent Assay; RBT: Rose Bengal Test; MRT: Milk Ring Test; IgG-ELISA: Immunoglobulin G Enzyme-Linked Immuno-Sorbent Assay; IgG/IgM-ELISA: Immunoglobulins G and M Enzyme-Linked Immuno-Sorbent Assay; q-PCR: Quantitative Polymerase Chain Reaction

2.4.3. Brucellosis prevalence in Rwanda

Rwanda is the smallest country in EAC located at the West of the regional block (Figure 1) and sharing borders with Uganda at the North, Tanzania at the East, Burundi at the South and Democratic Republic of Congo at the West. Rwanda has a total area of 26,338 square kilometers and the total population in the country is estimated at 12.0 million (National Institute of Statstics of Rwanda, 2019). Rwanda's important livestock are cattle, goats, sheep and pigs and the reported numbers of different livestock showed that the country had a total of 1,856,490 cattle, 2,283,445 goats, 499,316 sheep and 703,145 pigs. The Eastern province had the highest number of cattle (28.3 %) followed closely by the southern province with 27.3 % of cattle (National insititute of statistics of Rwanda, 2018). In Rwanda, cattle is raised mainly under the small scale zero grazing system which is practiced by 80 % of cattle keeping households. Semi-intensive and extensive/open grazing systems are practiced by 3 % and 17 % of the cattle keeping households, respectively (Land O' lakes, 2014).

Brucellosis studies have been conducted in Rwanda, although few and scattered over the years (Akayezu, 1984; Chatikoba et al., 2008; Gafirita et al., 2017; Manishimwe *et al*., 2015; Ndazigaruye *et al*., 2018; Rujeni & Mbanzamihigo, 2014; Thienpont *et al*., 1961). In the last decade (2010-2019), a total of four studies were published on animal and human brucellosis in the country (Table 3). For animal brucellosis, a study published in 2015 (Manishimwe *et al*., 2015) was focusing on comparing Rose Bengal Test (RBT) to competitive Enzyme Linked Immuno-Sorbent Assay (c-ELISA) in detecting *Brucella* antibodies in cattle serum. The study was conducted on a total of 2017 sera previously collected from 157 cattle farms in Kigali and reported a bovine brucellosis prevalence of 2.0 % using RBT and 1.7 % using c-ELISA. A second study, published in 2018, was conducted in Nyagatare district with the aim of analyzing the risk factors associated with brucellosis in cattle in the district (Ndazigaruye *et al*., 2018). The overall reported bovine brucellosis prevalence in Nyagatare district was 18.9 %. With these studies, brucellosis prevalence in the last decade varied between 1.7 % and 18.9 % among cattle in Rwanda.

No studies on brucellosis in small ruminants were found in searched literature for Rwanda and in the last decade.

Reported human brucellosis in Rwanda in the last decade varied between 6.1 to 25 % according to two studies (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014). In the first study conducted on women presenting with abortion or stillbirth of unknown origin at Huye district hospital, a prevalence of 25 % among those women was reported (Rujeni & Mbanzamihigo, 2014). The second study covered patients attending Nyagatare district hospital, willing to participate in the study and having any of the following symptoms: intermittent or persistent fever, headache, weakness, profuse sweating, chills, arthralgia, weight loss, and joint pain. The study reported a prevalence of 6.1 % (Gafirita *et al*., 2017).

Table 5: Studies on livestock and human brucellosis prevalence in Rwanda in the last decade (2010-2019)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Cattle</u>				
Subnational	2017	RBT	2.0[a]	(Manishimwe et al., 2015)
		c-ELISA	1.7[a]	
Subnational	604	RBT	18.9[a]	(Ndazigaruye et al., 2018)
<u>Humans</u>				
Subnational	198	RBT	6.1 (0.6–7.8)	(Gafirita et al., 2017)
Subnational	60	RBT	25[a]	(Rujeni & Mbanzamihigo, 2014)

[a]: Prevalence confidence interval not provided in the original article

RBT: Rose Bengal Test; c-ELISA: Competitive Enzyme-Linked Immuno-Sorbent Assay

2.4.4. Brucellosis prevalence in South Sudan

South Sudan is the newest EAC member country located at the north of the regional block (Figure 1) and bordered by Sudan at the North, Ethiopia at the East, Kenya, Uganda and Democratic Republic of Congo at the South and Central African Republic at the West. South

Sudan has an area of 644,329 square kilometres. South Sudan population was estimated at 12.2 million (United Nations Economic Commission for Africa, 2013).

Livestock, including cattle, goats and sheep, is a very important agricultural subsector in South Sudan where the livestock per capita holding is considered among the first in Africa. Cattle, goats, sheep, camel and pig populations were estimated at 17,729,188; 12,307,686; 11,682,172; 23,583 and 14,406 respectively (Onyango *et al*., 2015). Livestock is distributed all over South Sudan but more livestock populations are observed in the upper half of the country in the states of Northern Bhar El Ghazal, Warrap, Jonglei, Lakes, Western Bhar El Ghazal and Unit (Onyango *et al*., 2015). In South Sudan, livestock is reared under agro-pastoralism and pastoralism mainly with 85 % of South Sudanese households involved in livestock being agro-pastoralists and the remaining 15 % being pastoralists (Emmanuel et al., 2018; FAO, 2016).

As is the case for Burundi and Rwanda, studies on brucellosis in South Sudan are still few. Among the few studies undertaken and published in the period of 2010 to 2019 (Table 4), was a cross-sectional study conducted in peri-urban Juba and in Terekeka county on bovine brucellosis (Lita *et al*., 2016). In this study, an overall individual animal sero-prevalence of 31.9 % by RBT and 29.3 % by c-ELISA was reported. A series of three brucellosis sero-prevalence studies was also conducted by Madut and colleagues on febrile patients attending hospital, on cattle and their herders and on slaughter house workers in the region of Bahr el Ghazal (Madut *et al*., 2018; Madut *et al*., 2019, 2018). The prevalence among 416 febrile patients attending Wau hospital in Bahr el Ghazal region was 23.3 % after tests of blood samples by RBT and Serum Agglutination and confirmation of results by c-ELISA (Madut *et al*., 2018). Of the 893 bovine sera and 87 herders' sera tested using RBT and confirmation by c-ELISA, the reported overall prevalence was 31.0 % among cattle and 33.3 % among cattle herders (Madut *et al*., 2018).

In the same region of Bahr el Ghazal, a total 234 slaughterhouse workers were screened for brucellosis infection using RBT and the overall prevalence was 32.1 % following c-ELISA confirmation of results (Madut *et al*., 2019). With these few studies in the last decade, reported brucellosis prevalence in South Sudan varied from 29.3 to 31.9 % among cattle and from 23.3 to 33.3 % among humans.

Table 6: Studies on livestock and human brucellosis prevalence in South Sudan in the last decade (2010-2019)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Cattle</u>				
Subnational	160	RBT	31.9[a]	(Lita et al., 2016)
	147	c-ELISA	29.3[a]	
Subnational	893	RBT & c-ELISA	31.0 (28.0–34.2)	(Madut et al., 2018)
<u>Humans</u>				
Subnational	416	RBT, SAT & c-ELISA	23.3[a]	(Madut et al., 2018)
Subnational	87	RBT & c-ELISA	33.3 (23.9–44.3)	(Madut et al., 2018)
Subnational	234	RBPT & c-ELISA	32.1[a]	(Madut et al., 2019)

[a]: Prevalence confidence interval not provided in the original article

RBT: Rose Bengal Test; c-ELISA: Competitive Enzyme-Linked Immuno-Sorbent Assay; SAT: Serum Agglutination Test

2.4.5. Brucellosis prevalence in Tanzania

Tanzania is an EAC country located at the South of the regional block (Figure 1) and sharing borders with Uganda at the North, Kenya at the North-East, Mozambique at the South, Malawi at the South-West, Zambia and Democratic Republic of Congo at the West and Burundi and Rwanda at the North-West. According to Tanzania National Bureau of Statistics, Tanzania has a total area of 947,300 square kilometres and a population of 54.2 million (Tanzania National Bureau of Statistics, 2019). Tanzania's most important livestock is cattle at an estimated population of 30,672,001 heads followed by goats estimated at 19,055,651 heads and sheep estimated at 5,565,986 heads (Tanzania National Bureau of Statistics, 2017). In Tanzania, livestock is especially raised in Shinyanga, Mwanza and Tabora regions (Engida *et al.*, 2015).

Many brucellosis studies have been conducted in Tanzania and recent brucellosis studies in cattle in Tanzania used serological diagnostic tests, mostly the RBT to screen samples and c-ELISA to confirm the results (Table 5). They reported an individual cattle brucellosis prevalence varying mostly between 0.2 % and 11.7 % (Asakura *et al*., 2018; Assenga *et al*., 2015; Chitupila *et al*., 2015; Chota *et al*., 2016; Kayombo *et al*., 2017; Mathew, 2017; Shirima & John, 2016; Sagamiko *et al*., 2018; Shirima *et al*., 2010; Swai & Schoonman, 2012; Swai & Schoonman, 2010).

However, in one study which was conducted on animals from a single farm with a total of 350 cattle, a higher individual cattle brucellosis prevalence of 48 % was reported (Mathew *et al*., 2015). The dairy herd from which the high individual cattle brucellosis was reported is located in the southern highlands of Tanzania and had been experiencing abortions (Mathew *et al*., 2015). Another investigative study following an abortion storm on a research farm reported an individual cattle brucellosis prevalence of 28.9 % (Shirima *et al*., 2014). Brucellosis in this farm was eventually controlled through culling, among other measures, and brought to 0.0 % over a period of 5 years.

Human brucellosis studies in Tanzania included mostly hospital patients with symptoms, like fever and spontaneous abortions, and communities in pastoral and agro-pastoral areas. The reported human brucellosis prevalence in Tanzania from recent studies (2010-2019) varied mostly between 0 % and 28.2 % (Assenga *et al*., 2015; Bouley *et al*., 2012; Carugati *et al*., 2018; Cash-goldwasser *et al*., 2017; Chipwaza *et al*., 2015; Chota *et al*., 2016; Crump *et al*., 2013; Mngumi *et al*., 2016; Nonga & Mwakapeje, 2017; Shirima & John, 2016; Shirima *et al*., 2010).

It should, however, be mentioned that a prevalence as high as 58.1 % was reported from a study which investigated the association of *Brucella* seropositivity to abortion for a group of 148 women with spontaneous abortions and 250 women with full-term deliveries. The group of women with spontaneous abortions had a prevalence of 58.1 % while the group with full-term deliveries had a prevalence of 26 % (Mujuni *et al*., 2018). A different but also focused study on 250 abattoir workers and meat vendors in the city of Mwanza reported that 48.4 % of them were *Brucella*-seropositive (Mirambo *et al*., 2018).

Fewer studies were conducted on small ruminants in Tanzania in the last decade. Brucellosis prevalence in goats was between 0 % and 2.0 % while brucellosis prevalence in sheep was between 0 % and 5.7 % (Assenga *et al*., 2015; Mathew *et al*., 2015; Shirima & John, 2016). Apart from common livestock animals, a few recently published studies (2010-2019) in Tanzania covered other animals. A study on the epidemiology of *Brucella* infection in human, livestock and wildlife interface in the Katavi-Rukwa ecosystem included 38 buffaloes, 2 lions and 2 zebras and reported a brucellosis prevalence of 7.9 % in buffaloes while 1 out of the 2 lions was seropositive and none of the zebras was (Assenga *et al*., 2015). In a study to investigate a farm which had been experiencing cattle abortions, 6 dogs were included and none of the dogs tested positive to brucellosis (Mathew *et al*., 2015). Dogs were also covered in another study with a sample of 100 dogs randomly selected in the region of Morogoro and no dog was positive to *Brucella canis* (Muhairwa *et al*., 2012). A study conducted on camels in agro-pastoral communities of Northern Tanzania reported a prevalence of 2.1 % at an animal level for a sample of 193 camels selected from 14 traditional herds (Swai *et al*., 2011). A low prevalence of 0.7 % was reported in pigs from 5 selected pig slaughter facilities in Dar-Es-Salam (Simon *et al*., 2015).

A few recent studies (published from 2010 to 2019) covered also the prevalence of *Brucella* antibodies in raw marketed milk in Tanzania. One such study which sought to evaluate the microbiological quality and associated health risks for raw milk marketed in Tanga region reported that 56 % of 59 raw milk samples collected from selling points and deliverers were *Brucella* positive (Swai & Schoonman, 2011). The reported high milk contamination may be due to milk bulking and pooling from different cattle and farms. It presents a risk for transmission of brucellosis to consumers and calls for milk boiling or pasteurization prior to using. Another study which, in addition to animal sera, investigated the milk from dairy animals reported a prevalence of *Brucella* in 3.7 % and 0 % of cattle milk and goat milk respectively (Assenga *et al*., 2015). Herd-level brucellosis prevalence was, also, investigated in a study using bulk farm milk. The prevalence of *Brucella* in milk at herd level was 44.4 % out of 124 agro-pastoral farms investigated in Morogoro region (Asakura *et al*., 2018).

In addition to determining brucellosis prevalence in animals and humans, a few studies in Tanzania identified and characterized some prevalent *Brucella* species. In their investigative study on a single farm, Mathew and colleagues (Mathew *et al*., 2015) cultured, identified and characterized *Brucella* isolates from cattle to be *Brucella abortus* biovar 3. In another study published about the same time, molecular methods were used and *Brucella abortus* biovar 1 was identified and characterized from cattle milk (Assenga *et al*., 2015).

Table 7: Studies on livestock and human brucellosis prevalence in Tanzania in the last decade (2010-2019) (a)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
Cattle				
Subnational	667	RBT & c-ELISA	0.2 (0.0-1.1)[d]	(Asakura et al., 2018a)
	673	RBT & c-ELISA	7.0 (5.7-8.4)	
Subnational	1,103	RBT & c-ELISA	6.8 (5.4-8.5)	(Assenga et al., 2015)
Subnational	410	RBT & c-ELISA	5.6 (3.8-8.3)	(Chitupila et al., 2015)
Subnational	1,376	RBT & c-ELISA	1.0-11.4[a]	(Chota et al., 2016)
Subnational	192	RBT & c-ELISA	4.2[a]	(Kayombo et al., 2017)
Subnational	658	i-ELISA	5.4[a]	(Mathew, 2017)
Subnational	200	RBT	21.5 (16–27)	(Mathew et al., 2015)
		i-ELISA	48.0 (41–55)	
Subnational	296	RBT & c-ELISA	7.8[a]	(Shirima & John, 2016)
Subnational	929	RBT & c-ELISA	2.8 (1.4-5.6)	(Sagamiko et al., 2018)
	282	RBT & c-ELISA	11.3 (9.4-13.5)	
Subnational	2,723	c-ELISA	4.9[a]	(Shirima et al., 2010)
Subnational	51	RBT	11.7 (9.1-14.9)	(Swai & Schoonman, 2012)
Subnational	655	RBT	5.3 (3.1-7.8)	(Swai & Schoonman, 2010)
Subnational	483	RBT & c-ELISA	28.9[a]	(Shirima et al., 2014)

Table 8: Studies on livestock and human brucellosis prevalence in Tanzania in the last decade (2010-2019) (b)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
Goats				
Subnational	248	RBT & c-ELISA	1.6 (0.4-4.1)	(Assenga et al., 2015)
Subnational	50 goats	RBT	0	(Mathew et al., 2015)
		i-ELISA	2.0 (0.0-7.0)[d]	
Subnational	75	RBT & c-ELISA	0	(Shirima & John, 2016)
Subnational	[c]	RBT & c-ELISA	0	(Shirima et al., 2014)
Sheep				
Subnational	35	RBT	0	(Mathew et al., 2015)
		i-ELISA	5.7 (0.0–17.0)[d]	
Subnational	42	RBT & c-ELISA	0	(Shirima & John, 2016)

Table 9: Studies on livestock and human brucellosis prevalence in Tanzania in the last decade (2010-2019) (c)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Humans</u>				
Subnational	340	RBT, BAPA & Riv. T	0.6 (0.1-2.1)	(Assenga et al., 2015)
Subnational	455	MAT & Blood culture	3.5[a]	(Bouley et al., 2012)
Subnational	1095	MAT & Blood culture	2.9[a]	(Carugati et al., 2018)
Subnational	562	MAT	6.9[a]	(Cash-Goldwasser et al., 2017)
Subnational	370	IgM-ELISA , IgG-ELISA & MAT	7.0[a]	(Chipwaza et al., 2015)
Subnational	578	[b]	28.2[a]	(Chota et al., 2016)
Subnational	118	MAT	13.6[a]	(Crump et al., 2013)
Subnational	250	SAT	48.4 (42–54)	(Mirambo et al., 2018)
Subnational	382	Rapid *Brucella* serum agglutination	14.1 (10.6–17.5)	(Mngumi et al., 2016)
Subnational	148	SAT	58.1 (50–66)	(Mujuni et al., 2018)
Subnational	13,642	[b]	5.8[a]	(Nonga & Mwakapeje, 2017)
Subnational	82	RBT & c-ELISA	0	(Shirima & John, 2016)
Subnational	120	c-ELISA	10.0[a]	(Shirima et al., 2014)
Subnational	460	c-ELISA	8.3[a]	(Shirima et al., 2010)

[a]: Prevalence confidence interval not provided in the original article

[b]: Diagnostic test not specified (Study based on health center results' records)

[c]: Sample size not specified

[d]: The computed confidence interval value was < 0.0

RBT: Rose Bengal Test; c-ELISA: Competitive Enzyme-Linked Immuno-Sorbent Assay; i-ELISA: Indirect Enzyme-Linked Immuno-Sorbent Assay; BAPA: Buffered Acidified Plate

Antigen Test; Riv. T: Rivanol precipitation Test; MAT: Microscopic Agglutination Test; IgG-ELISA: Immunoglobulin G Enzyme-Linked Immuno-Sorbent Assay; IgM-ELISA: Immunoglobulin M Enzyme-Linked Immuno-Sorbent Assay; SAT: Slide Agglutination Test.

2.4.6. Brucellosis prevalence in Uganda

Uganda is an EAC country at the Central-West of the regional block (Figure 1). Uganda shares borders with South Sudan at the North, Kenya at the East, Tanzania at the South, Rwanda at the South-West and Democratic Republic of Congo at the West. As indicated by Uganda Bureau of Statistics, Uganda is 241,550.7 square kilometres in total area and has a human population of 41.0 million (Uganda Bureau Of Statistics, 2018). Uganda's important livestock are cattle, goats, sheep, pigs and poultry. There were 14,189,000 cattle, 16,034,000 goats, 4,445,000 sheep and 4,109,000 pigs in 2017 (Uganda Bureau Of Statistics, 2018). The most important livestock in Uganda is cattle. In Uganda, cattle is especially found in the "cattle corridor" which extends from the northeast of the country, with the highest concentration of cattle, to the southwest (Egeru et al., 2014). Cattle in Uganda is raised under commercial ranching, pastoral, agro-pastoral and semi-intensive production systems, the agro-pastoral system being the most common one (FAO, 2019).

In a recent report, FAO estimated brucellosis prevalence in Uganda at a national level to be 10 % in cattle and 5.5 % in cattle keepers (FAO, 2018). In addition to FAO's national estimations many other studies on brucellosis have been conducted in Uganda. According to most recent cattle brucellosis studies published from 2010 to 2019 (Table 6), brucellosis was diagnosed using mostly serological tests (Table 6). The reported brucellosis prevalence in cattle (animal level) varied a lot and was between 1.2 % and 43.8 % (Bugeza *et al*., 2019; Ezama *et al*., 2019; Kabi *et al*., 2015; Kashiwazaki *et al*., 2012; Lolli *et al*., 2016; Makita *et al*., 2011; Miller *et al*., 2016; Mugizi *et al*., 2015; Nanfuka, 2018; Nguna *et al*., 2019; Nina *et al*., 2017; Nizeyimana *et al*., 2013).

Small ruminants were also covered in recent brucellosis studies in Uganda and the reported brucellosis prevalence in goats was lower compared to cattle and varied between 0.3 % and 9.8 % (Dubad *et al*., 2015; Lolli *et al*., 2016; Miller *et al*., 2016; Nguna *et al*., 2019). The reported prevalence in sheep varied between 2.6 % and 10.5 % (Dubad *et al*., 2015; Lolli *et al*., 2016).

Among recent studies, one study investigated brucellosis in pigs from 3 districts in Uganda where pig keeping is commonly practiced. The reported prevalence in the districts varied from 0.0 to 0.2 % (Erume *et al*., 2016).

Human brucellosis was also reported in recent brucellosis studies in Uganda published from 2010 to 2019. The reported human brucellosis prevalence varied between 4.0 and 33.0 % (Ezama *et al*., 2019; Ezama *et al*., 2018; Frank *et al*., 2017; Kansiime *et al*., 2015; Majalija *et al*., 2018; Migisha *et al*., 2018; Miller *et a*l., 2016; Muloki *et al*., 2018; Nabukenya *et al*., 2013; Nanfuka, 2018; Nasinyama *et al*., 2014; Nguna *et al*., 2019; Tumwine *et al*., 2015). These reported human brucellosis results should, however, be interpreted considering that most of them were conducted on suspected patients attending hospitals with febrile illness or having prolonged fevers and on exposed cattle keepers and farm attendants in pastoralist and agro-pastoralist communities. Human brucellosis studies used serological diagnosis tests with only one study adding blood culturing to confirm the presence of *Brucella* spp. (Migisha *et al*., 2018).

A few recent studies in Uganda were also conducted to determine the prevalence of *Brucella* antibodies in cattle milk. Raw milk samples collected from dairy farms, milk shops, street vendors, milk deliverers, boiling points, dairies and milk collection centers were tested using serological diagnosis tests and the prevalence varied between 6.5 % and 40 % (Hoffman *et al*., 2016; Kamwine *et al*., 2017; Makita *et al*., 2010; Rock *et al*., 2016). While these reported results on prevalence of *Brucella* in milk are from bulk and pooled raw milks and cannot be related to individual cattle prevalence, they are still an indication of the presence of brucellosis in cattle.

Table 10: Studies on livestock and human brucellosis prevalence in Uganda in the last decade (2010-2019) (a)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Cattle</u>				
Subnational	728	RBT & i-ELISA	3.2 (1.9-4.5)	(Bugeza et al., 2019)
Subnational	839	RBT	34.7[a]	(Ezama et al., 2019)
National	925	i-ELISA & c-ELISA	8.64[a]	(Kabi et al., 2015)
Subnational	1,237	RBT & i-ELISA	21.5[a]	(Kashiwazaki et al., 2012)
	1,033	RBT & i-ELISA	3.4[a]	
Subnational	3,935	RBT	9.8 (8.9-10.7)	(Lolli et al., 2016)
Subnational	423	c-ELISA	5.0 (2.7-9.3)	(Makita et al., 2011)
Subnational	768	RBT (*Abortus* antigen)	12.9 (10.0-16.2)	(Miller et al., 2016)
		RBT (*Melitensis* antigen)	15.7 (12.4-19.3)	
Subnational	1,007	i-ELISA & c-ELISA	7.5 (6.1-9.4)	(Mugizi, Boqvist, et al., 2015)
Subnational	1,503	SAT & i-ELISA	23.0[a]	(Nanfuka, 2018)
Subnational	345	i-ELISA	1.2[a]	(Nguna et al., 2019)
Subnational	1,749	RBT & i-ELISA	43.8[a]	(Nina et al., 2017)
Subnational	149	i-ELISA	3.3[a]	(Nizeyimana et al., 2013)

Table 11: Studies on livestock and human brucellosis prevalence in Uganda in the last decade (2010-2019) (b)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
Goats				
Subnational	305	RBT	8.8[a]	(Dubad et al., 2015)
Subnational	729	RBT	8.8 (6.9-11.1)	(Lolli et al., 2016)
Subnational	315	RBT (*Abortus* antigen)	1.1 (0-6.0)[d]	(Miller et al., 2016)
		RBT (*Melitensis* antigen)	9.8 (3.8-15.7)	
Subnational	351	i-ELISA	0.3[a]	(Nguna et al., 2019)
Sheep				
Subnational	95	RBT	10.5[a]	(Dubad et al., 2015)
Subnational	306	RBT	2.6 (1.2-5.3)	(Lolli et al., 2016)

Table 12: Studies on livestock and human brucellosis prevalence in Uganda in the last decade (2010-2019) (c)

Species and study scope	Sample size	Diagnostic test	Prevalence in % (95 % Confidence Interval)	Reference
<u>Humans</u>				
Subnational	216	RBT	33 (27-39)	(Ezama et al., 2019)
Subnational	216	RBT & IgM-ELISA	13.4[a]	(Ezama et al., 2018)
Subnational	177	Rapid agglutination test	10.7[a]	(Frank et al., 2017)
Subnational	9,177	PAT	14.4[a]	(Kansiime et al., 2015)
Subnational	200	SAT and TAT	7.5[a]	(Majalija et al., 2018)
Subnational	235	RBT Blood culture	14.9 (10.6-20.1) 4.3[a]	(Migisha et al., 2018)
Subnational	236	IgG/IgM-LFA	8.1 (3.2-13.0)	(Miller et al., 2016)
Subnational	251	PAT	18.7[a]	(Muloki et al., 2018)
Subnational	232	MAT & TAT	10 (6-16)	(Nabukenya et al., 2013)
Subnational	113	i-ELISA	4-12[a]	(Nanfuka, 2018)
Subnational	161	Rapid Agglutination Test, TAT & c-ELISA	5.8 (3.3-8.3)	(Nasinyama et al., 2014)
	168	Rapid Agglutination Test, TAT & c-ELISA	9.0 (4.7-13.3)	
Subnational	451	i-ELISA	4.4[a]	(Nguna et al., 2019)
Subnational	235	SAT & RBT	17.0[a]	(Tumwine et al., 2015)

[a]: Prevalence confidence interval not provided in the original article

[b]: Diagnostic test not specified (Study based on health center results' records)

[c]: Sample size not specified

[d]: The computed confidence interval value was < 0.0 RBT: Rose Bengal Test; i-ELISA: Indirect Enzyme-Linked Immuno-Sorbent Assay; c-ELISA: Competitive Enzyme-Linked Immuno-Sorbent Assay; SAT: Serum Agglutination Test; IgM-ELISA: Immunoglobulin M Enzyme-Linked Immuno-Sorbent Assay; TAT: Tube Agglutination Test; IgG/IgM-LFA: Immunoglobulins G and M lateral flow assay; PAT: Plate Agglutination Test; MAT: Microplate Agglutination Test

2.4.7. Critique of brucellosis in East Africa

Brucellosis is considered endemic across the African continent (Franc *et al*., 2018). This review, which focused on recent literature (2010-2019), indicates that brucellosis is prevalent in EAC with prevalence ranges that are quite variable within individual EAC countries and between countries. The variation in brucellosis prevalence in EAC should, however, be looked at considering that the retained and reviewed studies used different serological diagnostic tests and different sampling techniques from populations of various sizes. The practiced livestock production systems were also different, from the traditional free open grazing system through mixed agro-pastoral system to zero grazing system. The cattle brucellosis prevalence range of 0.2 % to 43.8 % observed over EAC is comparable but higher than the prevalence in Sub-Saharan Africa (SSA). A review of brucellosis sero-prevalence studies published from 2003 to 2015 from 12 different SSA countries showed that brucellosis prevalence was between 1.0 % and 36.6 % among cattle raised under a variety of livestock production systems (Ducrotoy *et al*., 2015).

The cattle brucellosis prevalence range of 0. 2 % to 43.8 % is, also, comparable but higher than previous cattle brucellosis prevalences reported for specific EAC countries (McDermott & Arimi, 2002) or estimated for EAC as a region (International Livestock Research Institute, 2012). In their review on cattle brucellosis in SSA including specific EAC countries (Tanzania, Kenya, Burundi and Uganda), McDermott and Arimi reported a brucellosis prevalence range varying mostly from 1.8 % in Tanzania to 25.4 % in Burundi (McDermott & Arimi, 2002). Grace and colleagues also estimated a lower prevalence (8.2 %) among cattle in the East African region (International Livestock Research Institute, 2012). In the current review and across other reports (Ducrotoy *et al*., 2015; International Livestock Research Institute, 2012; McDermott & Arimi, 2002), higher cattle brucellosis prevalence is observed in cattle raised in larger pastoral and agro-pastoral herds and lower prevalence is observed in small holder farms.

The pastoral and agro-pastoral livestock production systems, in which cattle closely interact within herds and between different herds, are practiced by at least some communities or sub-regions in each of the EAC countries. In such livestock production systems, cattle share grazing areas, watering sources and bulls for natural breeding. Brucellosis is transmitted from animal to animal and has been reported to spread and stay in herds through contaminated grazing areas, contaminated water and through natural breeding by brucellosis positive bulls (Aparicio, 2013). In their efforts to control cattle brucellosis, EAC and EAC countries should, therefore, include and adapt brucellosis control strategies recommended for pastoral and agro-pastoral livestock production systems with high brucellosis prevalence.

Outside Africa, a number of developed countries including New Zealand, Sweden, Denmark, Norway, Finland, Germany, Switzerland, Canada, Japan, UK, Netherlands, Belgium, Luxembourg and Austria have controlled and eradicated brucellosis (Benkirane, 2006; Pappas *et al.*, 2006; Bosilkovski, 2015). Other developing countries in Central and South America, South and South-East Asia and Middle East are, like Africa, still facing bovine brucellosis although the reported prevalence is lower. In Central American countries of Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica and Panama, bovine brucellosis was estimated to be from 4 to 8 % and bovine brucellosis was 3 to 4 %, 2 to 2.5% and 0.04 to 0.28 % among cattle in Paraguay, individual Dairy cattle in Argentina and across federal Brazil (Lopes *et al*., 2010). Brucellosis estimates from South Asia and South East Asia regions were 16.0 % and 2.9 %, respectively (International Livestock Research Institute, 2012). Cattle individual sero-prevalence in Middle East was from 0.8 % to 12.2 % (Musallam *et al*., 2016).

As observed in the current literature review, studies on brucellosis in small ruminants are fewer compared to studies on cattle. Indeed, for three of the six covered EAC countries, brucellosis in small ruminants has not yet been studied or is yet to be reported. This is a similar situation across SSA where there is limited information on brucellosis studies on small ruminants (Ducrotoy et al., 2015). For the few reported studies on goats and sheep brucellosis in EAC, the prevalence range was 0 to 20.0 % among goats and 0 to 13.8 % among sheep. This EAC prevalence range is higher compared to the SSA prevalence which varied between 0 % and 4.8 % for sheep and between 0 and 5.5 % for goats (Ducrotoy *et al*., 2015).

Although studies on brucellosis in small ruminants are still few, there is a need to evaluate the significance of this infection among small ruminants in EAC and across Africa. Indeed goats and sheep are natural hosts and mainly infected by *Brucella melitensis* (Bruce, 1887) and among *Brucella* species, *B. melitensis* is the most virulent to humans resulting in a more acute infection (Mantur *et al*., 2007; Moreno, 2014). Outside Africa, brucellosis in small ruminants is still prevalent and remains a major problem in the Mediterranean region, the Middle East, Central Asia, South Asia and South-East (McDermott *et al*., 2013; Musallam *et al*., 2016).

The recent brucellosis studies in EAC indicate the prevalence of the infection among livestock and human in the region and call for plans in individual countries to control brucellosis. FAO has given general guidelines in controlling brucellosis, which can be very well adapted by the different individual EAC countries. In regions, like EAC, where brucellosis prevalence is higher than 10 %, FAO recommends a mass brucellosis vaccination until the prevalence is reduced to below 2 % (FAO, 2009). Once the prevalence is brought under control, further eradication strategies can, then, be considered. Prior to any brucellosis control strategy, epidemiological studies are needed to determine the prevalence taking into account the different regions within the same country or different countries within a same region (Blasco, 2010).

Most of the studies considered in the current review were targeting livestock and humans in identified suspected agro-pastoral communities within individual EAC countries. In designing their approach to control brucellosis, EAC countries could take advantage of such studies to determine the epidemiological units of intervention and design appropriate brucellosis control strategies. In designing any brucellosis control strategy, there are, however, important considerations for the strategy to be implemented successfully. In countries where brucellosis control in livestock was, for example, implemented and attained success, adequate veterinary infrastructure were in place or put in place, awareness campaigns conducted to concerned farmers and economic resources availed by government and government donors (Blasco, 2010; FAO, 2014).

It is also critical that the country's or region's political will to control brucellosis is demonstrated and maintained throughout the implementation of any brucellosis control strategy. In developed countries where brucellosis was controlled and eradicated, a strong political support and a legal framework to enforce control measures were important to the success of the control and eradication programs (FAO, 2014). With a brucellosis control strategy in place, the invested efforts and economic resources have to be maintained over the course of the strategy implementation which is usually long. In countries where brucellosis is high, FAO recommends to start by long-term mass vaccination to control the disease, vaccination taking up to ten years to sustainably bring brucellosis prevalence to low levels (FAO, 2014).

Once a brucellosis control strategy is designed and needed resources availed, the implementing country needs to also ensure that good farm management is practiced to enable the control strategy to be effective and successful. The control of livestock movement, screening of replacement livestock prior to their introduction to the farms, hygienic disposal of abortive materials are all good farm management practices that have been reported as additional elements to the success of any brucellosis control strategy (Avila-Granados *et al*., 2019; Bamaiyi *et al*., 2014; Perez-Sancho *et al*., 2015; Zamri-Saad & Kamarudin, 2016).

2.5. Risk factors of milk contamination with *Brucella*

The natural hosts for *Brucella spp* are animal hosts and the humans are accidental hosts (Mukhtar, 2010). When animal hosts such as cows are infected, *Brucella* are shed into milk and abortive products (Mukhtar, 2010). Indeed, in infected animals, *Brucellae* localize in the supra-mammary lymph nodes and mammary glands and can then be secreted into milk (Hamdy & Amin, 2002).

Shedding of *Brucella* into milk and, therefrom, milk contamination with *Brucella* have been studied and reported to be associated with a number of risk factors. In their study to identify risk factors affecting *Brucella* contamination of raw milk, Ning and colleagues examined risk factors that were supposedly influencing raw milk contamination with *Brucella* and reported brucellosis disease in the animal, abortion rate and animal polyculture to be the most important risk factors for milk contamination with *Brucella* (Ning *et al*., 2013). In a different study conducted among bovine dairy herds in peri-urban dairy zones across West and Central Africa, regular introduction of new animals into herds was found to be strongly associated with *Brucella* seropositivity in farm bulk milk samples (Musallam *et al*., 2019).

In a study conducted to assess the potential risk factors of Brucellosis in cattle in Niger, it was reported that animals aged from 1 to 4 years were more susceptible to contracting brucellosis than animals of less than 1 year. The same study identified farm location in rural areas, transhumance, occurrence of abortions and herd size as the other main risk factors for brucellosis in cattle (Boukary et al., 2013). Other reports from Sub-Saharan Africa have associated management factors, herd size, pastoral grazing system, population density, type of animal breed and biological features such as herd immunity to the incidence of brucellosis in cattle (Mekonnen *et al*., 2010; Makita *et al*., 2011; McDermott & Arimi, 2002; Megersa *et al*., 2011; Muma *et al*. , 2007). In addition to abortion, older age, sex, species or breed, herd size, contact with wild animals, type of animal production, farm management, other studies on cattle brucellosis reported the lack of farmers' knowledge about brucellosis as a potential risk factor of the disease (Hossain *et al*., 2014; Coelho *et al*., 2015).

2.6. Exposure to *Brucella* through milk consumption

One way through which humans are exposed to *Brucella* is through the consumption of animal sourced foods (Corbel, 2006; Estradaa *et al*., 2016). Unpasteurized milk and milk products from unpasteurized milk, in particular, have been associated with the transmission of brucellosis from animals to humans (Dadar *et al*., 2019). A study conducted to determine the seroprevalence of *Brucella* in bovine and ovine anaimals as well as in people who raise the animals, reported a higher prevalence among people who made cheese from raw milk compared to people who made cheese from boiled milk, suggesting a higher exposure to *Brucella* through the consumption of raw milk cheese (Ürünlerinin *et al*., 2013). Increased risk of brucellosis among humans in Uganda was associated with milk consumption and consumption of locally processed milk products (Tumwine *et al*., 2015).

In a study conducted in Kenya to assess sero-prevalence and risk factors for brucellosis among humans and their livestock, there was a strong association between human and animal *Brucella* seropositivity and one of the risk factors for human brucellosis was regular ingestion of raw milk (Osoro *et al*., 2015). A study in Bangladesh, also, reported higher brucellosis cases among populations with a history of consuming raw milk (Rahman *et al*., 2012). Findings from other studies reported milk consumption as an important route of humans exposure to *Brucella* (Hosek *et al*., 2006; Jennings *et al*., 2007; Mishal *et al*., 1999).

Exposure to *Brucella* through milk consumption has, also, been assessed. Consuming unpasteurized in Kajiado, Kenya, was linked with a high risk of exposure to *Brucella* for household members living in rural areas while the risk was very low to low for household members living in peri-urban areas (Onono *et al*., 2020). In a different study conducted in Kenya to assess zoonotic milkborne health risks, milk sold in small units and originating from stall feeding farms hardly posed any risk of exposure to *Brucella* compared to milk from extensive cattle production areas sold after bulking. With most consumers boiling milk before consumption, exposure to *Brucella* was greatly reduced (Arimi *et al*., 2005).

CHAPTER THREE: KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING BRUCELLOSIS AMONG CATTLE FARMERS PRACTICING OPEN AND ZERO GRAZING SYSTEMS IN RWANDA

Abstract

Beyond simple awareness, information on farmers' knowledge, attitudes and practices in regard to brucellosis is limited in Rwanda. Therefore, a study was conducted to assess knowledge, attitudes and practices regarding brucellosis among cattle farmers practicing open and zero grazing systems in Rwanda. A cross-sectional study was designed in which a total of 330 rural cattle keeping households were included: 198 from zero grazing areas and 132 from open grazing areas. To collect and assess data on brucellosis knowledge, attitudes and practices, a questionnaire was administered to households' respondents and obtained data analyzed using SPSS descriptive statistics. More than half of the respondents (63.6 %; 210/330) had heard about brucellosis with significantly ($p < 0.05$) more respondents from open grazing study areas (75.8 %; 100/132) having heard about brucellosis compared to respondents from zero grazing areas (55.6%; 110/198). Of the respondents who had heard about brucellosis, 3.8 %; 10.0 % and 4.3 % could correctly name at least two ways through which cattle contract brucellosis, at least two brucellosis clinical signs in cattle and at least two farm animals which can contract brucellosis. Very few among farmers, 5.2 % (6.6 % in zero grazing study areas and 3.0 % in open grazing study areas) were using PPE while assisting cattle in parturition or handling aborted materials. Artificial insemination was practiced by 66.2 % (131/198) among zero grazing farms while no farm among open grazing farms reported using artificial insemination. Majority among respondents (63.9 %; 211/330) indicated they would seek veterinary help if their animal had or was suspected of having brucellosis. Only a total of 8 farms, all of which were zero grazing, had vaccinated their cattle against brucellosis. Majority among respondents (63.9 %; 211/330) indicated they would seek veterinary help if their animal had or was suspected of having brucellosis. Brucellosis knowledge among surveyed respondents was generally poor across both zero grazing and open grazing study areas and many risky practices regarding brucellosis were recorded. An intervention training program is needed to raise farmers' knowledge and improve farmers' practices and attitudes regarding brucellosis.

3.1. Introduction

Brucellosis is a bacterial zoonotic disease which can affect livestock animals and humans (Corbel, 2006). Livestock animals contract brucellosis when they are infected by the causative bacteria agent, *Brucella* spp. (Godfroid *et al*., 2014). The sources of infection are infected animals which transfer the bacteria to healthy animals through aborted materials, fetal membranes, vaginal discharges, milk and manure (Corbel, 2006; Poester *et al*., 2013). These highly infected materials, also, end up in water, soils and on vegetation and become environmental sources of contamination (Aune *et al*., 2012; Kaden *et al*., 2018). Brucellosis clinical signs in animals include abortions in females (Lampel *et al*., 2012) and other reporoductive disorders such as stillbirths, weak calves, retained placenta, longer calving intervals (Acha *et al*., 2001) and epididymitis in male animals (Parkinson & McGowan, 2019).

Brucellosis in humans is acquired through contact with infected animals' aborted materials, fetuses, placental materials, vaginal discharges, urine, manure and animal tissues during slaughter (Pappas *et a*l., 2006; Tsegay *et al*., 2017). Humans, also, contract brucellosis through the consumption of unpasteurized milk and improperly cooked meat from infected animals (Casalinuovo et al., 2016; Dadar et al., 2019). Brucellosis symptoms in humans include intermittent fevers, sweats, chills, weakness, malaise, headache, insomnia, anorexia and joint and muscle pain (Corbel, 2006; Pappas *et al*., 2006; Lampel *et al*., 2012, Acha & Szyfres, 2003).

Brucellosis can be prevented or controlled in both animals and humans, if farmers and other animals' caretakers have the knowledge and implement good practices and attitudes in regards to brucellosis disease. Sourcing replacing animals from brucellosis free farms or areas, screening replacement animals prior to their addition to the herd, using PPE (Personal Protective Equipment) while assisting in parturition or handling abortions, practicing artificial insemination, vaccinating animals against brucellosis, boiling milk before consumption and seeking veterinary help in case brucellosis is suspected are some known good farm practices and attitudes towards brucellosis prevention and control (Alhaji *et al*., 2016; Cárdenas *et al*., 2019; Dadar *et al*., 2019; Earhart *et al*., 2009; Kadohira *et al*., 1997; Poester *et al*., 2013; Tsegay *et al*., 2017). In any country, brucellosis prevention or control depends on the knowledge, practices and attitudes towards brucellosis among farmers.

In Rwanda, information on farmers' brucellosis knowledge and their practices and attitudes towards the disease is limited. Previous studies which were focusing on brucellosis sero-prevalence and risk factors in Rwanda have reported brucellosis awareness levels of 42.5 % (Ndazigaruye *et al*., 2018) and 63.6 % (Djangwani *et al*., 2021) among dairy farmers and 14.1 among cattle keepers (Ntivuguruzwa *et al*., 2020). Beyond brucellosis awareness, however, information on farmers' knowledge in terms of brucellosis transmission routes, symptoms as well as farm practices and attitudes in regards to brucellosis is lacking. Such information is a good foundation on designing and implementing effective intervention program against brucellosis in a country like Rwanda where animal and human and brucellosis are prevalent (Chatikoba et al., 2008; Djangwani *et al*., 2021; Gafirita *et al*., 2017; Manishimwe *et al*., 2015; Ndazigaruye *et a*l., 2018; Ntivuguruzwa *et al*., 2020; Rujeni & Mbanzamihigo, 2014).

The aim of the current study is, therefore, to assess knowledge, attitudes and practices regarding brucellosis among cattle farmers in the open and zero grazing systems in Rwanda. It is expected that findings from the study will help guide future brucellosis control programs in Rwanda.

3.2. Materials and methods

3.2.1. Study design

The study was a cross-sectional survey. Cattle keeping households/farms from five selected study districts across Rwanda were visited and a mobile based electronic questionnaire (Appendix) was administered to households' respondents to collect information on knowledge, practices and attitudes regarding brucellosis.

3.2.2. Study sites

The study was conducted in Rwanda. Rwanda is an African country located in Central-Eastern Africa at 1º 04' to 2º 51' of latitude below the equator and at 28º 45' to 31º 15' of longitude at the east of the prime meridian. The country has a surface area of 26,338 square kilometers and a total of 30 administrative districts. The total population in Rwanda was estimated at 12,089,721 with an urban population of 2,484,438 and a rural population of 9,605,283 (National Institute of Statstics of Rwanda, 2019).

The study target population was cattle keeping households/farms in rural areas of Rwanda practicing the two main cattle production systems, zero grazing and open grazing. It was estimated that up to 68.8 % of rural households in Rwanda keep cattle (Ojango *et al*., 2012) and typical cattle keeping households in Rwanda own small farms with an average land size of about 0.7 ha per farm (TechnoServe Rwanda, 2008; Mutimura, 2010) and two to three cows per farm (Bishop & Pfeiffer, 2008; Kamanzi & Mapiye, 2012; Miklyaev *et al*., 2017). Cattle is mainly kept under zero grazing and open grazing production systems with 80 %, 17 % and 3 % of farms practicing zero-grazing, open grazing and semi-grazing, respectively (Land O' lakes, 2014).

To assess knowledge, attitudes and practices regarding brucellosis among rural cattle farmers in the open and zero grazing systems in Rwanda, cattle keeping households were selected from five study districts (Nyanza, Gicumbi, Rwamagana, Nyagatare and Nyabihu) across Rwanda (Figure 2) . The five districts were selected to represent the two main grazing cattle production systems (zero grazing and open field grazing) practiced in Rwanda: in three of the selected districts (Nyanza, Gicumbi and Rwamagana), the zero grazing system is practiced, while in two of the selected districts (Nyagatare and Nyabihu), the open field grazing system is practiced (Land O' lakes, 2014; Mazimpaka, 2017).

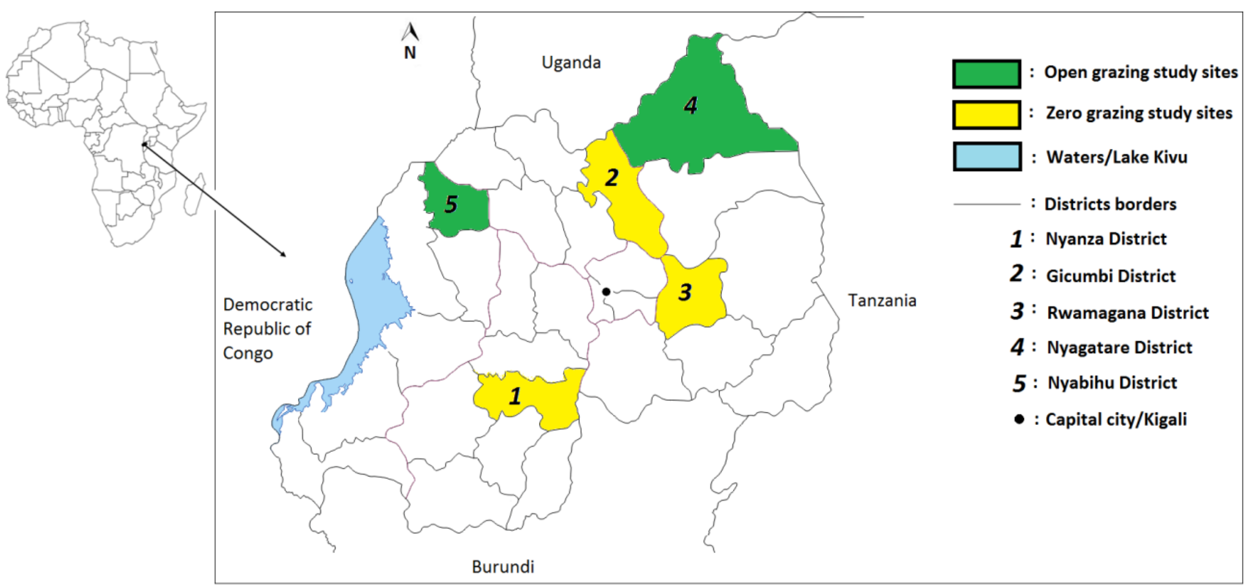

Figure 2: Map of the study districts (Nyanza, Gicumbi, Rwamagana, Nyagatare and Nyabihu) in Rwanda.

3.2.3. Study population

The study population consisted of cattle keeping farmers.

3.2.4. Sample size

The sample size for the number of rural cattle keeping households included in the study, and therefore the number of respondents to interview on knowledge, attitudes and practices regarding brucellosis, was determined using Fischer's formula (Fisher *et al.*, 1991),

$n = \{z_\alpha{}^2 * P * (1-P)\} / d^2$, where:

- n: is the sample size
- z_α: is 1.96 which is the statistic corresponding to a level of confidence of 95 %
- P: is 68.8 %, the percentage of cattle keeping households among rural households in Rwanda (Ojango *et al.*, 2012).
- d: is the level of precision set at 5 %

A total sample size of 330 cattle keeping households was, then, determined. An equal sample size of 330/5 = 66 of cattle keeping households was then considered per study district (Table 7).

Table 13: Surveyed cattle keeping households

Cattle production system and Study districts	Number of cattle keeping HHs surveyed with questionnaire	Number of interviewed respondents
Zero-grazing system		
Nyanza	66	66
Gicumbi	66	66
Rwamagana	66	66
Total/Zero-grazing system	**198**	**198**
Open grazing system		
Nyagatare	66	66
Nyabihu	66	66
Total/Open grazing system	**132**	**132**
TOTAL	**330**	**330**

HHs: Households

3.2.5. Sampling

Selection of cattle keeping households was conducted in all five study districts with 66 cattle keeping households being sampled per district. The sampling was done randomly and systematically by selecting the first household, skipping the next household and selecting the next one until required sample size was reached.

To be considered, the randomly selected household had to be cattle keeping, to be willing to provide needed information, to have a household member available to provide the needed information for the questionnaire and to be geographically located within the district of interest. A household was excluded if the household was not keeping cattle. A cattle keeping household was, also, excluded if no one in the household was willing to give needed information or did not have a member available to respond to the questionnaire. For each selected cattle keeping household fulfilling the criteria, a questionnaire (Appendix) was administered to collect information on knowledge, attitudes and practices regarding brucellosis.

3.2.6. Data collection

3.2.6.1. Questionnaire

To collect data on Knowledge, practices and attitudes regarding brucellosis, a structured questionnaire with closed questions was developed, pre-tested on farmers not included in the study and adjusted into a final questionnaire (Appendix) that was used. The questionnaire comprised questions about socio-demographic characteristics of respondents and surveyed households. Questions to inquire about the respondent's relationship to the household head, gender, age, education level as well as questions about the household's farm size and practiced grazing system were asked. The questionnaire also included questions to assess the respondent's brucellosis knowledge.

Respondents were, in the first place, asked whether they had heard about brucellosis. Respondents who had heard about brucellosis were further asked about ways through which cattle contract brucellosis, brucellosis clinical signs in cattle, farm animal species that can contract brucellosis and whether brucellosis could be transmitted from animals to humans. Respondents' brucellosis knowledge was then assessed by determining the proportion of respondents who could correctly name at least two ways through which cattle contract brucellosis, two brucellosis clinical signs in cattle and two farm animals which can contract brucellosis. Respondent's knowledge was also determined by assessing whether the respondent knew whether brucellosis could be transmitted from animals to humans.

The questionnaire (Appendix) was also developed to cover farm practices and farmers/respondents' attitudes towards brucellosis and included questions on cattle origins, assisting parturition and handling abortions, insemination methods and brucellosis vaccination status. Attitudes were assessed by inquiring from respondents what they would do in case of suspected case of brucellosis among their animals. To collected data more efficiently, the pre-tested final questionnaire was built into the data collection tool Open data kit (ODK). Brucellosis knowledge, practices and attitudes were then collected using ODK with https://ona.io as the server.

3.2.6.2. Interview procedure

At each surveyed household, the respondent to be interviewed was the household head who was considered as the most informed among household members. In the case the household head was not present at the time of interview, the spouse of the household was interviewed. Where both household head and household head's spouse were not available for the interview, the household member designated as the member with the most information about the household characteristics and brucellosis knowledge, practices and attitudes was interviewed. Before conducting the interview, respondents were informed about the objectives of the study and their right to participate or not in the study. To answer questions during the interview, the respondent was allowed to and would inquire information from other household members also involved in the farms' daily activities.

3.2.7. Data analysis

Collected questionnaire data on cattle keeping households' knowledge, attitudes and practices regarding brucellosis were exported from ODK to Microsoft Excel for data cleaning and coding. Cleaned and coded data was then exported to SPSS (IBM SPSS Statistics version 20) for analysis. Data was first analyzed using SPPS descriptive statistics to determine the frequencies and percentages and mean and standard deviations on socio-demographic characteristics of respondents and their respective surveyed households/farms.

To assess the knowledge of respondents regarding brucellosis, collected data was analyzed using SPSS descriptive statistics to determine the proportions of respondents with knowledge on different investigated brucellosis aspects (having heard about brucellosis, ways of contracting brucellosis for cattle, clinical signs of brucellosis in cattle, farm animals that can contract brucellosis and brucellosis transmission from farm animals to humans). Knowledge of respondents regarding brucellosis was, also, assessed using SPSS chi square to compare the proportions of respondents who had heard about brucellosis by different respondents' characteristics.

To estimate the general knowledge regarding brucellosis and among all surveyed cattle keeping households, the proportions of respondents who had heard about brucellosis and the proportions of respondents who could correctly name at least two ways through which cattle contract brucellosis, two brucellosis clinical signs in cattle and two farm animals which can contract brucellosis was computed over the total surveyed population of respondents.

To estimate the overall knowledge of regarding brucellosis, respondents from surveyed household were given scores based on their answers to all of the asked questions about brucellosis knowledge (having heard about brucellosis, ways of contracting brucellosis for cattle, clinical signs of brucellosis in cattle, farm animals that can contract brucellosis and brucellosis transmission from farm animals to humans). Having heard about brucellosis was given a score of 1 while having not heard about brucellosis was given a score of 0. Out of 4 considered (and reported in literature) ways through which cattle can contract brucellosis, each cited correct way of contracting brucellosis for cattle was given a score of 1. Out of 6 considered (and reported in literature) brucellosis clinical signs, each cited and correct brucellosis clinical sign was given a score of 1. Out of 3 considered (and reported in literature) common farm animals that can contract brucellosis, each cited and correct farm animal was given a score of 1. Knowing whether brucellosis can be transmitted from farm animals to humans was given a score of 1 while not knowing whether brucellosis can be transmitted from farm animals to humans was given a score of 0.

To assess the practices and attitudes regarding brucellosis, data collected on selected brucellosis associated practices and attitudes towards brucellosis management disease at the farm were analyzed using SPSS descriptive statistics to compute proportions of respondents and their actual brucellosis related practices and attitudes.

3.2.8. Ethical considerations

Data coll at the Directorate of Research and Innovation, College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda. Ethical clearance number 20/DRI/January 2020 was obtained and used during data collection. Prior to administering the questionnaire, respondents in the cattle keeping households got verbal explanation on the purpose of data collection and their rights to participate or not to participate in the study. Those willing to participate in the study were further assured that their identities will remain confidential for any future presentation or publication of results.

3.3. Results

3.3.1. Socio-demographic characteristics of respondents and surveyed households/farms

A total of 330 farms/households were surveyed, of which 198 were practicing zero grazing cattle production system and were located in the districts of Nyanza, Gicumbi and Rwamagana while 132 practiced open grazing cattle production system and were located in the district of Nyagatare and Nyabihu. Most interviewed respondents (78.5 %; 259/330) were household heads. Most respondents (76.4 %; 252/330) were of the male gender (Table 8). More than half of respondents (56.4 %; 186/330) had between 41 and 60 years of age and an overall average age of 46.0 ± 13.0 years.

The majority of respondents (60.3 %; 199/330) had some or complete primary school education while 24.5 % (81/330) and 15.2 % (50/330) had some or complete secondary school and above education and no formal education, respectively (Table 8). The average number of cows owned and present in surveyed households' farms was high (17.7 ± 5.8 cows per farm) in open grazing farms compared to zero grazing farms (2.2 ± 1.2 cows per farm) (Table 8). Overall, the farm with the smallest number of cows had 1 cow while the farm with the highest number of cows had 58 cows.

Table 14: Socio-demographic characteristics of respondents and surveyed households/farms

Respondents' and households/farms' characteristics	Zero grazing (N=198): Nyanza, Gicumbi and Rwamagana districts	Open grazing (N=132): Nyagatare and Nyabihu districts	TOTAL (N=330)
	n (%)	*n (%)*	*n (%)*
Respondent's relationship to HHH*			
HHH	168 (84.8)	91 (69.0)	259 (78.5)
Wife	21 (10.6)	0 (0.0)	21 (6.4)
Other (Child or Relative or Worker)	9 (4.5)	41 (31.1)	50 (15.2)
Respondent's gender			
Female	67 (33.8)	11 (8.3)	78 (23.6)
Male	131 (66.2)	121 (91.7)	252 (76.4)
Respondent's age group			
Less than (≤) 40	56 (28.3)	50 (37.9)	106 (32.1)
41 to 60	113 (57.1)	70 (53.0)	183 (55.5)
More than 60	29 (14.6)	12 (9.1)	41 (12.4)
Respondent's age (Average)	47.8 ± 12.3	43.4 ± 13.8	46.0 ± 13.0
Respondent's level of education			
No formal education	40 (20.2)	10 (7.6)	50 (15.2)
Some or full primary education	117 (59.1)	82 (62.1)	199 (60.3)
Some or full secondary and above education	41 (20.7)	40 (30.3)	81 (24.5)
Herd size range			
1 to 2 cows	151 (76.3)	0 (0.0)	151 (45.8)
3 to 6 cows	43 (21.7)	1 (0.8)	44 (13.3)
More than 6 cows	4 (2.0)	131 (99.2)	135 (40.9)
Number of cows per household/farm (Average)	2.2 ± 1.2	17.7 ± 5.8	8.5 ± 8.5

*HHH: Household head

3.3.2. Respondents' knowledge regarding brucellosis disease

More than half of the respondents (63.6 %; 210/330) had heard about brucellosis, while 36.4 % had not. The majority (86.2 %; 181/210) among those who had heard about brucellosis had gotten the information from fellow farmers, neighbors, family members or friends. The proportion of respondents from open grazing study areas who had heard about brucellosis (75.8

%; 100/132) was significantly higher ($p < 0.05$) than the proportion of respondents from zero grazing study areas who had heard about brucellosis (55.6 %; 110/198) (Table 9).

The location district of a respondent was significantly ($p < 0.05$) associated with whether the respondent had heard about brucellosis or not and Nyagatare district had the highest proportion of respondents (95.5 %; 63/66) who had heard about brucellosis while Gicumbi district had the lowest proportion of respondents who had heard about brucellosis (31.8 %; 21/66) (Table 9). The respondent's relationship to the household head, the respondent's age and the respondent's farm herd size were also significantly ($p < 0.05$) related to whether the respondent had heard about brucellosis or not (Table 9). Highest proportions of respondents who had heard about brucellosis were among respondents who happened to be household heads themselves, respondents who were older and respondents who were associated to farms with bigger herd size (Table 9).

Respondent's gender was not significantly ($p > 0.05$) associated with whether a respondent had heard about brucellosis or not. More among males (66.3 %; 167/252) had, however, heard about brucellosis compared to those among females (55.1 %; 43/78) who had heard about brucellosis. The level of education did not have any significant ($p > 0.05$) effect on respondents having heard about brucellosis or not and more among respondents without any formal education had heard about brucellosis compared to those among respondents with primary school education who had heard about brucellosis (Table 9).

Table 15: Proportions of respondents who had heard about brucellosis by different respondents and households'/farms' characteristics

Characteristic	Respondents (Respondents ***who heard about*** brucellosis); proportion (%)	Respondents (Respondents ***who did not hear about*** brucellosis); proportion (%)	p-value
Study districts			0.000*
Nyanza	66 (39); 59.1	66 (27); 40.9	
Gicumbi	66 (21); 31.8	66 (45); 68.2	
Rwamagana	66 (50); 75.8	66 (16); 24.2	
Nyagatare	66 (63); 95.5	66 (3); 4.5	
Nyabihu	66 (37); 56.1	66 (29); 43.9	
Cattle production system			0.000*
Zero grazing	198 (110); 55.6	198 (88); 44.4	
Open grazing	132 (100); 75.8	132 (32); 24.2	
Respondent's relationship to HHH			0.000*
HHH	259 (191); 73.7	259 (68); 26.3	
Wife	21 (5); 28.8	21 (16); 76.2	
Other (Child or Relative or worker)	50 (14); 28.0	50 (36); 72.0	
Respondent's gender			0.074
Female	78 (43); 55.1	78 (35); 44.9	
Male	252 (167); 66.3	252 (85); 33.7	
Respondent's age group			0.000*
Less than (≤) 40	106 (43); 40.6	106 (63); 59.4	
41 to 60	183 (137); 74.9	183 (46); 25.1	
More than (>) 60	41 (30); 73.2	41 (11); 26.8	
Respondent's level of education			0.095
No formal education	50 (33); 66.0	50 (17); 34.0	
Some or full primary education	199 (118); 59.3	199 (81); 40.7	
Some or full secondary and above education	81 (59); 72.8	81 (22); 27.2	
Herd size range			
1 to 2 cows	151 (77); 51.0	151 (74); 49.0	0.000*
3 to 6 cows	44 (32); 72.7	44 (12); 27.3	
More than 6 cows	135 (101); 74.8	135 (34); 25.2	

*: Significant association

HHH: Household head

Respondents who indicated they had heard about brucellosis (63.6 %; 210/330) were further asked about ways through which cattle contract brucellosis, brucellosis clinical signs in cattle, farm animals that can contract brucellosis and whether or not brucellosis could be transmitted from farm animals to humans. On the question about ways through which cattle contract brucellosis, 21.0 % (44/210) of respondents who had heard about brucellosis indicated natural insemination as a way through which cattle contract brucellosis from infected bulls (Table 10). Other indicated ways through which cattle can contract brucellosis were feed/water, faeces/urine from infected animals and contact with infected animals cited by 0.5 %, 0.5 % and 2.9 % of respondents who had heard about brucellosis, respectively (Table 3). On the same question about ways through which cattle contract brucellosis, the majority (79.0 %; 166/210) of respondents who had heard about brucellosis said they did not know and could not indicate any way through which cattle contract brucellosis.

On the question about clinical signs of brucellosis in cattle, 22.9 % (48/210) of respondents who had heard about brucellosis indicated abortion as a clinical sign of brucellosis while 1.4 % (3/210) indicated abortion during late gestation. Of the 210 respondents who had heard about brucellosis, 1.0 %, 2.4 %, 11.9 % and 5.7% cited weak calves at birth, still birth, low calving rate and retained placenta as brucellosis clinical signs, respectively (Table 10). The majority of respondents who had heard about brucellosis (74.8; 157/210) did not know and could not cite any brucellosis clinical sign in cattle.

When asked to name which farm animals could contract brucellosis, almost all respondents who had heard about brucellosis (99.5 %; 209/210) could identify cattle as the farm animal which can contract brucellosis. Only 3.8 % (8/210) of respondents who had heard about brucellosis could indicate sheep and goats as other farm animals which can contract brucellosis (Table 10). Respondents who had heard about brucellosis were also asked whether or not brucellosis could be transmitted from infected animals to humans and 36.7 % (77/210) knew brucellosis could be transmitted from animals to humans while 63.3 % did not know (Table 10).

Table 16: Responses about brucellosis knowledge from respondents who had heard about brucellosis

Questions and responses	Zero grazing study areas (N=110)	Open grazing study areas (N=100)	TOTAL (N=210)
	n (%)	***n (%)***	***n (%)***
Through which ways cattle contract brucellosis?			
Feed/Water	1 (0.9)	0 (0.0)	1 (0.5)
Dung/faeces/urines from infected animals	0 (0.0)	1 (1.0)	1 (0.5)
Contact with infected animals	1 (0.9)	5 (5.0)	6 (2.9)
Bulls (natural insemination)	8 (7.3)	36 (36.0)	44 (21.0)
Don't know	102 (92.7)	64 (64.0)	166 (79.0)
Respondents who could indicate at least 2 ways through cattle contract brucellosis	2 (1.8)	6 (6.0)	8 (3.8)
What are the clinical signs of brucellosis in cattle?			
Abortion	13 (11.8)	35 (35.0)	48 (22.9)
Abortion during late gestation	2 (1.8)	1 (1.0)	3 (1.4)
Weak calves at birth	1 (0.9)	1 (1.0)	2 (1.0)
Still birth	2 (1.8)	3 (3.0)	5 (2.4)
Low calving rate	11 (10.0)	14 (14.0)	25 (11.9)
Retained placenta	5 (4.5)	7 (7.0)	12 (5.7)
Don’t know	93 (84.5)	64 (64.0)	157 (74.8)
Respondents who could indicate at least 2 brucellosis clinical signs in cattle	7 (6.4)	14 (14.0)	21 (10.0)
Which farm animals can contract brucellosis?			
Cattle	109 (99.1)	100 (100)	209 (99.5%)
Sheep	5 (4.5)	3 (3.0)	8 (3.8)
Goats	4 (3.6)	4 (4.0)	8 (3.8)
Don't know	1 (0.9)	0 (0.0)	1 (0.5)
Respondents who could name at least 2 farm animals which can contract brucellosis	5 (4.5)	4 (4.0)	9 (4.3)
Can brucellosis be transmitted from farm animals to humans?			
Yes	35 (31.8)	42 (42.0)	77 (36.7)
No	75 (68.2)	58 (58.0)	133 (63.3)

When compared, proportions of respondents from zero grazing areas who could correctly name at least two ways through which cattle contract brucellosis (1.8 %; 2/110) and two brucellosis clinical signs in cattle (6.4 %; 7/110) were lower than the proportions of respondents from open grazing areas who could correctly name at least two ways through which cattle contract brucellosis (6.0 %; 6/100) and two brucellosis clinical signs in cattle (14.0; 14/100) (Table 10).

Overall and out of a possible maximum score of 15 points attributed based on answers given following the four asked knowledge related questions, only 1.8 % (6/330) of all interviewed respondents had a knowledge score above 7.5 and were considered knowledgeable on brucellosis. Of the 330 interviewed respondents, 120 (36.4 %) had a score of zero as they had not heard about brucellosis (Figure 3). Overall and out of the total interviewed 330 respondents across all study sites, 63.6 % (210/330) had heard about brucellosis and only 2.4 % (8/330) could correctly name at least two ways through which cattle contract brucellosis, 6.4 % (21/330) could correctly name two brucellosis clinical signs in cattle and 2.7 % (9/330) could correctly name two farm animals which can contract brucellosis.

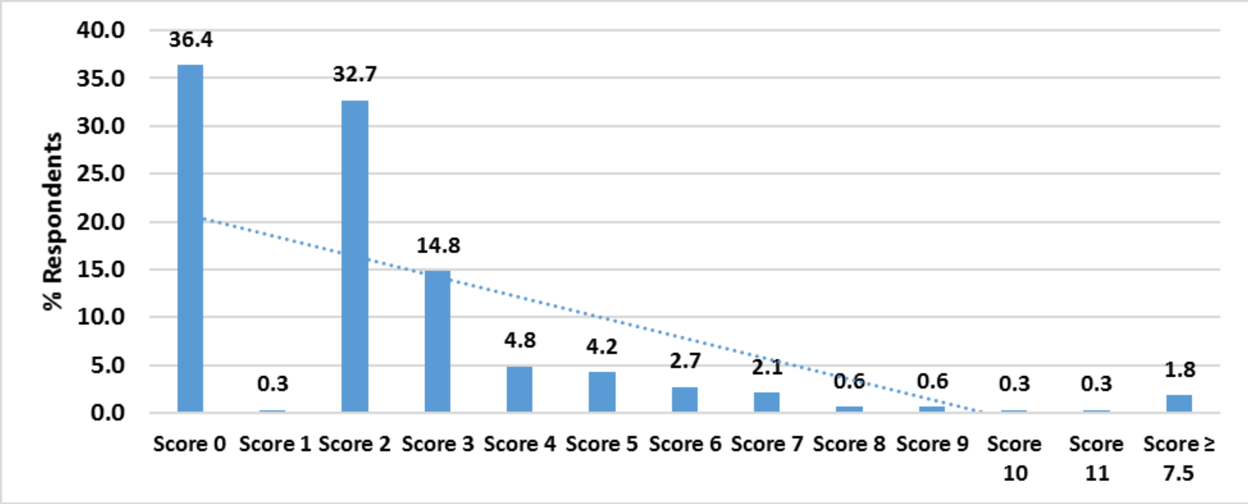

Figure 3: Knowledge score for the interviewed respondents

3.3.3. Farm management practices regarding brucellosis

Cattle at surveyed farms originated from farm breeding, purchasing from markets and neighbors in the community, donations from government and non-governmental cattle donating programs, gifts and keeping cattle for family and friends. The majority of farms (77.0 %; 254/330) owned at least one cow that originated from own farm breeding (Table 11). Zero grazing farms had cows that originated from donating programs with 63 farms (31.8 %) among zero grazing farms owning at least one cow that originated from government “Girinka” or non-governmental cattle donating programs while zero farm among surveyed open grazing farms had a donated cow (Table 11).

In total and across both zero grazing and open grazing surveyed farms, very few (5.2 %; 17/330) farmers used PPE (Personal Protective Equipment) while assisting cattle in parturition or handling aborted materials (Table 11). For the handling of abortions, 3.3 % (11/330) of farms indicated they buried the aborted materials while in 17.0 % (56/330) of the surveyed farms, aborted materials were given or taken or stolen by dogs. Most farms (79.7; 263/330) had not yet had abortion cases.

The use of artificial insemination for cattle breeding was practiced by 66.2 % (131/198) among zero grazing farms while no farm among open grazing farms reported using artificial insemination (Table 11). The use of bulls for natural breeding was especially observed in open grazing study areas where 100 % of surveyed farms used bulls for breeding. In zero grazing study areas, 34 % of farms used bulls while 67 %used artificial insemination. In open grazing where all farms practiced natural breeding, more than half of the farms (66.0 %; 87/132) owned an adult bull while in zero grazing study areas, only 3.5 % of the farms owned an adult bull (Table 11).

The rate of vaccination against brucellosis was low with only 2.4 % (8/330) of all surveyed farms having vaccinated their cattle against brucellosis (Table 11). All the 8 farms which reported having vaccinated their cattle against brucellosis were zero grazing farms. On the same inquiry to know whether farm cattle were vaccinated against brucellosis, 26.7 % (88/330) of all surveyed farms indicated they did not know or did not remember whether their cattle were vaccinated against brucellosis or not (Table 11).

The consumption of raw was especially recorded in households practicing open grazing where in 95.5 % of households, raw milk was consumed. In households zero grazing households, the practice of drinking raw milk was recorded in 18.2 % households (Table 11).

Table 17: Responses about farm management practices regarding brucellosis

Questions and responses	Zero grazing study areas (N=198)	Open grazing study areas (N=132)	TOTAL (N=330)
	n (%)	n (%)	n (%)
Cattle origin			
At least 1 cattle is farm bred	122 (61.6)	132 (100)	254 (77.0)
At least 1 cattle is purchased from the community	52 (26.3)	73 (55.3)	125 (37.9)
At least 1 cattle is from “Girinka”	42 (21.2)	0 (0.0)	42 (12.7)
At least 1 cattle is from Donating NGOs	21 (10.6)	0 (0.0)	21 (6.4)
At least 1 cattle is a gift	3 (1.5)	38 (28.8)	41 (12.4)
At least 1 cattle is kept for others	3 (1.5)	24 (18.2)	27 (8.2)
Are PPE used when assisting cattle during parturition or handling aborted materials?			
Yes	13 (6.6)	4 (3.0)	17 (5.2)
No	185 (93.4)	128 (97.0)	313 (94.8)
What was done with aborted materials?			
N/A	176 (88.9)	87 (65.9)	263 (79.7)
Buried	6 (3.0)	5 (3.8)	11 (3.3)
Given to/Taken by/Stolen by dogs	16 (8.1)	40 (30.3)	56 (17.0)
What is the used insemination method?			
Artificial insemination	131 (66.2)	0 (0.0)	131 (39.7)
Natural insemination (bull)	67 (33.8)	132 (100)	199 (60.3)
Do you own an adult bull?			
Yes	7 (3.5)	87 (66.0)	94 (28.5)
No	191 (96.5)	45 (34.0)	236 (71.5)
Are your cattle vaccinated against brucellosis?			
Yes	8 (4.0)	0 (0.0)	8 (2.4)
No	139 (70.2)	95 (72.0)	234 (70.9)
Don't know/Don't remember	51 (25.8)	37 (28.0)	88 (26.7)
Is raw milk consumed in the household?			
Yes	36 (18.2)	126 (95.5)	162 (49.1)
No	162 (81.8)	6 (4.5)	168 (50.9)

N/A: Not applicable

3.3.4. Attitudes towards management of cattle brucellosis disease at the farm

Asked what they would do if one of their animal had or was suspected of having brucellosis, the majority among respondents (63.9 %; 211/330) said they would seek help from vets (Table 12). Other reported actions in case an animal has or is suspected of having brucellosis were selling the animal, slaughtering the animal, separating the animal, leaving the animal to heal itself and traditionally treating the animal (Table 12). Out of 330 interviewed respondents, 100 respondents (30.3 %) did not know what they would do if their animal had or was suspected of having brucellosis (Table 12).

Table 18: Responses about attitudes towards cattle brucellosis management

Questions and responses	Zero grazing study areas (N=198)	Open grazing study areas (N=132)	TOTAL (N=330)
	n (%)	n (%)	n (%)
What would you do if your animal had or was suspected of having brucellosis?			
Selling	6 (3.0)	3 (2.3)	9 (2.7)
Slaughtering	1 (0.5)	0 (0.0)	1 (0.3)
Seek help from vets	155 (78.3)	56 (42.4)	211 (63.9)
Separate animal	0 (0.0)	2 (1.5)	2 (0.6)
Leave animal to heal itself	0 (0.0)	37 (28.0)	37 (11.2)
Treat traditionally	4 (2.0)	39 (29.5)	43 (13.0)
Don't know	41 (20.7)	59 (44.7)	100 (30.3)

3.4. Discussion

This study revealed that more than half of farmers in rural cattle keeping households in Rwanda heard about brucellosis. In the current study, it was, also found out that more farmers from open grazing areas had heard about brucellosis compared to farmers from zero grazing areas. This could be related to a higher brucellosis prevalence reported in areas practicing open grazing cattle production system in Rwanda (Djangwani *et al*., 2021) which has resulted in more farmers encountering and becoming aware of the disease. Nyagatare district, in which open grazing is practiced, was, for example, the study district with the highest proportion of respondents who had heard about brucellosis. The high awareness about brucellosis in Nyagatare, in particular, could, also, be explained by brucellosis endemicity in the area (Chatikoba *et al*., 2008).

The current study, also, revealed that higher proportions of respondents who had heard about brucellosis were among older respondents and respondents whose households/farms had bigger herds. Respondents' older age translates into more experience and longer times of cattle production during which older respondents have had more chances of encountering and becoming aware of brucellosis. Having a bigger herd size could mean more chances of encountering brucellosis disease and becoming aware of the disease. Indeed, a study previously conducted in Rwanda reported that the occurrence of brucellosis was significantly higher in larger herds of 40 to 70 cattle compared to smaller herds of 10 to 39 cattle (Ndazigaruye *et al*., 2018).

In addition to grazing system, location district, respondent's age and herd size, the respondent's relationship to the household holder, also, influenced whether the respondent had heard about brucellosis or not. Many among respondents who happened to be household head themselves were aware of brucellosis compared to when the respondent happened to be the wife, child, relative or worker. This could be explained by the household heads being the most responsible in a household and the most invested in taking care of the cattle.

Contrary to previous studies (Lindahl et al., 2015; Njuguna *et al*., 2017b), our findings indicate that the level of education did not have any influence on whether a respondent had heard about brucellosis. In line with our findings, however, it was found that knowledge about common endemic zoonotic diseases including brucellosis existed among surveyed nomadic pastoralists despite lack of formal education (Zinsstag *et al*., 2006). In our study in particular, the level of education was generally low across all interviewed respondents with three quarters of respondents having primary school education or below. The generally observed low education level among respondents and the popular source of information about brucellosis, which was fellow farmers and neighbors in the community, could explain why education level did not show any influence on whether a respondent had heard about brucellosis or not.

Although more than half of all respondents in this study had heard about brucellosis, further knowledge about brucellosis was poor. Very few among respondents who claimed to have heard about brucellosis could name at least two ways through which cattle contract brucellosis and at least two resulting clinical signs. A number of previous studies have, similarly, reported poor brucellosis knowledge among farmers who claimed to know or to have heard about brucellosis. (Buhari *et al*., 2015; Cloete *et al*., 2019; Lindahl *et al*., 2015).

The poor knowledge of brucellosis transmission routes and clinical signs reported in this study could be explained by the source of information about brucellosis. The majority among respondents stated that they had about brucellosis from fellow farmers, neighbors, family members or friends from the surrounding community. The persons in the community passing on the information may, themselves, be having limited or incomplete information on brucellosis compared to, for example, veterinary officers and animal health works who have received specific education in animal health. The poor knowledge about brucellosis could, also, be attributed to a weak agricultural extension service. Without reliable and more complete information on brucellosis from veterinary officers in charge of extension, farmers rely on informal information from fellow farmers who may have not necessarily been educated on brucellosis.

The most correctly cited brucellosis transmission routes by the few respondents who could identify ways through which brucellosis is contracted was natural insemination while the most cited brucellosis clinical sign was abortion. The majority of respondents in other studies have, also, indicated abortion and miscarriages as the main signs of brucellosis in livestock (Holt *et al*., 2011; Njuguna *et al*., 2017). Also, the few respondents who could correctly name brucellosis transmission routes and clinical signs in this study were more from open grazing study areas than from zero grazing study areas and this may be due to farmers from open grazing areas having been more exposed to brucellosis cases compared to farmers from zero grazing study areas who are relatively new or not as experienced in cattle farming. Indeed, higher brucellosis prevalence rates in Rwanda have been reported in open grazing areas or in areas predominantly practicing open grazing (Djangwani *et al*., 2021; Ndazigaruye et al., 2018). The relatively higher knowledge from open grazing farmers could also be explained by the longer history and culture of raising cattle compared to zero grazing farmers, many of whom had started raising cattle following the governmental and non-governmental cattle donating programs which distributes dairy cattle to poor families.

Almost all respondents could correctly name cattle as the farm animal species which can contract brucellosis but very few could name other farm animals, such as sheep and goats, which can contract brucellosis as well. This resulted in only 4.3 % of all interviewed respondents being able to name at least two farm animal species which can contract brucellosis. The common knowledge that cattle is the farm animal that can contract brucellosis observed in this study may be linked to the fact that cattle was, also, the most common livestock raised at visited farms and very few farms had other farm animal species such as sheep and goats in addition to cattle.

About only a third of respondents knew brucellosis can be transmitted from animals to humans. This in line with a previous human brucellosis study conducted in Rwanda in which 88.4 % of study participants did not know how brucellosis is transmitted (Gafirita *et al*., 2017). The finding about low awareness among farmers that brucellosis can be transmitted from animals to humans could explain some of the practices reported by visited farmers and discussed below including the no use of PPE while assisting in parturition or handling aborted materials.

This study, also, assessed practices regarding brucellosis and it was revealed that only a small percentage of cattle are sourced from sources (such as the government and non-governmental cattle donating programs) where cattle are screened for brucellosis prior to distribution to farmers (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2019). While this study did not record farmers who practiced brucellosis screening privately, a previous study in Nyagatare district, Rwanda, has reported that 85.8 % of surveyed farmers did not screen new additions to the herd for brucellosis (Ndazigaruye *et al*., 2018). Not screening new cattle is a risky practice and can result in the introduction and spread of brucellosis in a herd which was brucellosis free (Cárdenas *et al.*, 2019).

Not using PPE when assisting cattle during parturition or when handling aborted materials is a known brucellosis risk factor for humans (Earhart *et al*., 2009) which was observed in this study with 94.8 % of respondents stating that they do not use PPE. The risky practice of not using PPE during cattle parturition and aborted materials disposal was also reported in other studies in Tajikistan (Lindahl *et al*., 2015) and in Egypt (Holt *et al*., 2011). The low awareness that brucellosis can be transmitted from animals to humans observed in this study and the lack or limited access and cost of PPE could explain the high percentage of farmers who do not use PPE.

While the majority of respondents in this study indicated to have not yet encountered abortion cases, many among those who experienced abortion in their cattle gave or left the aborted materials to the dogs and a very small percentage of respondents buried the aborted materials. In line with this finding, a study conducted on prevalence and risk factors for brucellosis in Nyagatere, Rwanda, reported that 62.5 % fed fetal membranes to dogs (Ndazigaruye *et al*., 2018). While burying the aborted materials is a good practice which prevents cattle and other animals to pass their tongue over or feed from potentially brucellosis containing materials, leaving the aborted materials to dogs results in dogs dragging the aborted materials across the ground spreading the disease to many other animals (Hegazy *et al*., 2016) and is a risky practice.

Although natural breeding and especially bulls sharing among herds are major risk factors for cattle brucellosis (Alhaji *et al*., 2016; Berhe *et al*., 2007; Ebrahim *et al*., 2016), all open grazing farms visited in this study practiced natural breeding and overall more than half of respondents stated they practiced natural breeding. Bull sharing could also be expected as less than a third of all surveyed farms owned an adult bull. This finding was interesting given that the most brucellosis transmission route stated by respondents who knew about brucellosis was natural insemination. This would imply that although natural insemination was known as a potential way of brucellosis transmission from infected bulls, farmers continue practicing natural breeding. The cost of artificial insemination and the traditional preference of using bulls for breeding could be the reasons for the risky practice of natural insemination.

Vaccination against brucellosis was not practiced in open grazing study areas while only 8 out of the visited 198 zero grazing farms had vaccinated their cattle against brucellosis. Similar results have been reported in Rwanda, where none of the surveyed farmers had vaccinated their cattle (Ndazigaruye *et al*., 2018). Vaccination is a known brucellosis cost effective control measure especially in free grazing large herds with high brucellosis prevalence (Kadohira *et al*., 1997) and not vaccinating healthy cattle was another risky practice in regards to brucellosis in this study.

Raw milk was consumed in almost half of all surveyed farms and in 95.5 % of surveyed open grazing farms. In a recent brucellosis study conducted at the wildlife-livestock-human interface in Rwanda, the consumption of raw milk was also reported in more than 21.7 % of cattle keepers

(Ntivuguruzwa *et al*., 2020). Elsewhere, studies on brucellosis knowledge, practices and attitudes in Kenya, Uganda, Tajikstan have, also, reported the consumption of un-boiled milk and unpasteurized dairy products (Lindahl *et al*., 2015; Nabirye *et al*., 2017; Njenga *et al*., 2020). Human brucellosis cases in Rwanda have, also, been associated with the consumption of un-boiled milk (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014). Raw milk consumption habit recorded in this study and especially in households/farms practicing zero grazing was, therefore, a high risk practice with regards to human brucellosis.

The majority among respondents in this study indicated they would seek help from vets if their animals had or were suspected of brucellosis. In other studies, also, the majority of the farmers resorted to seeking help form a veterinarian when their cattle showed brucellosis symptoms (Holt *et al*., 2011; Lindahl *et al*., 2015). Veterinarians are educated animal health professionals and seeking veterinarians help for any cattle disease is a good attitude that should be encouraged among farmers. Although the majority in this study had the good attitude of consulting animal health professionals in case of brucellosis or suspected brucellosis disease in their cattle, the cost of veterinary services could be a hindrance to the rather good attitude.

Some respondents, although very few, indicated they would sell their animal if it had or was suspected of brucellosis while others said they would slaughter the animal. Trading or selling for slaughter animals that are suspected of brucellosis are bad attitudes towards managing brucellosis and would only result in spreading the disease to more herds and even to humans through abattoir's workers. Introducing a *Brucella* infected animal in a herd would be a source of infection in the herd as brucellosis causing bacteria are transferred to healthy animals through physical contact and aborted materials, fetal membranes, vaginal discharges, milk and manure from the infected animal (Corbel, 2006; Poester *et al*., 2013). Also, brucellosis is an occupational disease and some occupational groups including butchers are at high risk of getting brucellosis during animal slaughter (Tsegay *et a*l., 2017).

Only a very small number of respondents in this study indicated they would separate the brucellosis diseased or suspected animal while the majority among respondents would not. This finding is consistent with a previous brucellosis study conducted in Rwanda in which 82.5 % did not remove *Brucella*-positive animals from the herd (Ndazigaruye *et al*., 2018). Although, separating diseased or suspected animals may be hindered by the lack of space or isolation facilities at the farm, keeping diseased and healthy animals together is one of the major risk factors of brucellosis transmission between animals (Corbel, 2006) and is therefore a bad attitude towards managing brucellosis at the farm.

Interestingly in this study, some few respondents indicated that they would leave a *Brucella* infected animal to heal itself while some few others said they would treat the animal traditionally. Leaving the animal to heal itself would only contribute to spreading brucellosis more in the herd via contact with healthy animals. While the effectiveness of traditional treatments is usually not scientifically established, treating brucellosis with known and established antibiotics is still questionable given the observed treatment failures and high relapse rates (Seleem *et al*., 2010). Leaving the animal to heal itself from brucellosis or treating the animal would, therefore, not be good attitudes towards management of brucellosis disease at the farm.

3.5. Conclusions

Brucellosis knowledge is poor among cattle keeping farmers in Rwanda. Farm practices regarding brucellosis are risky but the attitude towards brucellosis is good.

There is, therefore, a need to tackle the recorded poor knowledge and the observed risky practices through awareness campaigns conducted by veterinarians or other trained and knowledgeable animal health workers.

CHAPTER FOUR: PREVALENCE AND RISK FACTORS OF *BRUCELLA* OCCURRENCE IN FARM BULK MILK FROM OPEN AND ZERO GRAZING CATTLE PRODUCTION SYSTEMS IN RWANDA

Abstract

Even though, animal and human brucellosis have been reported in Rwanda as well as drinking inadequately heat-treated milk being implicated in the latter, information on the risk factors of *Brucella* presence in milk is still scarce. A cross sectional study was therefore conducted to determine the sero-prevalence and risk factors of *Brucella* in farm bulk milk from zero and open grazing cattle production systems in Rwanda. A total of 330 farm bulk milk samples were collected from 198 zero grazing farms and 132 open grazing farms across Rwanda. Sero-prevalence of *Brucella* in milk was analyzed using indirect Enzyme-Linked Immuno-Sorbent Assay. A questionnaire was also administered to farmers to determine the risk factors of milk contamination with *Brucella*. Anti-*Brucella* antibodies were prevalent in 19.7 % (95 % CI, 15.5-24.4) of the 330 collected farm bulk milk. Sero-prevalence was significantly higher ($p < 0.05$) in open grazing farms (37.9 % [50/132]) than in zero grazing farms (7.6 % [15/198]). Practicing open grazing system (OR = 69.5; 95 % CI = 1.6 - 3033.6), history of abortion (OR = 19.5; 95 % CI = 8.1 - 46.8), and placenta retention (OR=4.2; 95 % CI = 1.7 - 10.3) were the significant risk factors for the presence of anti-*Brucella* antibodies in milk. Notably, more than a third of milk from open grazing farms in Rwanda contains *Brucella* antibodies. Considering the zoonotic nature of *Brucella*, there is a need to reinforce brucellosis control programs in the country.

4.1. Introduction

Brucella species (spp) are bacteria which cause the infection known as brucellosis in different livestock such as cattle, which are among natural hosts of *Brucella* spp (Hull & Schumaker, 2018). The most frequent clinical symptoms exhibited by brucellosis positive animals are reproductive disorders of abortion, still births, weak calves, retained placenta and longer calving intervals in female animals such as dairy cattle (Acha & Szyfres, 2001; Boukary *et al*., 2013; McDermott *et al*., 2013). The reproductive disorders associated with brucellosis in animals result further into animal infertility and reduction to absence of milk production (Mangen *et al*., 2002; Corbel, 2006; Ul-Islam *et al*., 2013) which translate into economic losses for the farmer.

Being zoonotic, brucellosis can be transmitted from animals to humans causing a febrile illness with intermittent undulating fevers, sweats, chills, weakness, malaise, headache, insomnia, anorexia and joint and muscle pain (Pappas *et al*., 2006; Lampel *et al*., 2012). Brucellosis is transmitted to humans when the causative agent, *Brucella*, infect humans through contact with infected animals or infected animal's excretions or through the consumption of animal products from infected animals (Corbel, 2006; Estradaa *et al*., 2016). The consumption of animal sourced foods is a common way of transmission of brucellosis from infected natural host animals to humans (Corbel, 2006; Estradaa *et al*., 2016). Furthermore, among animal sourced foods, unpasteurized milk and milk products are the main routes of brucellosis transmission (Dadar *et al*., 2019).

In Rwanda, brucellosis studies have been conducted focusing on animal health (Chatikoba *et al*., 2008; Manishimwe *et al*., 2015; Ndazigaruye *et al*., 2018; Ntivuguruzwa *et al*., 2020). Using animal sera and serological diagnostic methods, these studies reported the occurrence of animal brucellosis in Rwanda with a cattle brucellosis prevalence of 0.0 to 2.0 % in peri-urban areas of Kigali City (Manishimwe *et al*., 2015; Ntivuguruzwa et al., 2020), 8.3 % in districts bordering the national parks (Ntivuguruzwa *et al*., 2020) and 9.9 to 18.9 % in the district of Nyagatare in which the high prevalence was associated to the practiced extensive grazing with higher risks of brucellosis transmission between and within herds of different brucellosis statuses(Chatikoba et al., 2008; Ndazigaruye et al., 2018). One study which investigated the general microbiological quality of milk from farms to milk collection centers in Rwanda detected anti-*Brucella* antibodies in milk from two milk collection centers (Ndahetuye *et al*., 2020).

The few studies conducted in Rwanda on human brucellosis, targeted patients attending district hospitals (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014) and reported a prevalence of 25 % among women presenting with abortion or still birth at Huye district hospital (Rujeni & Mbanzamihigo, 2014) and 6.1 % among patients attending Nyagatare district hospital with brucellosis symptoms (Gafirita *et al*., 2017). In both studies, the consumption of un-boiled or inadequately heat treated milk was reported as a risk factor for human brucellosis (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014).

It is in the backdrop of the zoonotic nature of *Brucella*, public health complications, burdens resulting from brucellosis, previous detection of *Brucella* antibodies at two milk collection centers in Rwanda and the reported associations of milk to transmission of brucellosis from animals to humans, that the current aim of the study was created. Therefore, the current study aimed at investigating *Brucella* sero-prevalence in milk produced across Rwanda, focusing on farm bulk milk from zero- and open-grazing cattle production systems using ELISA methods while at the same time determining the risk factors associated with *Brucella* prevalence in farm bulk milk.

4.2. Materials and methods

4.2.1. Study design

A cross-sectional study design was carried out where farm bulk cow milk samples were collected from open and zero grazing farms from five selected districts across Rwanda. In addition, a mobile based electronic questionnaire (Appendix) was used to gather information on farms characteristics, farm management practices, cow reproduction disorders and farmers' brucellosis awareness.

4.2.2. Study sites

Rwanda is a landlocked country located in Central Eastern Africa, between 1° 04' and 2° 51' of latitude below the equator and between 28° 45' and 31° 15' of longitude at the East. The country is 26,338 square kilometers and is administratively divided in five provinces and 30 districts with a total population of 12.0 million people (National Institute of Statstics of Rwanda, 2019). Rwanda is a highland country with altitudes varying between 900 and 4,507 meters above sea level (Ilunga *et al*., 2004). The climate is tropical but moderated by the high altitude (Haggag *et al*., 2016).

About 80.2 % of all households in Rwanda are agricultural households involved in crop production, livestock production or both (National insititute of statistics of Rwanda, 2018). Among livestock, cattle is the most common and up to 68.8 % of rural households in Rwanda keep cattle (Ojango *et al*., 2012). Cattle is observed in all five provinces of the country with some regions keeping more cattle than others (National insititute of statistics of Rwanda, 2018). Along the important cattle keeping regions in Rwanda are also the main milk sheds. The five main milk sheds in the country are the Eastern milk shed, Southern milk shed, Northern milk shed, North-Western milk shed and Kigali milk shed (Miklyaev *et al*., 2017).

In Rwanda, the main practiced cattle production systems are zero grazing and open grazing. In the East and part of the North-West of the country with higher cattle populations and relatively more land for grazing, cattle are raised mainly under the open grazing systems in which cattle are left to graze on fenced farms. In the rest of the country with lower cattle populations and smaller holder farms, cattle are mainly raised under the zero grazing system in which cows are kept in-doors and farmers cut and carry forage, crop residues and water to feed the cows (Feed the Future Innovation Lab, 2016a; Mazimpaka, 2017). Countrywide, the zero-grazing system is the most common with 80 %, 17 % and 3 % of farms practicing zero-grazing, open grazing and semi-grazing, respectively (Land O' lakes, 2014).

To determine the sero-prevalence of *Brucella* in farm bulk milk and the risk factors of milk contamination with *Brucella* spp in Rwanda, five study Districts (Nyanza, Gicumbi, Rwamagana, Nyagatare and Nyabihu) were selected across Rwanda (Figure 2). The five Districts were selected based on their location in cattle production and milk shed areas in the country: Nyanza and Gicumbi Districts are located in the Southern and Northern milk sheds, respectively. Rwamagana and Nyagatare districts are located in the largest Eastern milk shed while Nyabihu District is located in the North-Western milk shed. The five districts were also selected to represent the two main grazing cattle production systems (zero grazing and open field grazing) practiced in Rwanda: in three of the selected districts (Nyanza, Gicumbi and Rwamagana), the zero grazing system is practiced, while in two of the selected districts (Nyagatare and Nyabihu), the open field grazing system is practiced (Land O' lakes, 2014; Mazimpaka, 2017).

4.2.3. Study population

The study population consisted of cattle farms randomly selected from the five study districts.

4.2.4. Sample size

The sample size for the number of rural cattle keeping households/farms included in the study to determine the sero-prevalence of *Brucella* in farm bulk milk and the risk factors of milk contamination with *Brucella* spp was determined as previously described in section 3.2.4.

4.2.5. Sampling

Selection of study farms or households was conducted as previously described in section 3.2.5. For each selected cattle keeping household/farm fulfilling the criteria, a questionnaire (Appendix) was administered to collect information on farm/household characteristics and potential brucellosis risk factors. After questionnaire administration, farm/household bulk raw milk was sampled and collected in duplicate sterile 15-ml conical sampling tubes.

4.2.6. Data collection

4.2.6.1. Farmers' interviews with a questionnaire

During the questionnaire (Appendix) administration at selected cattle keeping households, data was collected on the farm/household characteristics and on the potential risk factors of milk contamination with *Brucella* spp. including farm/herd management practices, cattle reproduction and farmer's awareness about brucellosis. Questionnaire data was collected using Open Data Kit (ODK) with https://ona.io as the server.

4.2.6.2. Serology with indirect ELISA on farm bulk milk samples

The collected farm bulk milk samples were submitted to laboratory analysis to determine contamination with *Brucella* spp. The presence or absence of anti-*Brucella* antibodies in collected farm bulk milk samples was determined using the SVANOVIR® *Brucella*-Ab Indirect ELISA (i-ELISA) kit. According to the manufacturer, SVANOVIR® *Brucella*-Ab I-ELISA kit detects antibodies to major species of *Brucella* (*B. abortus* and *B. melitensis*) in cattle. According to the manufacturer, the test kit's specificity with milk samples is 99 % when compared to the reference complement fixation test.

Ninety-six (96) well micro-plates coated with *Brucella* antigen were used according to detailed manufacturer's kit protocol for milk samples. For each used plate, milk samples were tested in duplicate together with a positive serum control (in duplicate) and a negative serum control (in duplicate). Following instructed additions of reagents, incubation periods and washing steps, optical densities of individual wells with milk samples and controls were measured using a micro-plate photometer (Thermo Scientific Multiscan FC, Finland) at 450 nm according to manufacturer's instructions. To determine whether a sample is positive or negative, optical density (OD) values were calculated into percent positivity (PP) values according to manufacturer's instructions:

$$\mathbf{PP = (OD_{\text{Milk sample or negative control}} / OD_{\text{Positive control}}) \times 100}$$

A used micro-plate was considered valid if (1) the duplicate OD values of the positive serum control did not differ more than 25 % from the mean value of the two duplicates, (2) the OD value of the positive serum control was > 1.0 and (3) the PP of the negative serum control was < 10. A milk sample was considered negative if its calculated PP value was < 1 or considered positive if its calculated PP value was ≥ 10.

4.2.7. Data analysis

Collected questionnaire data was exported from ODK to Microsoft Excel for data cleaning. Indirect ELISA data on prevalence of anti-*Brucella* antibodies in collected farm bulk milk samples was also entered into Microsoft Excel. Questionnaire and i-ELISA prevalence data were then coded and exported from Excel into SPSS for analysis. IBM SPSS Statistics version 20 was used to analyze data by descriptive statistics, univariate and multivariate logistic regressions. The level of significance was set at 5%.

Farm/households characteristics and farm management practices were analyzed by descriptive statistics to obtain proportions and compute averages where needed. Comparisons of characteristics and farm management practices between zero grazing and open grazing farms/households were drawn using independent samples t-test (for means comparisons) or Pearson's chi square (for proportions' comparisons). Farm/households characteristics and farm management practices were also compared to the proportions of anti-*Brucella* antibodies detection in farm bulk milks using Pearson's chi square.

To understand the associations between the different surveyed potential risk factors and farm bulk milk contamination with *Brucella* spp, binary logistic regression was used. The potential surveyed risk factors (farm characteristics and management practices) were set as independent/predictor variables while the presence/detection of anti-*Brucella* antibodies in milk (negative or positive) was set as the dependent/outcome variable. Each individual surveyed potential risk factor was run against the dependent/outcome variable using a univariable binary logistic regression model to determine the significance of association between that individual independent variable and the presence/detection of anti-*Brucella* antibodies in farm bulk milk. A risk factor was considered to be statistically significant for the presence of anti-*Brucella* antibodies in farm bulk milk, if the p-value for that association was ≤ 0.05. Following the Pearson's chi-square and univariable analyses, a multivariable logistic regression model was also used to get the effect of combination of risk factors (significant from the univariable logistic regression model) had on farm bulk milk contamination with *Brucella* and to determine which risk factors best predicted the presence of anti-*Brucella* antibodies in milk.

The odds (odds ratio, OR) of presence of anti-*Brucella* antibodies in farm bulk milk in relation to each individual risk factor were also determined with a 95 % Confidence Interval (CI). A factor was considered a risk if the OR was greater than one while also being cognizant of the level of significance being less than 0.05.

4.3. Results

4.3.1. Farm characteristics, management practices and reproductive disorders

A total of 330 farms/households were enrolled in this study. Most farms/households' respondents (76.4 %; 252/330) were male. The majority of respondents (56.4 %; 186/330) were in the age range of 41 to 60 years old with an overall mean (± standard deviation [SD]) age of 46 ± 13.0 years. The mean (SD) herd size per farm/household in the open grazing farms was 17.7 ± 5.8 cows and was significantly higher ($P < 0.05$) than the mean (SD) herd size of 2.2 ± 1.2 cows in the zero grazing farms.

Seventy-seven per cent (77.0 %; 254/330) of all visited farms/households owned at least a farm bred cow (born and raised on the farm). In the study sites (Nyanza, Gicumbi and Rwamagana districts) practicing zero-grazing cattle production system, more of the owned cows were from government and non-government donating programs with 21.2 % (42/198) of zero grazing farms having at least one cow from the government "Girinka" program which has been donating cows to poor families since 2006 (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2009a) and 10.6 % (21/198) of the same zero-grazing farms/households having at least one cow from other donating non-government programs supporting the "Girinka" program. Visited farms/households in the open grazing areas had no cows from donors, whether from the government or from non-government organizations. Natural breeding with bulls was practiced in 100 % (132/132) of visited open grazing farms and in significantly ($p < 0.05$) less zero-grazing farms (33.8 %; 67/198) (Table 13).

Reproductive disorders were recorded in farms across both zero and open grazing study sites, but at different proportions. Histories of abortion, still births and weak calves at birth were recorded significantly more ($p < 0.05$) in open grazing farms than in zero grazing farms (Table 13). Calving intervals of more than a year were recorded significantly more ($p < 0.05$) in zero-grazing farms (73.2 %; 145/198) than in open grazing farms (39.4 %; 52/132) (Table 13). The history of placenta retention was recorded in both zero grazing and open grazing farms with no significant difference ($p > 0.05$) between proportions (Table 13). External clinical signs of arthritis or hygromas that have been linked to cattle brucellosis (Musa *et al*., 1990) were observed in only one zero-grazing farm at which a cow had arthritis in the leg joints.

Among respondents from all visited farms, 63.6 % (210/330) indicated they had heard about brucellosis. Significantly ($p < 0.05$) more farmers from open grazing cattle production areas had heard about brucellosis with 75.8 % [100/132] of the respondents having heard about brucellosis compared to farmers from zero-grazing cattle production areas with 55.6 % (110/198) having heard about brucellosis (Table 13). Across all study sites, only 2.4 % (8/330) of farms had their cows vaccinated against brucellosis while 70.9 % (234/330) had not vaccinated their cows and 26.7 % (88/330) did not know whether their cows were vaccinated against brucellosis or not. The few farms which had vaccinated cows were, all, from study sites practicing the zero-grazing cattle production system (Table 13).

Table 19: Farm management practices and reproductive disorders

Description	Response	TOTAL (N=330) Proportion (%)	Zero-Grazing (N=198) Proportion (%)	Open Grazing (N=132) Proportion (%)	Comparisons: Zero grazing and Open grazing p-value
Breeding method	Bull	60.3	33.8	100	0.000*
	Artificial insemination	39.7	66.2	0.0	
History of abortion	Yes	20.9	11.1	35.6	0.000*
	No	79.1	88.9	64.4	
History of placenta retention	Yes	27.9	26.3	30.3	0.423
	No	72.1	73.7	69.7	
History of longer calving intervals (>1 year)	Yes	59.7	73.2	39.4	0.000*
	No	40.3	26.8	60.6	
History of still births	Yes	22.1	3.0	50.8	0.000*
	No	77.9	97.0	49.2	
History of weak calves at birth	Yes	2.1	0.5	4.5	0.013*
	No	97.9	99.5	95.5	
History/presence of arthritis or hygromas	Yes	0.3	0.5	0.0	
	No	99.7	99.5	100.0	
Respondent heard about brucellosis	Yes	63.6	55.6	75.8	0.000*
	No	36.4	44.4	24.2	
Vaccination against brucellosis	Yes	2.4	4.0	0.0	
	No	70.9	70.2	72.0	
	Don’t know	26.7	25.8	28.0	

*: Significant difference between compared zero grazing and open grazing proportions

4.3.2. Prevalence of anti-*Brucella* antibodies in farm bulk milks by different farm management practices and reproduction disorders

Anti-*Brucella* antibodies were detected using i-ELISA and were found to be prevalent in 19.7 % (95 % CI, 15.5 - 24.4) of all 330 collected farm bulk raw milk samples (Table 14).

Table 20: Prevalence of anti-Brucella antibodies in collected farm bulk milks

Cattle production system and Study districts	Farm bulk milk samples (Positive samples by i-ELISA)	Anti-*Brucella* antibodies sero-prevalence (proportion, %)	Anti-*Brucella* antibodies sero-prevalence (proportion, %); 95% Confidence interval
Zero-grazing			
Nyanza	66 (3)	4.5	
Gicumbi	66 (4)	6.1	
Rwamagana	66 (8)	12.1	
Total/Zero-grazing	**198 (15)**	**7.6**	**7.6 (4.3-12.2)**
Open grazing			
Nyagatare	66 (34)	51.5	
Nyabihu	66 (16)	24.2	
Total/Open grazing	**132 (50)**	**37.9**	**37.9 (29.6-46.7)**
Both systems combined	330 (65)	19.7	**19.7 (15.5-24.4)**

Farm bulk milks from farms practicing open grazing cattle production system were contaminated at a significantly ($p < 0.05$) higher proportion (37.9 %, 50/132) compared to farm bulk milks from farms practicing the zero-grazing cattle production system (7.6 %; 15/198) (Table 15).

Table 21: Proportions of *Brucella* sero-positive farm bulk milk samples by potential risk factors

Risk factors	Level	Samples (Positive); proportion	Samples (Negative); proportion	p-value
Study district	Nyanza	66 (3); 4.5	66 (63); 95.5	0.000*
	Gicumbi	66 (4); 6.1	66 (62); 93.9	
	Rwamagana	66 (8); 12.1	66 (58); 87.9	
	Nyagatare	66 (34); 51.5	66 (32); 48.5	
	Nyabihu	66 (16); 24.2	66 (50); 75.8	
Cattle production system	Zero-grazing	198 (15) ; 7.6	198 (183) ; 92.4	0.000
	Open grazing	132 (50) ; 37.9	132 (82) ; 62.1	
Herd size	1 to 2	151 (11); 7.3	151 (140) ; 92.7	0.000*
	3 to 6	44 (3); 6.8	44 (41) ; 93.2	
	> 6	135 (51); 37.8	135 (84) ; 62.2	
Breeding method	Artificial insemination	131 (14); 10.7	131 (117) ; 89.3	0.001
	Bull	199(51); 25.6	199(148) ; 74.4	
History of abortion	Yes	69 (47); 68.1	69 (22) ; 31.9	0.000
	No	261 (18); 6.9	261 (243) ; 93.1	
History of placenta retention	Yes	92 (35); 38.0	92 (57) ; 62.0	0.000
	No	238 (30); 12.6	238 (208) ; 87.4	
History of longer calving intervals (>1 year)	Yes	197 (48); 24.4	197 (149); 75.6	0.009
	No	133 (17); 12.8	133 (116); 87.2	
History of still births	Yes	73 (27); 37.0	73 (46); 63.0	0.000
	No	257 (38); 14.8	257 (219); 85.2	
History of weak calves at birth	Yes	7 (6); 85.7	7 (1); 14.3	0.000
	No	323 (59); 18.3	323 (264); 81.7	
History of arthritis or hygroma	Yes	1 (1); 100	1 (0); 0	0.043
	No	329 (64); 19.5	329 (265); 80.5	
Respondent heard about brucellosis	Yes	210 (56); 26.7	210 (154); 73.3	0.000
	No	120 (9); 7.5	120 (111); 92.5	
Vaccination against brucellosis	Yes	8 (2); 25.0	8 (6) ; 75.0	0.071
	No	234 (53); 22.6	234 (181) ; 77.4	
	Don't know	88 (10); 11.4	88 (78) ; 88.6	

*Comparisons of seropositivity between the different groups (levels) are shown below in Table 4

In terms of study districts, Nyagatare district had the highest prevalence with 51.5 % (34/66) of farm bulk milk samples containing anti-*Brucella* antibodies while Nyanza district had the lowest prevalence with 4.5 % (3/66) of farm bulk milk samples containing anti-*Brucella* antibodies (Table 14). The proportions of anti-*Brucella* antibodies prevalence in farm bulk milks from zero-grazing study districts (Nyanza, 4.5 %; Gicumbi, 6.1 % and Rwamagana, 12.1 %) were not significantly different ($p > 0.05$) when compared to each other (Table 16). Open grazing study districts had significantly ($p < 0.05$) higher *Brucella* seropositivity proportions than zero grazing study districts (Table 16). In particular, Nyagatare District had a significantly higher proportion ($p < 0.05$) of *Brucella* seropositive farm bulk milks compared to any other study district (Table 16).

Table 22: Comparisons of *Brucella* seropositivity proportions in farm bulk milks from different locations and from different herd size groups

Compared groups/levels	p-value
Location/Study districts	
Nyanza versus Gicumbi	0.698
Nyanza versus Rwamagana	0.115
Gicumbi versus Rwamagana	0.226
Nyanza versus Nyagatare	0.000*
Gicumbi versus Nyagatare	0.000*
Rwamagana versus Nyagatare	0.000*
Nyanza versus Nyabihu	0.001*
Gicumbi versus Nyabihu	0.004*
Rwamagana versus Nyabihu	0.071
Nyagatare versus Nyabihu	0.001*
Herd size groups	
1 to 2 cows versus 3 to 6 cows	0.916
1 to 2 cows versus > 6 cows	0.000*
3 to 6 cows versus > 6 cows	0.000*

*Significant difference between *Brucella* seropositivity of the compared groups/levels

The proportion of farm bulk milks contaminated with anti-*Brucella* antibodies increased as the herd size increased (Table 15) and farms with more than six cows had a significantly higher ($p < 0.05$) proportion of farm bulk milk *Brucella* seropositivity (Table 16). The proportion of Anti-*Brucella* antibodies prevalence in farm bulk milks from farms using natural breeding (25.6 %;

51/199) was significantly higher ($p < 0.05$) than the proportion of prevalence in farm bulk milks from farms using artificial insemination (10.7 %; 14/131) (Table 15). Anti-*Brucella* antibodies were detected in significantly higher proportions in farms with histories of reproductive disorders ($p < 0.05$) compared to farms with no histories of reproductive disorders (Table 3). Anti-*Brucella* antibodies were also detected in the farm bulk milk from one zero-grazing farm at which a cow presented with external brucellosis clinical sign of arthritis (Table 15).

A significantly ($p < 0.05$) higher proportion of farm bulk milks (26.7 %; 56/210) from respondent farmers who had heard about brucellosis contained anti-*Brucella* antibodies compared to the proportion of sero-positive farm bulk milks (7.5 %; 9/120) from respondent farmers who had not heard about brucellosis (Table 15). Respondents from only eight farms (8/330) indicated that their cattle were vaccinated against brucellosis, although no vaccination records were kept or shown. Out of these eight farms, anti-*Brucella* antibodies were detected in farm bulk milks from two farms (Table 15).

4.3.3. Risk factors of farm bulk milk contamination with *Brucella*

Potential risk factors for milk contamination with *Brucella* spp on which data were collected were first individually analyzed using univariable binary logistic regression to determine their associations with the prevalence anti-*Brucella* antibodies in farm bulk milks. Ten (10) potential risk factors (out of 12) were found to be statistically significant factors ($p < 0.05$) for the presence/detection of anti-*Brucella* antibodies in farm bulk milk (Table 17). They are location/study district, cattle production system, herd size, breeding method, history of abortion, history of placenta retention, history of longer calving intervals (> 1 year), history of still births, history of weak calves at birth and the respondent having heard about brucellosis.

In particular, history of reproductive disorders such as abortion and weak calves at birth were strong predictors of detection of anti-*Brucella* antibodies in farm bulk milk. The odds of detecting anti-*Brucella* antibodies in milk from a farm/household with a history of abortion were, for example, 28.8 times more (OR=28.8; 95 % CI, 14.3-57.9) than the odds of detecting anti-*Brucella* antibodies in milk from a farm/household with no history of abortion (Table 17). Practiced cattle production system was also a strong predictor and the odds of detecting anti-*Brucella* antibodies in milk from a farm/household practicing open grazing cattle production system were also 7.4 times more (OR=7.4; 95 % CI, 3.9-14.0) than the odds of detecting anti-*Brucella* antibodies in milk from a farm/household practicing zero-grazing cattle production system (Table 17).

Table 23: Univariable binary logistic regression analysis of associations between risk factors and the prevalence of anti-*Brucella* antibodies in farm bulk raw milk

Risk factor	**Level**	**p-value**	**Odds ratio (95 % Confidence Interval)**
Study district		0.000*	
	Nyanza	0.004	0.1 (0.0-0.5)
	Gicumbi	0.007	0.2 (0.0-0.6)
	Rwamagana	0.076	0.4 (0.1-1.1)
	Nyagatare	0.002	3.3 (1.6-6.9)
	Nyabihu	[a]	
Cattle production system	Zero-grazing	[a]	
	Open grazing	0.000*	7.4 (3.9-14.0)
Herd size		0.000*	
	1 to 2	0.000	0.1 (0.0-0.2)
	3 to 6	0.001	0.1 (0.0-0.4)
	> 6	[a]	
Breeding method	Artificial insemination	[a]	
	Bull	0.001*	2.8 (1.5-5.4)
History of abortion	Yes	0.000*	28.8 (14.3-57.9)
	No	[a]	
History of placenta retention	Yes	0.000*	4.2 (2.4-7.5)
	No	[a]	
History of longer calving intervals (>1 year)	Yes	0.011*	2.2 (1.2-4.0)
	No	[a]	
History of still births	Yes	0.000*	3.3 (1.8-6.0)
	No	[a]	
History of weak calves at birth	Yes	0.003*	26.8 (3.1-227.2)
	No	[a]	
History of arthritis or hygroma	Yes	1.000	6689075610 (0.0)
	No	[a]	
Respondent heard about brucellosis	Yes	0.000*	4.4 (2.1-9.4)
	No	[a]	
Vaccination against brucellosis		0.079	
	Yes	0.279	2.6 (0.4-14.6)
	No	0.026	2.2 (1.1-4.7)
	Don’t know	[a]	

*Significant risk factor; [a]: Reference value

Following univariable logistic regression analyses, a multivariable logistic regression model was used with all ten (10) significant risk factors to determine which risk factors best predicted the presence of anti-*Brucella* antibodies in farm bulk milks (Table 18). Multivariable logistic regression showed that practicing open grazing system, history of abortion, history of placenta retention and history of longer calving intervals (> 1 year) were the significant risk factors ($p < 0.05$) which better predicted the presence of anti-*Brucella* antibodies in farm bulk milk (Table 18).

Practicing open grazing system and having a history of abortion at the farm were associated with the highest odds (OR =69.5; 95 % CI, 1.6 - 3033.6 and OR =19.5; 95 % CI, 8.1 - 46.8, respectively) for the presence of anti-*Brucella* antibodies in farm bulk milk when compared to practicing zero-grazing system and having no history of abortion, respectively (Table 18). To determine the predictability of the multivariable logistic regression model, the goodness of fit of the model was tested using Hosmer-Lemeshow test and was 0.161. The overall ability of prediction of the model was 91.2 %.

Table 24: Multivariable binary logistic regression analysis of associations between all significant risk factors and the prevalence of anti-*Brucella* antibodies in farm bulk raw milk

Risk factor	Level	Multivariable logistic regression p-value	Odds ratio (95 % Confidence Interval)
Study district		0.922	
	Nyanza	0.530	0.6 (0.1-3.0)
	Gicumbi	0.907	0.9 (0.1-4.6)
	Rwamagana		
	Nyagatare	0.766	1.2 (0.3-3.9)
	Nyabihu	[a]	
Cattle production system	Zero-grazing	[a]	
	Open grazing	0.028*	69.5 (1.6-3033.6)
Herd size		0.895	
	1 to 2	0.646	1.8 (0.1-24.6)
	3 to 6	0.660	1.8 (0.1-24.8)
	> 6	[a]	
Breeding method	Bull	0.053	0.1 (0.0-1.0)
	Artificial insemination	[a]	
History of abortion	Yes	0.000*	19.5 (8.1-46.8)
	No	[a]	
History of placenta retention	Yes	0.002*	4.2 (1.7-10.3)
	No	[a]	
History of longer calving-intervals (>1 year)	Yes	0.007*	3.8 (1.4-10.2)
	No	[a]	
History of still births	Yes	0.845	1.1 (0.4-2.9)
	No	[a]	
History of weak calves at-birth	Yes	0.635	4.2 (0.0-1739.6)
	No	[a]	
Respondent heard about-brucellosis	Yes	0.584	1.3 (0.4-4.0)
	No	[a]	

*Significant risk factor; [a]: Reference value

4.4. Discussion

This study was conducted to determine *Brucella* sero-prevalence in farm bulk milk in Rwanda and to determine the risk factors associated with farm bulk milk contamination with *Brucella* spp. To determine *Brucella* prevalence in farm bulk milk, the serological method, i-ELISA, was the preferred method due to its commercial availability, sensitivity and specificity. According to the manufacturer, the used i-ELISA kit is highly sensitive and specific for *Brucella abortus* and *Brucella melitensis* (Boehringer Ingelheim Svanova) and *Brucella abortus* is the main *Brucella* species affecting cattle (Godfroid *et al*., 2014). High i-ELISA sensitivity (varying from 96 % to 100 %) and high specificity (varying from 93.8 % to 100 %) have also been reported (Gall & Nielsen, 2004; Gall *et al*., 2001).

ELISA-based tests are also known to be the most sensitive among serological tests (Geresu & Kassa, 2015; Smirnova *et al*., 2013; Zhao *et al*., 2014). However, most ELISA methods, including i-ELISA used in this study, detect antibodies against the *Brucella* smooth lipopolysaccharide and, therefore, may also detect antibodies due to vaccine strains S19 and Rev1 (Ko *et al*., 2012; Lim *et al*., 2012). This was not a setback in this study as cattle brucellosis vaccination is not yet widespread in Rwanda and in this study, very few farms (only 8/330 representing 2.4 %) indicated their cattle were vaccinated against brucellosis, although no vaccination records were kept or shown.

This study revealed that anti-*Brucella* antibodies were prevalent in farm bulk milk in Rwanda and especially in milk from open grazing farms. Previous studies investigating brucellosis in Rwanda have also reported prevalence of the disease in the country. A recent study which was conducted on cattle brucellosis in Nyagatare district using cattle sera and the Rose Bengal Test reported a prevalence of 18.9 % at individual cow level in the district (Ndazigaruye *et al*., 2018). A different study which also investigated brucellosis at an individual cow level in Kigali city reported a much lower prevalence of 2.03 % using Rose Bengal Plate test and 1.7 % using competitive ELISA (Manishimwe *et al*., 2015).

Findings in this study on anti-*Brucella* antibodies prevalence in farm bulk milk reflected, however, brucellosis prevalence at herd level (and not at individual cow level) and would be better compared against herd level cattle brucellosis prevalence. A herd level investigation on bovine brucellosis in Nyagatare district, Rwanda, reported a prevalence of 30.2 % using Rose Bengal Test on cattle sera collected from 998 cows from 205 herds in the district (Chatikoba *et al*., 2008). The current study findings in Nyagatare district (*Brucella* sero-prevalence in 51.5 % of farm bulk milks), are, therefore, higher than the cattle herd level prevalence of 30.2 % reported by Chatikoba and colleagues (Chatikoba *et al*., 2008). This indicates an increase of cattle brucellosis prevalence at herd level in Nyagatare district over the past 12 years. Sample types and sensitivities of the serological diagnostic methods used in both studies were, however, different, Chatikoba and colleagues having used animal sera and the Rose Bengal Plate Test which is less sensitive compared to ELISA-based tests (Geresu & Kassa, 2015; Smirnova *et al*., 2013; Zhao *et al*., 2014). The apparent increase in herd level brucellosis could, also, be due to the elapsed time (about 12 years) with no control measures known to have been put in place to control brucellosis. This may have, then, led to further transmission between herds over time, especially in Nyagatare district where, although most farms are fenced, the majority of farms (89.7 %) have no water at the farm or near the farm and use shared watering points (Mazimpaka, 2017). Sharing drinking water and interactions between cows from different herds cause transmission of the disease between herds (Alhaji *et al*., 2016; Aparicio, 2013; Mekonnen *et al*., 2010).

While this study is among the first study on *Brucella* prevalence in milk in Rwanda, data from a recent study on microbiological quality and safety of milk from farm to milk collection centers in Rwanda (Ndahetuye *et al*., 2020) detected anti-*Brucella* antibodies in milk samples from two milk collection centers in the Eastern province of Rwanda. Other studies carried out on human brucellosis in Rwanda have, also, associated the prevalence in humans to raw milk consumption and implied milk contamination with *Brucella* in the country (Gafirita et al., 2017; Rujeni & Mbanzamihigo, 2014).

Outside Rwanda and in the East African region, studies on *Brucella* prevalence in cattle milk have been conducted. Using diagnosis tests including MRT, i-ELISA and Real time PCR, the reported prevalence in raw milk samples from dairy farms, milk shops, street vendors, milk deliverers, boiling points, milk collection centers and dairy factories in Uganda, varied between 6.5 % and 49.45 % (Hoffman *et al*., 2016; Kamwine *et al*., 2017; Makita *et al*., 2010; Rock *et al*., 2016). Compared to this study's findings, there were similar trends between *Brucella* prevalence in milk in Uganda and in Rwanda. Results in Nyagatare district (51.5 %; 34/66) where the open grazing cattle production system is predominant are, for example, similar to the results of 49.45 % prevalence obtained from raw milk collected from dairy farms and dairy factories in Southwestern Uganda where the extensive cattle production system is predominantly practiced (Kamwine *et al*., 2017). This study's results of low prevalence of anti-*Brucella* antibodies in farm bulk milk from districts practicing zero grazing system (4.5 %, 6.1 %, 12.1 % in Nyanza district, Gicumbi district and Rwamagana district, respectively) are also similar to the prevalence of 11 % reported in Gulu district, in Uganda, where the zero-grazing cattle production was predominant (Rock *et al*., 2016). Similar studies in Tanzania investigating *Brucella* herd level sero-prevalence, using farm bulk milk and using i-ELISA to test the milk, reported a herd level prevalence of 44.4 % in Morogoro region, where cattle are mostly raised in semi-extensive and extensive production systems (Asakura *et al*., 2018).

It is obvious in this study findings that the practiced cattle production system (open grazing or zero grazing) had an effect on the level of *Brucella* sero-prevalence in farm bulk milk and practicing open grazing cattle production system was found to be a significant risk factor associated with anti-*Brucella* antibodies presence in farm bulk milk. This clear finding of significantly higher *Brucella* sero-prevalence in cattle farms of Nyagatare and Nyabihu practicing open grazing system compared to cattle farms of Nyanza, Gicumbi and Rwamagana practicing zero-grazing system was also reported in several studies on cattle brucellosis (Boukary et al., 2013; de Alencar Mota et al., 2016; Makita et al., 2011; Sagamiko et al., 2018; Shahid et al., 2014; Tadesse, 2016). The transmission and spread of cattle brucellosis is favored in areas practicing open grazing in which cattle freely interact within a herd and between herds. The spread is realized through shared grazing areas, shared bulls (if natural breeding is practiced), shared water sources, contaminated and contaminating aborted materials, vaginal discharges and manure (Aparicio, 2013; Kaur *et al*., 2018; Tekle *et al*., 2019). With regard to findings in this study, however, the high proportion of contaminated herds in open grazing areas may not be due to shared grazing areas as farms are predominantly fenced, but it may be explained by shared water sources and shared bulls. The high proportions of contaminated herds in both open grazing areas of Nyagatare and Nyabihu covered in this study were, however, significantly different when compared to each other. This may be explained by the water shortage and water sources sharing reported in Nyagatare (Mazimpaka, 2017) where the higher proportion of contaminated herds was found. Indeed, Nyagatare is faced with more water scarcity being located in the lower drier eastern lands of the country with an annual rainfall of 700 to 1100 mm and an annual average temperature of up to and beyond 30°C (Haggag *et al*., 2016; Muhire *et al*., 2014). Nyagatare district, also, experiences longer dry periods per year during which only 6 % of farmers have water on farm and the remaining majority have to trek their cattle to the nearest valley dams or rivers where the water source is shared by different herds (Mazimpaka, 2017). Nyabihu, on the other hand, has more water sources being located in the higher, more humid western lands of the country with an annual rainfall of 1300 to 1550 mm and an annual average temperature of 15 to 17°C (Haggag *et al*., 2016; Muhire *et al*., 2014). The water shortage and water sharing in Nyagatare district could, therefore, be contributing to the significantly higher prevalence proportion compared to the prevalence proportion in Nyabihu district where open grazing is also practiced.

Concerning zero grazing areas, the transmission and spread of cattle brucellosis is limited due to low level of herd-to-herd contact and small confined herds (McDermott & Arimi, 2002). In the case of Rwanda, in particular, the low sero-prevalence (7.6 %; 15/198) reported, in this study, from zero-grazing study sites could also be explained by the origin of the one to two cows per farm widely observed in the smallholder zero grazing farms. Indeed, a number of the zero grazing smallholder farms (31.8 %; 63/198) had cows from cow donating governmental ("Girinka") and non-governmental programs which have been distributing cattle to poor families while none (0 %) of the visited open grazing larger farms had cows from the donating programs. The distributed heifers from cow donating programs are screened by conducting a Rose Bengal brucellosis test for each heifer prior to distribution to farmers (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2019). Although data on animal age was not collected in this study, the one to two cows per farm observed in zero grazing farms are relatively younger. The younger cattle in the zero grazing system have, therefore, been less exposed for brucellosis contamination compared to older cows raised in open grazing farms. The young age of animals in zero grazing farms could, therefore, explain the low prevalence in zero grazing farms. Recent studies in Rwanda (Ndazigaruye *et al*., 2018; Ntivuguruzwa *et al*., 2020) have, also, found older animal age to be significantly associated with brucellosis prevalence in cows. Other factors such as zero grazing and stall feeding with limited cattle movements, preference and use of artificial insemination and additional follow-up veterinary services offered by the cattle donating programs to the benefiting farmers (Rwanda Ministry of Agriculture and Animal Resources (MINAGRI), 2019) may also contribute to preventing new brucellosis infections and would explain the low sero-prevalence in zero-grazing farms. Some studies have, however, found no statistically significant associations between cattle production systems and brucellosis prevalence. No significant difference was found in the overall prevalence of brucellosis in cattle from different grazing systems in Nyagatare, Rwanda, although more cattle brucellosis seropositive cases were reported in farms practicing extensive open grazing system than in the few farms which practice zero grazing in the district (Ndazigaruye *et al*., 2018). A higher prevalence of cattle brucellosis was reported from zero-grazing cattle production systems in Nigeria but it was argued that the higher prevalence was due to most zero grazing farms sourcing their cattle from open markets with high risks of contamination (Mai *et al*., 2012). A study in Ethiopia reported lower prevalence of cattle brucellosis in the extensive production system and this low prevalence was attributed to reduced animal-to-animal contact and to reduced contamination of pastures under dry conditions (Elemo & Geresu, 2018).

In terms of herd size and proportionally, this study detected anti-*Brucella* antibodies more in farms with more than six cows than in smallholder farms with six or less cows. Independently, the herd size was also a significant risk factor associated with anti-*Brucella* antibodies detection in farm bulk milk. Findings of this study are in line with a study conducted in Nyagatare District, Rwanda, which found that the occurrence of cattle brucellosis in herds with 40-70 cattle was 26.9 % and was significantly greater than the occurrence (14.9 %) in herds with 10-39 cattle (Ndazigaruye *et al*., 2018). Several other studies have also found herd size to have a significant effect on herd and individual cattle brucellosis prevalence and the sero-prevalence increased with herd size (Awah-Ndukum et al., 2018; Boukary et al., 2013; Makita et al., 2011; Miller et al., 2016; Sagamiko et al., 2018; Sanogo et al., 2012; Tasiame et al., 2016). In larger herds, high stocking densities and associated poor hygiene contribute to within herd brucellosis infection (Ibrahim *et al*., 2010; Omer *et al*., 2000) and the larger the herd, the more likely there will be at least one infected cow per herd causing the pooled farm bulk milk to contain anti-*Brucella* antibodies. Once a herd is infected, the infection is also likely to stay in the herd as more and more cows are exposed through common grazing lands, common water sources and contaminating aborting materials and through other interactions within the herd. Although it is generally observed that the large stocking densities in larger herds result in higher level of prevalence among larger herds, some studies found that increasing herd size did not have a significant effect on cattle brucellosis in herds (Asgedom *et al*., 2016; Elemo & Geresu, 2018).

In the present study, farms using natural breeding were proportionally and significantly more contaminated with *Brucella* than farms using artificial insemination. Natural breeding, the use of community bulls and the exchange of bull for mating between herds and cattle have been reported as major risk factors for cattle brucellosis (Alhaji *et al*., 2016; Berhe *et al*., 2007; Ebrahim *et al*., 2016). The possible contribution of bulls to *Brucella* infection in herds, in the present study, was, also, supported by the preference and use of bulls in open grazing farms in which *Brucella* antibodies prevalence in farm bulk milk was the highest. Indeed, all visited open grazing farms indicated they preferred and used bulls for breeding. Some studies have, however, reported that artificial insemination can, as well, contribute to brucellosis spreading. In a case control study involving 98 newly infected farms and 93 farms which remained brucellosis free in Colombia, for example, natural breeding with bulls from certified brucellosis free farms was safer than the use of artificial insemination, whether with frozen semen (frozen semen coming from insemination centers certified as brucellosis-free by veterinary services) or with fresh semen from un-controlled herds (Cárdenas *et al*., 2019).

In this study, anti-*Brucella* antibodies were detected in significantly higher proportions in farms with history of reproductive disorders such as abortion and placenta retention compared to farms with no history of reproductive disorders. Several other studies have also associated reproductive disorders to cattle brucellosis at animal and herd levels (Alhaji et al., 2016; Boukary et al., 2013; Hossain et al., 2014; Makita et al., 2011; Mufinda et al., 2015; Tasiame et al., 2016) and cows infected with *Brucella* have been reported to be three to four times more likely to abort than un-infected and un-exposed cows (Boukary et al., 2013; Muma et al., 2007; Schelling et al., 2003). Reproductive disorders such as abortion are known symptoms and most frequent clinical signs of brucellosis in animals including cattle (Acha & Szyfres, 2001; McDermott *et al*., 2013; Schmutz *et al*., 1996). Following infection, *Brucellae* spread in different areas and especially in the animal's reproductive system where they cause placentitis and metritis (Poester *et al*., 2013) which in turn results in abortions (Ul-Islam *et al*., 2013). Contrary to our findings, however, some studies did not find significant associations between reproductive disorders and brucellosis but did report higher brucellosis prevalences in cattle with history of reproductive disorders (Al-Majali *et al*., 2009; Ibrahim *et al*., 2010; Kebede *et al*., 2008; Makita *et al*., 2011).

The reproductive disorder of longer calving intervals (> 1 year) was a significant risk factor for anti-*Brucella* antibodies presence in farm bulk milk, as computed by both univariate and multivariate analyses of risk factors. However, it is interesting to note that longer calving intervals were more recorded in zero-grazing system farms (with significantly lower anti-*Brucella* antibodies presence in farm bulk milks) than in open grazing system (with significantly higher anti-*Brucella* antibodies presence in farm bulk milks). This means that the high occurrence of longer calving intervals reported across zero grazing and open grazing farms could be due to other factors such as irregularities in carrying out artificial insemination where it is practiced, insufficient training or experience for identifying a cow in heat and other poor husbandry management practices.

Farmers were, in general, aware of cattle brucellosis (by having heard of the infection from fellow farmers mainly). In open grazing farms where anti-*Brucella* were detected in a significantly higher proportion of farm bulk milks, farmers were even more aware of cattle brucellosis. Having heard about brucellosis by the respondent farmer did not, therefore, reduce the risk of farm bulk milk being contaminated with anti-*Brucella* antibodies. This finding is not in line with the study by Awah-Ndukum and colleagues which associated high prevalence of cattle brucellosis to farmers being not aware or not knowing about the infection (Awah-Ndukum *et al*., 2018). Findings in this study could indicate that brucellosis is known among farmers but is neglected and not considered a serious cattle infection that should be dealt with. Indeed, the WHO has classified brucellosis as one of the top neglected zoonotic diseases (WHO, 2012). Endemic zoonotic diseases, including brucellosis, are also reported to be especially neglected in low income countries (Halliday *et al*., 2015). This study's findings implying the negligence of brucellosis among farmers were, also, supported by the rate of vaccination which is still very low

(2.4 % of all visited farms) and the practice by 85.8 % of farmers of not screening replacement cows for brucellosis prior to addition to existing herds as previously reported in Nyagatare district in Rwanda (Ndazigaruye *et al*., 2018). Majority of farmers who had heard about brucellosis in this study (86.2 %), indicated they heard about the infection from fellow farmers. Hearing about brucellosis in rather informal ways from fellow farmers may also contribute to the lightness with which farmers consider brucellosis.

In this study, very few farms (eight [2.4 %] of all visited farms) indicated they had vaccinated their cattle against brucellosis, although vaccination records could not be provided and concerned farmers could not recall the specific vaccine that was used. Upon contacting and consulting local veterinary officers and Rwanda Agriculture Board (RAB) which is in charge of brucellosis vaccination program in the country, it was established the RB51, which is still the only vaccine used in Rwanda, was the vaccine administered at the eight farms. Following risk factors' analysis, vaccination status was not a significant risk factor for the presence of anti-*Brucella* antibodies in farm bulk milk. Also, the proportion of seropositive farms among vaccinated farms was not significantly different from the proportion of seropositive farms among non-vaccinated farms. The overall small number of farms with vaccinated cattle (eight out of 330 farms) in this study could be the reason for the statistically non-significant difference between the proportion of seropositivity among farms with vaccinated cattle and the proportion of seropositivity among farms with non-vaccinated cattle. Similar results of no significant difference between the prevalence of brucellosis among vaccinated and cattle and the prevalence among non-vaccinated cattle were reported by others (Nguna *et al*., 2019). Among the eight farms (out of 330 farms) which reported having vaccinated their cattle, anti-*Brucella* antibodies were detected in two farms. Antibodies produced following vaccination with brucellosis vaccines such as S19 and Rev1 can be detected by i-ELISA (Ko et al., 2012; Lim *et al*., 2012). However, antibodies produced from RB51 vaccine (which is the vaccine that was used) are different from antibodies induced by natural infection and do not interfere with brucellosis serological diagnostic methods (Dorneles *et al.*, 2015) including i-ELISA used in this study. The detection of anti-*Brucella* antibodies at the two farms, which indicated (by recalling) they had vaccinated their cattle, was not expected but could suggest a natural infection rather than a positive reaction due to vaccine's antibodies.

4.5. Conclusion

This study indicates that *Brucella* is prevalent in farm bulk milk in Rwanda as evidenced by the detection of anti-*Brucella* antibodies in 19.7 % of all farm bulk milk collected from all study sites across the country. The prevalence is especially high in farm bulk milk from open grazing farms in Nyagatare district. Beyond the prevalence of *Brucella* in farm bulk milk, there is risk of human infection as a result of consumption of raw or inadequately heat treated milk especially milk from open field grazing farms and milk from cattle with a history of reproductive disorders of abortion and placenta retention. An urgent need to plan for or reinforce animal brucellosis control measures in Rwanda is recommended.

CHAPTER FIVE: RISK OF EXPOSURE TO *BRUCELLA* AMONG MILK CONSUMERS IN CATTLE KEEPING HOUSEHOLDS OF RWANDA

Abstract

An important portion of milk produced in Rwanda is consumed in cattle keeping households and, being un-regulated, it poses un-documented food safety risks including the transmission of the zoonotic brucellosis from animals to humans. The aim of this study was, therefore, to assess the risk of exposure to *Brucella* among milk consumers in zero grazing and open grazing cattle keeping households in Rwanda. The study was a cross-sectional study which involved 198 and 132 households practicing zero grazing and open grazing cattle production systems, respectively. To assess the risk of exposure to *Brucella* through milk consumption, a questionnaire was used and collected data were analyzed using SPSS descriptive statistics and logistic regression. In nearly half (49.1 %; 162/330) of all surveyed households, raw milk was consumed. And overall, 14.2 % (47/330) of all surveyed households were exposed to *Brucella* by having at least one household member consuming raw milk while the farm bulk milk sample had turned *Brucella* seropositive. Notably and significantly ($p < 0.005$), raw milk was consumed in more open grazing households with a *Brucella* seropositive farm bulk milk sample (34.8 %; 46/132) than in zero grazing households with a *Brucella* seropositive farm bulk milk sample (0.5 %; 1/198). The proportion of open grazing households in which raw milk was consumed and from which the farm bulk milk sample was seropositive to *Brucella* (34.8 %; 46/132) was significantly higher ($p < 0.05$) compared the proportion of zero grazing households consuming raw milk and having a *Brucella* seropositive farm bulk milk sample (0.5 %; 1/198). While in total 4.8 % (77/1589) of all surveyed individual household members were exposed to *Brucella* by consuming raw milk in a household for which the farm bulk milk sample had turned *Brucella* seropositive, the cattle keeper was the household member most exposed (OR=19.9; 95 % CI, 5.9-66.2). Practicing open grazing cattle production system was significantly associated with raw milk consumption and raw milk consumption in a household with a *Brucella* seropositive farm bulk milk. The risk of exposure to *Brucella* through milk consumption exists and is high in households practicing open grazing cattle production in Rwanda. Educational campaigns are needed to raise awareness about the dangers of drinking raw milk in regards to zoonotic brucellosis.

5.1. Introduction

Brucella is a zoonotic bacteria which causes brucellosis in both animals and humans (Corbel, 2006). In animals, brucellosis causes abortion and other reproductive disorders including stillbirths, weak calves, retained placenta and longer calving intervals (Acha *et al*., 2001). Human brucellosis results in an illness and patients experience symptoms of intermittent fevers with high body temperatures, sweats, chills, weakness, malaise, headache, insomnia, anorexia and joint and muscle pain (Corbel, 2006; Pappas et al., 2006; Lampel *et al*., 2012, Acha & Szyfres, 2003).

Animals are natural hosts of *Brucella* and can become infected by *Brucellae* from different sources including aborted materials, fetal membranes, vaginal discharges, milk, manure from infected animals (Hamdy & Amin, 2002; Kaur *et al*., 2018; Langoni *et al*., 2000; Mugizi *et al*., 2015; Tekle *et al*., 2019). Human brucellosis originates from animals and one way through which brucellosis is transmitted from infected animals to humans is through the consumption of unpasteurized milk from infected animals (Dadar *et al*., 2019).

In Rwanda, an important portion of produced milk is consumed on producing farms. It was estimated that that 36.7 % of the total milk production is own-consumed by producing farmers (National institute of statistics of Rwanda, 2018). Milk consumed on farms and milk sold through other informal channels is not monitored by regulators (Kamana *et al*., 2014) and pose a risk of causing foodborne infections including the zoonotic brucellosis. Animal brucellosis exist in Rwanda with a reported individual animal prevalence varying between 0.0 % and 18.9 % (Chatikoba *et al*., 2008; Manishimwe *et al*., 2015; Ndazigaruye *et al*., 2018; Ntivuguruzwa *et al*., 2020). The authors' recent study in Rwanda and a different study on milk microbiological quality in Rwanda have, also, reported *Brucella* seroprevalence in raw milk (Djangwani *et al*., 2021; Ndahetuye *et al*., 2020).

Although figures on the consumption of raw milk and raw milk products in Rwanda are limited, a recent study reported that more than 21.7 % of cattle keepers at the wildlife-livestock-human interface indicated they drank raw milk (Ntivuguruzwa et al., 2020). More interestingly in Rwanda, human brucellosis was associated with raw milk consumption in studies conducted in two different districts (Gafirita *et al*., 2017; Rujeni & Mbanzamihigo, 2014). With the reported animal and human brucellosis, prevalence of *Brucella* in raw milk, raw milk consumption and association of human brucellosis to raw milk consumption in Rwanda, the aim of this study was to assess the risk of exposure to *Brucella* among milk consumers in zero grazing and open grazing cattle keeping households.

5.2. Materials and methods

5.2.1. Study design

A cross-sectional study was carried out where a mobile electronic structured questionnaire (Appendix) was used to collect information on households' and household members' milk consumption habits in order to assess the risk of exposure to *Brucella* through milk consumption.

5.2.2. Study sites

The study was conducted in selected districts across Rwanda. The target population was rural cattle keeping households. About 68.8 % of rural households in Rwanda keep cattle (Ojango *et al.*, 2012). Typical cattle keeping households in Rwanda own farms of an average land size of about 0.7 ha per farm (TechnoServe Rwanda, 2008; Mutimura, 2010) and two to three cows are kept on the farm (Bishop & Pfeiffer, 2008; Kamanzi & Mapiye, 2012; Miklyaev *et al.*, 2017). The rural cattle keeping households are the main producers of milk with 38.75% of all of produced milk being directly consumed on the producing dairy farms (Rutamu, 2010).

To determine the risk of exposure to *Brucella* among milk consumers in cattle keeping households in Rwanda, cattle keeping households were selected from five study districts (Nyanza, Gicumbi, Rwamagana, Nyagatare and Nyabihu) across Rwanda. The five districts were selected based on their location in the targeted milk shed areas in the country: Nyanza and Gicumbi districts are located in the Southern and Northern milk sheds, respectively. Rwamagana and Nyagatare districts are located in the largest Eastern milk shed while Nyabihu district is located in the North-Western milk shed. The five districts were also selected to represent the two main grazing cattle production systems (zero grazing and open field grazing) practiced in Rwanda: in three of the selected districts (Nyanza, Gicumbi and Rwamagana), the zero grazing system is practiced, while in two of the selected districts (Nyagatare and Nyabihu), the open field grazing system is practiced (Land O' lakes, 2014; Mazimpaka, 2017).

5.2.3. Study population

The study population consisted of milk consumers in rural cattle keeping households randomly selected from the five study districts.

5.2.4. Sample size

The sample size for rural cattle keeping households to be included in the study to determine the risk of exposure to *Brucella* through milk consumption was determined using Fischer's formula (Fisher *et al*., 1991) and as described previously in section 3.3.4. A total sample size of 330 cattle keeping households was determined. An equal sample size of 330/5 = 66 of cattle keeping households was then considered per study district (Table 7).

5.2.5. Sampling

Selection of study cattle keeping households was conducted randomly as previously described in section 3.3.5. To be considered, the randomly selected cattle keeping had to have at least one lactating cow, to have at least one household member consuming milk and/or milk products, to be willing to provide needed information, to have a household member available and able to provide the needed information for the questionnaire and to be geographically located within the district of interest. A cattle keeping household was excluded if they did not have a lactating cow, if they were not willing to provide needed information, if they did not have at least a member consuming milk and/or milk products and if there was no household member available and able to provide needed information. For each selected cattle keeping household fulfilling the criteria, a questionnaire (Appendix) was administered to collect information on household characteristics and household and household members' milk consumption habits.

5.2.6. Data collection

Questionnaire

Using a pre-prepared and pre-tested structured questionnaire (Appendix), data was collected on the household characteristics and on the milk consumption habits of the household and household's individual members. Key information collected included household's location, practiced grazing system, herd size and milk production; forms in which milk is consumed in the household, raw milk consumption at the household and household member level; forms in which individual household members consume milk and individual household members' relationships to the household head m Questionnaire data was collected using Open Data Kit (ODK) with https://ona.io as the server.

Interview

To collect data on milk consumption habits, the respondents to be interviewed at each household consisted of the household head, his/her spouse and all household members present at the time of interview. Where female spouses were the most involved in managing the produced milk and its use, the female spouse was then considered the main respondent. Answering questions related to milk consumption habits relied on re-call and during the interview the respondents (household members in this case) were allowed to consult among themselves. Before conducting the interview, respondents were informed about the objectives of the study and their right to participate or not in the study.

5.2.7. Data analysis

Collected questionnaire data on households' characteristics and milk consumption habits were exported from ODK to Microsoft Excel for data cleaning. Indirect ELISA data on prevalence of anti-*Brucella* antibodies collected in a previous study by the same authors (Djangwani et al., 2021) were also entered into Microsoft Excel and considered for risk of exposure analysis. Data on households' characteristics and milk consumption habits of the household and household members were then analyzed by Microsoft Excel for descriptive statistics to obtain proportions and compute averages where needed.

Obtained analyses on consumption proportions (in percentages) of households and household members were then presented in graphs and tables. Comparisons of raw milk consumption habits between households and household members from different study districts and study cattle production systems were drawn using Pearson's chi square and logistic regression with SPSS (IBM SPSS Statistics version 20).

The risk of exposure to *Brucella* for milk consumers in the studied households was estimated with regard to households' and household members' characteristics influencing drinking raw milk and drinking raw milk from households with *Brucella* seropositive farm bulk milk samples.

Practiced cattle production system; location of the household; gender, age, education level of the household head and household size are the characteristics that were analyzed to determine their influence on raw milk being consumed or not consumed in a given household.

To further evaluate the risk of exposure, the odds of consuming raw milk and the odds of consuming raw milk in a household with a *Brucella* seropositive farm bulk milk sample were determined for households and households' members by their different characteristics. The odds were determined using binary logistic regression (where consumption or not of raw milk and consumption or not of raw milk in a household with *Brucella* seropositive farm bulk milk were set as the dependent/outcome variables) with a 95 % confidence interval (CI).

5.2.8. Ethical considerations

Data collection from human subjects required an ethical clearance which was applied for and obtained from the Directorate of Research and Innovation, College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda. Prior to administering the questionnaire, respondents in the cattle keeping households were also explained verbally the purpose of data collection and their rights to participate or not to participate in the study. Those willing to participate in the study were further assured that their identities will remain confidential for any future presentation or publication of results.

5.3. Results

5.3.1. Cattle keeping households' characteristics

A total of 1,589 members resided in the 330 surveyed households. The average household size (members per household) was 4.8 ± 1.6 members. More than half of all households' members (61.8 %; 982/1589) were under 30 years of age. Each household was also a dairy farm and of the surveyed 330 households, 198 practiced zero grazing cattle production in their farm while 132 practiced open grazing. The average herd size was significantly high ($p < 0.05$) in households practicing open grazing (17 ± 5.8) compared to the average herd size in households practicing zero grazing (2.2 ± 1.2).

The average milk production per farm and per day was higher in open grazing households/farms (78.4 ± 27.3 liters) compared to the average milk production per farm and per day in zero grazing farms of only 7.1 ± 5.2 liters. From the daily average milk production in households practicing open grazing, 70.8 ± 27.0 liters (≈ 90.3 %) was sold while the remaining 7.6 ± 3.4 liters (≈ 9.7 %) was kept for home consumption. In households practicing zero grazing, 5.0 ± 4.8 liters (≈ 70.0 %) of the daily average milk production was sold and the remaining 2.1 ± 1.7 liters (≈ 30 %) was kept for home consumption.

5.3.2. Milk and milk products' consumption patterns

Milk and milk products were consumed in 329 of the 330 surveyed cattle keeping households. Milk was consumed as fresh raw milk, fresh boiled milk, tea milk, porridge milk, fermented milk from raw milk and fermented milk from boiled milk. In Nyagatare and Nyabihu districts, milk was also consumed in other forms including the traditional ghee and butter used for cooking and "kawunga", a maize dough preparation in which boiled milk is used to cook the dough. The most popular milk product consumed at the level of households was fresh boiled milk being consumed by at least one member in 57.3% (189/330) of all surveyed households. Fresh boiled milk was closely followed by tea milk and fresh raw milk which were consumed by at least one household member in 53.6% and 49.1% of surveyed households, respectively (Figure 4).

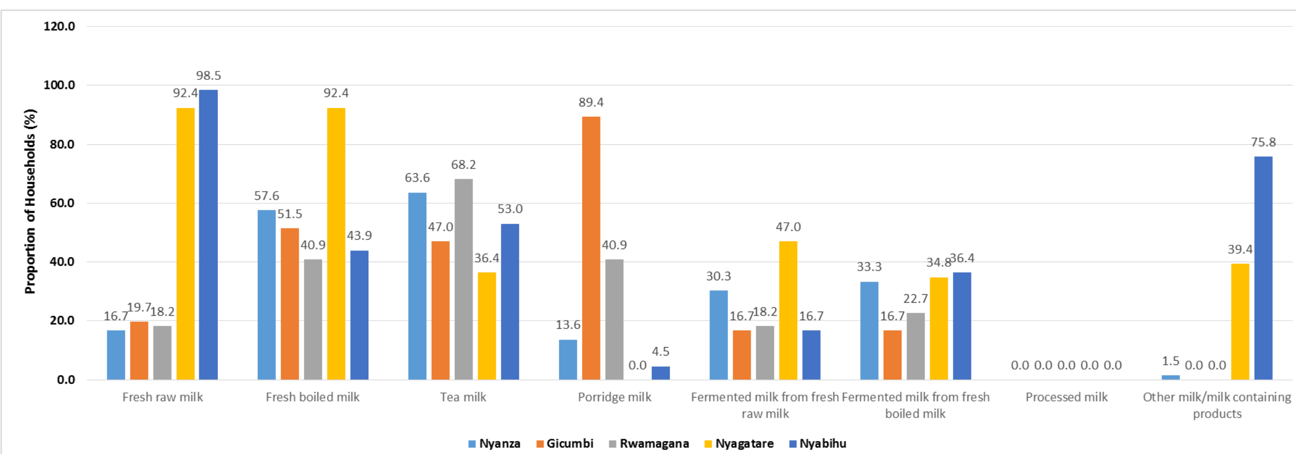

Figure 4: Proportions (%) of cattle keeping households consuming milk and milk products in different study areas in Rwanda.

The consumption of raw milk was recorded across all five study districts. The districts practicing the open grazing cattle production system (Nyagatare and Nyabihu) had the highest proportions (92.4%and 98.5%, respectively) of households in which fresh raw milk was consumed by at least one household member. The district of Nyagatare was also the district in which fresh boiled milk was consumed in most households (92.4%; 61/66). Porridge milk was popular in households from Gicumbi district with 89.4 % of households having at least a member consuming porridge milk.

The consumption of fermented milk from both fresh raw milk and fresh boiled milk was reported across all study districts with Nyagatare district having a higher proportion (47.0 %; 31/66) of households in which fermented milk from fresh raw milk was consumed. No cattle keeping household across all five study districts reported consuming industrially processed milk or milk product. Milk was also consumed in other forms, especially in the open grazing study districts of Nyagatare and Nyabihu. In Nyabihu districts, cattle keepers from 75.8 % (50/66) of surveyed households reported consuming milk in other form including mainly using fresh boiled milk for the preparation of "kawunga", a maize dough (Figure 4).

All 330 surveyed households across the five study districts had a total of 1589 members. The majority of this study population (93.8 %; 1490/1589) consumed milk or milk products. At the level of individual household members, the most popular milk products were, again, fresh boiled milk and tea milk which were consumed by 46.2 % and 44.1 % of all surveyed household members across all study households, respectively (Table 19) while raw milk was consumed by 18.3% of all household members. The proportion of household members consuming fresh raw milk was the highest in open grazing study districts with 29.8 % and 26.0 % of surveyed household members in Nyagatare and Nyabihu districts, respectively, reporting to consume fresh raw milk (Table 19).

Table 25: Proportions (%) of household members consuming milk and milk products in different study areas

	Zero Grazing			Open grazing		
	Nyanza	**Gicumbi**	**Rwamagana**	**Nyagatare**	**Nyabihu**	**TOTAL**
% HH members who consume milk or milk products	95.5	95.4	96.2	99.7	79.4	93.8
% HH members who consume:						
Fresh raw milk	11.9	15.2	6.6	29.8	26.0	18.3
Fresh boiled milk	42.8	45.8	39.9	67.2	29.2	46.2
Tea milk	59.2	40.4	62.2	20.8	44.0	44.1
Porridge milk	11.6	78.8	40.2	0.0	2.5	27.2
Fermented milk from fresh raw milk	21.5	12.3	15.4	36.6	9.7	19.8
Fermented milk from fresh boiled milk	28.3	15.2	21.0	22.1	26.7	22.4
Processed milk	0.0	0.0	0.0	0.0	0.0	0.0
Other milk/milk containing products	1.3	0.0	0.0	22.1	27.4	10.1

5.3.3. Risk of exposure to *Brucella* through milk consumption

5.3.3.1. Risk of consuming raw milk

At the level of households, raw milk was consumed by at least one household member in nearly half (49.1 %; 162/330) of all surveyed households. Raw milk was especially consumed in households practicing open-grazing cattle production system. The proportion of households practicing open grazing in which raw milk was consumed by at least one household member (95.5 %; 126/132) was significantly ($p < 0.05$) higher compared to the proportion of households practicing zero grazing in which raw milk was consumed (18.2 %; 36/198).

The practiced cattle production system; the location of the household and the gender of the household head were individually and significantly associated ($p < 0.05$) with the consumption of raw milk at the household level. The risk of raw milk consumption by at least one member in a household was especially high in households from open grazing study districts. The odds of consuming raw milk in a household practicing open grazing were, for example, 94.5 times (OR = 94.5; 95 % CI, 38.6-231.2) the odds of consuming raw milk in a household practicing zero grazing (Table 20).

Table 26: Univariable logistic regression analysis of associations between household characteristics and raw milk consumption

Household's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.000*	94.5 (38.6-231.2)
	Zero grazing	[a]	
Study district		0.000*	
	Nyanza	0.000	0.0 (0.0-0.0)
	Gicumbi	0.000	0.0 (0.0-0.0)
	Rwamagana	0.000	0.0 (0.0-0.0)
	Nyagatare	0.132	0.1 (0.0-1.6)
	Nyabihu	[a]	
HHH's gender	Female	0.005*	0.4 (0.2-0.7)
	Male	[a]	
HHH age group		0.193	
	Less than 30 years	0.045	0.1 (0.0-0.9)
	31-50 years	0.148	0.4 (0.1-1.3)
	51-70 years	0.261	0.5 (0.1-1.6)
	More than 70 years	[a]	
HHH level of education		0.016	
	No formal education	0.094	0.2 (0.0-1.2)
	Some or full primary school	0.423	0.4 (0.0-2.7)
	Some or full secondary/TVET school	0.702	0.7 (0.1-4.1)
	Some or tertiary (university or IPRC)	[a]	
HH size range		0.304	
	1-2 members	0.152	0.4 (0.1-1.3)
	3-6 members	0.700	0.8 (0.5-1.5)
	7-9 members	[a]	

*Significant risk factors

[a] Reference value

TVET: Technical and Vocational Education and Training

IPRC: Integrated Polytechnic Regional College

HHH: Household head

The individually three significant household's characteristics influencing the consumption of raw milk at household level were further analyzed with multivariable logistic regression (Table 21) to determine which household characteristics were better predictors of whether raw milk would be consumed in a cattle keeping household. The practiced cattle production system was the best household characteristic in predicting the consumption of raw milk at household level and households in which open grazing was practiced had the highest risk (OR=274.1; 95 % CI, 34.4-2181.0) of raw milk consumption by at least one household member.

Table 27: Multivariable logistic regression analysis of associations between household characteristics and raw milk consumption

Household's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.000*	274.1 (34.4-2181.0)
	Zero grazing	[a]	
Study district		0.470	
	Nyanza	0.749	0.8 (0.3-2.1)
	Gicumbi	0.919	0.9 (0.3-2.3)
	Rwamagana		
	Nyagatare	0.120	0.1 (0.0-1.5)
	Nyabihu	[a]	
HHH's gender	Female	0.191	0.5 (0.2-1.3)
	Male	[a]	

*Significant risk factors; [a] Reference value; HHH: Household head

At the level of individual household members, 18.3 % (290/1589) of all household members consumed raw milk (Table 19). The proportions of individual household members consuming raw milk were significantly higher ($p < 0.05$) in study sites practicing open grazing system (28.1 %; 181/643) compared to households' members consuming raw milk in study sites practicing zero grazing system (11.5 %; 109/946). Higher proportions of raw milk consumers were, also, recorded in the open grazing districts (Nyagatare, 29.8 % and Nyabihu, 26.0 %) compared to zero grazing study districts (Nyanza, 11.9 %; Gicumbi, 15.2 % and Rwamagana, 6.6 %) (Table 19).

The practiced grazing system, the household member's location, the household member's relationship to the household head, the household member's gender and age group were all characteristics that were significantly ($p < 0.05$) associated to the consumption of raw milk by a given household member (Table 21). The odds of consuming raw milk for household member were especially high (OR=168.0; 95 % CI, 61.4-421.0) if the household was a cattle keeper in the household (Table 22).

Table 28: Univariable logistic regression analysis of associations between household members' characteristics and household members' raw milk consumption

Household member's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.000*	3.0 (2.3-3.9)
	Zero grazing	[a]	
Study district		0.000*	
	Nyanza	0.000	0.3 (0.2-0.5)
	Gicumbi	0.001	0.5 (0.3-0.7)
	Rwamagana	0.000	0.2 (0.1-0.3)
	Nyagatare	0.290	1.2 (0.8-1.7)
	Nyabihu	[a]	
HHH's gender	Female	0.051	0.6 (0.4-1.0)
	Male	[a]	
Household member's relationship to HHH		0.000*	
	Household head	0.010	3.1 (1.3-7.7)
	Wife	0.671	1.2 (0.4-3.3)
	Child	0.001	4.2 (1.8-9.9)
	Cattle keeper	0.000	168.0 (61.4-421.0)
	Relative	[a]	
Household member's gender	Female	0.000*	0.1 (0.1-0.2)
	Male	[a]	
Household member's age group		0.000*	
	0-15 years	0.115	5.0 (0.6-37.0)
	16-30 years	0.001	27.3 (3.7-199.7)
	31-50 years	0.087	5.7 (0.7-43.2)
	51-70 years	0.173	4.1 (0.5-31.4)
	More than 70 years	[a]	

*Significant risk factors; [a] Reference value; HHH: Household head

All household member’s characteristics significantly associated with raw milk consumption by household members were further analyzed with multivariate logistic regression (Table 23) and all the household member’s significant characteristics from univariable logistic regression remained significantly associated and good predictors of raw milk consumption by a given household member. The odds of raw milk consumption household members remained the highest (OR=50.8; 95 % CI, 17.9-143.9) if the household member is a cattle keeper (Table 23).

Table 29: Multivariable logistic regression analysis of associations between household members' characteristics and household members' raw milk consumption

Household member's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.009*	2.4 (1.2-4.6)
	Zero grazing	[a]	
Study district		0.000*	
	Nyanza	0.026	2.0 (1.0-3.9)
	Gicumbi	0.000	3.4 (1.8-6.2)
	Rwamagana		
	Nyagatare	0.073	1.5 (0.9-2.6)
	Nyabihu	[a]	
Household member's relationship to HHH		0.000*	
	Household head	0.200	2.1 (0.6-6.5)
	Wife	0.405	1.6 (0.4-5.7)
	Child	0.032	2.6 (1.0-6.4)
	Cattle keeper	0.000	50.8 (17.9-143.9)
	Relative	[a]	
Household member's gender	Female	0.000*	0.3 (0.2-0.5)
	Male	[a]	
Household member's age group		0.007*	
	0-15 years	0.267	3.3 (0.3-28.4)
	16-30 years	0.079	6.6 (0.8-55.2)
	31-50 years	0.212	3.7 (0.4-30.6)
	51-70 years	0.295	3.0 (0.3-25.2)
	More than 70 years	[a]	

*Significant risk factors; [a] Reference value; HHH: Household head

5.3.3.2. Risk of exposure to *Brucella* by consuming raw milk in a household with *Brucella* seropositive farm bulk milk

At household level, at least a household member in 14.2 % (47/330) of all surveyed households was exposed to *Brucella* by consuming raw milk while the farm bulk milk sample had turned *Brucella* seropositive. The proportion of open grazing households in which raw milk was consumed and from which the farm bulk milk sample was seropositive to *Brucella* (34.8 %;

46/132) was significantly high ($p < 0.05$) compared the proportion of zero grazing households consuming raw milk and having a *Brucella* seropositive farm bulk milk sample (0.5 %; 1/198). The proportions of households consuming raw milk and having a *Brucella* seropositive farm bulk sample were also high in open grazing study districts (Nyagatare, 45.5 %, 30/66; Nyabihu, 24.2 %, 16/66) compared to zero grazing study districts (Nyanza, 0 %; Gicumbi, 0 %; Rwamagana, 1.5 %).

The household's practiced cattle production system and household location/district were significantly ($p < 0.05$) associated with consuming raw milk in a household with *Brucella* seropositive farm bulk milk (Table 24). The odds of raw milk consumption in an open grazing household with *Brucella* seropositive farm bulk milk were 105 times (OR=105.3; 95 % CI, 14.2-776.4) the odds of consuming raw milk in a zero grazing household with *Brucella* seropositive farm bulk milk (Table 24).

Table 30: Univariable logistic regression analysis of associations between household characteristics and consumption of raw milk in a household with *Brucella* seropositive farm bulk milk

Household characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.000*	105.3 (14.2-776.4)
	Zero grazing	[a]	
Study district		0.001*	
	Nyanza	0.997	0.0 (0.0)
	Gicumbi	0.997	0.0 (0.0)
	Rwamagana	0.004	0.0 (0.0-0.3)
	Nyagatare	0.012	2.6 (1.2-5.4)
	Nyabihu	[a]	
HHH's gender	Female	0.186	0.5 (0.2-1.3)
	Male	[a]	
HHH's age group		0.903	
	Less than 30 years	0.999	0.0 (0.0)
	31-50 years	0.637	0.7 (0.1-2.7)
	51-70 years	0.490	0.6 (0.1-2.3)
	More than 70 years	[a]	
HHH's level of education		0.435	
	No formal education	0.999	ND
	Some or full primary school	0.999	ND
	Some or full secondary/TVET school	0.999	ND
	Some or tertiary (university or IPRC)	[a]	
HH's size		0.660	
	1-2 members	0.998	0.0 (0.0)
	3-6 members	0.362	0.7 (0.3-1.4)
	7-9 members	[a]	

*Significant risk factors

[a] Reference value

ND: Not done

TVET: Technical and Vocational Education and Training

IPRC: Integrated Polytechnic Regional College

HHH: Household head

When the household's practiced cattle production system and the household's location were run in a multivariable model, the practiced cattle production system was the best predictor of the risk of ingesting raw milk in a household with *Brucella* seropositive farm bulk milk (Table 25).

Table 31: Multivariable logistic regression analysis of associations between household characteristics and consumption of raw milk in a household with *Brucella* seropositive farm bulk milk

Household characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.004*	20.8 (2.6-162.1)
	Zero grazing	[a]	
Study district		0.095	
	Nyanza	0.997	0.0 (0.0)
	Gicumbi	0.997	0.0 (0.0)
	Rwamagana		
	Nyagatare	0.012	2.6 (1.2-5.4)
	Nyabihu	[a]	

*Significant risk factors

[a] Reference value

At individual household member level, 4.8 % (77/1589) of all surveyed individual household members were exposed to *Brucella* by consuming raw milk in a household for which the farm bulk milk sample had turned *Brucella* seropositive. The proportion of household members in open grazing study areas who consumed raw milk in a household with a *Brucella* seropositive farm bulk milk sample (11.7 %, 75/643) was significantly higher ($p < 0.05$) than the proportion of household members in zero grazing study areas who consumed raw milk in a household with a *Brucella* seropositive farm bulk milk sample (0.2 %, 2/946).

In open grazing study districts, the proportions of individual household members consuming raw milk in households with *Brucella* seropositive farm bulk milk samples were 15.3 % (56/366) and 6.9 % (19/277) in Nyagatare and Nyabihu districts, respectively. In zero grazing study districts, there were no or very few raw milk consuming household members in households with *Brucella* seropositive farm bulk milk samples (Nyanza, 0 %; Gicumbi, 0 %; Rwamagana, 0.7 %, 2/286).

Following univariable logistic regression analysis of household member's characteristics, practiced cattle production system, household's location, household member's relationship to the household head, household member's gender and household member's age group were significantly ($p < 0.05$) associated with the risk of a household member consuming raw milk in a household with *Brucella* seropositive farm bulk milk (Table 26). With the univariable logistic regression, the odds of a household member consuming raw milk in a household with *Brucella* seropositive farm bulk milk were the highest (OR=19.9; 95 % CI, 5.9-66.2) if the household member was a cattle keeper (Table 26).

Table 32: Univariable logistic regression analysis of associations between household members' characteristics and consumption of raw milk in a household with *Brucella* seropositive farm bulk milk

Household member's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.000*	62.3 (15.2-254.7)
	Zero grazing	[a]	
Study district		0.000*	
	Nyanza	0.993	0.0 (0.0)
	Gicumbi	0.993	0.0 (0.0)
	Rwamagana	0.002	0.0 (0.0-0.4)
	Nyagatare	0.001	2.4 (1.4-4.2)
	Nyabihu	[a]	
Household member's relationship to Household head		0.000*	
	Household head	0.306	1.9 (0.5-6.9)
	Wife	0.483	0.5 (0.1-2.8)
	Child	0.764	1.2 (0.3-4.1)
	Cattle keeper	0.000	19.9 (5.9-66.2)
	Relative	[a]	
Household member's gender	Female	0.000*	0.0 (0.0-0.1)
	Male	[a]	
Household member's age group		0.000*	
	0-15 years	0.998	ND
	16-30 years	0.997	ND
	31-50 years	0.998	ND
	51-70 years	0.998	ND
	More than 70 years	[a]	

*Significant risk factors

[a] Reference value

ND: Not done

With multivariable logistic regression analysis of household member's characteristics, practiced cattle production system, household's location, household member's relationship to the household head and household member's gender remained significantly ($p < 0.05$) associated with the risk of a household member consuming raw milk in a household with *Brucella* seropositive farm bulk milk (Table 27).

Table 33: Multivariable logistic regression analysis of associations between household members' characteristics and consumption of raw milk in a household with *Brucella* seropositive farm bulk milk

Household member's characteristic	Level	p-value	Odds ratio (OR; 95 % CI)
Cattle production system	Open grazing	0.027*	5.7 (1.2-26.5)
	Zero grazing	[a]	
Study district		0.011*	
	Nyanza	0.994	0.0 (0.0)
	Gicumbi	0.994	0.0 (0.0)
	Rwamagana		
	Nyagatare	0.001	2.7 (1.5-5.0)
	Nyabihu	[a]	
Household member's relationship to Household head		0.015*	
	Household head	0.155	0.2 (0.0-1.5)
	Wife	0.614	0.5 (0.0-5.1)
	Child	0.317	0.4 (0.1-2.0)
	Cattle keeper	0.772	1.2 (0.2-5.7)
	Relative	[a]	
Household member's gender	Female	0.002*	0.0 (0.0-0.3)
	Male	[a]	
Household member's age group		0.280	
	0-15 years	0.997	ND
	16-30 years	0.997	ND
	31-50 years	0.997	ND
	51-70 years	0.997	ND
	More than 70 years	[a]	

*Significant risk factors

[a] Reference value

ND: Not done

5.4. Discussion

The study results showed that a number of milk products are consumed in cattle keeping households, all of which were not industrially processed but home prepared. Indeed, no cattle keeping household was consuming industrially processed milk products. A previous study also reported a similar trend whereby rural milk consumers use locally processed milk due to the availability of raw milk and locally processed milk products from local milk shops and neighbors; the unavailability of processed milk products in rural areas trade centers and the lack of refrigeration means needed for some processed milk products (Njarui *et al*., 2011). In the case of surveyed households in this study, the preference and exclusive use of locally or home prepared milk and milk products could, also, be due to lack of financial means to afford the processed milk products and the resulting price increase when milk is processed. Elsewhere, the increasing price of milk products when processed has, also, been reported as a reason for the low consumption of industrially processed milk products in low income households (Melesse & Beyene, 2009).

The surveyed households in this study were, all, cattle keeping implying direct availability of milk within the household. This could, also, explain the lack of processed milk products from shops and the use and preference of home prepared milk and milk products. The other possible reason to explain the absence of industrially processed milk in surveyed households is that consumers in rural households tend to prefer locally made dairy products over industrially processed ones. In a study conducted on the Rwandan traditional fermented milk "ikiviguto", for example, it was reported that Rwandan consumers prefer the locally made "ikivuguto" over related dairy products such as yoghurt and the industrialized form of "ikivuguto" due to the consistency and flavor of the traditional 'ikivuguto" (Karenzi *et al*., 2013).

With no industrially processed milk products consumed in surveyed cattle keeping households, a number of home prepared milk products were consumed. Among the milk products consumed at both household and household member levels, fresh boiled milk and tea milk were the most recorded being consumed by at least one household member in more than half of all surveyed households and by nearly half of all surveyed individual household members. The fact that boiled milk and tea milk are easy to prepare with a short amount of time could be contributing to the popularity of such milk products recorded in surveyed households. In line with this study's findings, a study conducted in Eldoret, Kenya, reported that tea milk was the predominant use of milk among households who were using milk for one or more domestic purposes (Namanda *et al*., 2009).

In this study, the consumption of raw milk was also important and the third in popularity after fresh boiled milk and tea milk. Raw milk consumption by at least one household member was recorded in almost half of all surveyed households and, interestingly, in nearly all households in which open grazing was practiced. At the level of all combined individual household members, nearly 1 in 5 household members consumed raw milk. A very similar rate of milk consumption among study population was reported in Ethiopia where 20 % of the study population indicated consuming raw milk (Deneke *et al*., 2021). Concerning the widespread raw milk consumption observed in rural open grazing households in this study, a study conducted on milk consumers in rural and urban households in Semi-arid in Kenya, also, reported that 99 % of rural households were consuming raw milk (Njarui *et al*., 2011).

Consumption of raw milk is generally not recommended due to food safety related risks and foodborne infections that have been associated with raw milk (LeJeune & Rajala-Schultz, 2009; Oliver *et al*., 2009). Despite the associated food safety risks, there are different reported reasons for consuming raw milk including preferring raw milk for its freshness, higher nutritional value, and superior taste (Amenu *et al*., 2019; Bigouette *et al*., 2018) but also constraints related to time, cost of charcoal or wood used to boil milk in rural areas have been reported as hindrances to boiling or not properly boiling milk in some rural households in Rwanda (Miklyaev *et al*., 2017). Furthermore, in the case of this study and in the same logic with other recorded popular products such as boiled milk and tea milk, raw milk could also be preferred due to its easy and quick availability.

It was interesting to observe, in this study, that some products are specifically and almost exclusively consumed in some specific areas. Porridge milk, for example, was very popular in Gicumbi district where zero grazing was practiced and un-recorded or almost un-recorded in households in Nyagatare and Nyabihu districts where open grazing was practiced. Similarly, butter, ghee and a dough preparation made of boiled milk and flour popular among cattle keepers were un-reported or almost un-reported from zero grazing study areas. This study did not investigate further to know the reasons of such different milk consumption habits between zero grazing and open grazing households. Different culture and traditions between historically cattle keepers in open grazing areas and relatively new cattle owners in zero grazing areas could explain such observed differences in milk products preferences.

The risk of raw milk consumption in a given household, in this study, was best predicted by the practiced grazing system and the risk was high if the household/farm was practicing open grazing in which cattle are left to graze on large open lands. This finding is consistent with other studies which have, also, observed that milk is widely consumed raw in pastoralist communities where open grazing is, also, practiced (Amenu *et al*., 2019; Onyango *et al*., 2021).The fact that, in this study, the risk of raw milk consumption in a given household was high if the household was practicing open grazing could be explained by quantity and availability of raw milk in households and traditions and milk consumption habits. Farmers from open grazing areas have more cattle, produce more milk, have easy access to milk and are, therefore, more exposed to milk, in general, and raw milk, in particular. Farmers practicing open grazing like pastoralists tend to, also, have a tradition of raw milk consumption and a belief that raw milk is wholesome, more nutritious and tastier (Amenu *et al*., 2019; Deneke *et al*., 2021). In addition to traditions and beliefs, raw milk requires no preparation and may therefore be opted for.

On the other hand, and as recorded in this study, households practicing zero grazing have smaller farms with about 2 cows per farm (compared to about 18 cows per open grazing farm), with a daily milk production of only 7 liters (compared to 78 liters per open grazing farm). With 70 % of the little daily milk production sold, zero grazing households remain about 2 liters for home consumption. This amount of milk may be insufficient for direct consumption as raw milk and may instead be boiled and left for smaller children to consume or be made into tea milk or porridge milk for the whole family to share. This, therefore, limits the risk of raw milk consumption in zero grazing household, not necessarily because in the household they are aware of dangers associated with milk consumption but because of insufficient available raw milk.

At the level of individual household members, more household members consuming raw milk were from open grazing areas. In this study, it was also revealed that the odds of consuming raw milk were especially high if the household member was the cattle keeper. High rates of raw milk consumption among cattle keepers have been reported in other studies. The high risk of raw milk consumption if the household member is the cattle keeper is consistent with how, among milk products, raw milk is the most available and most accessible for the cattle keeper who is usually in charge of milking the cows. Raw milk, also, requires no other energy and time consuming preparations like boiling or fermentation. The easy accessibility and no preparation could, therefore, explain why the cattle keepers were found to have the highest risk of raw milk consumption compared to other household members.

In order to further assess the risk of exposure to *Brucella*, the risk of consuming raw milk in a household which had a *Brucella* seropositive farm bulk milk sample was determined. The author of the current study studied and reported the prevalence of anti-*Brucella* antibodies in households' farm bulk milks in a previous and recent study (Djangwani *et al*., 2021). Of the 330 raw milk samples collected from the same study households/farms as in the current study, 19.7 % contained anti-*Brucella* antibodies and cattle keeping households practicing the open grazing cattle production system had a significantly ($p < 0.05$) higher proportion of farm bulk milk samples containing anti-*Brucella* antibodies compared to the proportion of farm bulk milk samples (Djangwani *et al*., 2021).

The current study revealed that grazing system was the best predictor of whether a household was exposed to *Brucella* by consuming raw milk while having farm bulk milk which had turned positive to anti-*Brucella* antibodies. The risk of consuming potentially *Brucella* containing raw milk was significantly higher in households/farms practicing open grazing system compared to household practicing zero grazing system. At the level of individual household members, the risk of consuming potentially *Brucella* containing raw milk was the highest if the member was the cattle keeper. The high exposure to *Brucella* through milk consumption established in open grazing areas in this study, is consistent with the author's previously reported data on *Brucella* prevalence in farm bulk milk and previously reported data on brucellosis prevalence in Rwandan cattle reported by others. Indeed previous cattle brucellosis studies in Rwanda reported higher rates of prevalence in areas where open grazing was predominantly practiced (Chatikoba *et al*., 2008; Ndazigaruye *et al*., 2018; Ntivuguruzwa et al., 2020) and lower prevalence rates in areas where zero grazing was predominantly practiced (Manishimwe *et al*., 2015; Ntivuguruzwa *et al*., 2020). The study by Ndazigaruye et al. which focused on Nyagatare district, for example, reported an individual cattle brucellosis rate of 19.1 % in extensively grazed cattle and 0.0 % in intensively grazed cattle. The author's previous study (Djangwani *et al*., 2021), also, reported open grazing as a significant risk factor in *Brucella* prevalence in milk and brucellosis prevalence in cattle.

In this study, being a cattle keeper was associated with the highest risk of ingesting potentially *Brucella* containing raw milk. This finding is consistent with the observation, in this study, that in households where raw milk was consumed, it was the cattle keeper who was the household member most likely to be consuming the raw milk. Not all surveyed households/farms had dedicated paid cattle keepers. The finding about the cattle keeper being the most exposed to *Brucella* contaminated milk is, also, in line with the observation that farms which had cattle keepers were open grazing farms in which *Brucella* prevalence in milk and brucellosis prevalence in cattle were high (Djangwani *et al*., 2021; Ndazigaruye *et al*., 2018). Indeed none of the zero grazing households/farms in which *Brucella* prevalence in milk and brucellosis prevalence in cattle were low (Djangwani *et al*., 2021; Ndazigaruye *et al*., 2018) had a dedicated cattle keeper. This study focused on the exposure to *Brucella* through milk consumption. It should, however, be mentioned that cattle keepers, who were found to be the most exposed in this study, can also become infected with *Brucella* due to their occupational exposure (Lytras *et al*., 2016).

5.5. Conclusions

Milk consumers in cattle keeping households in Rwanda are exposed to *Brucella* through milk consumption, especially if the consumers are from households practicing open grazing cattle production and especially if the consumer is the cattle keeper in the household. Educational campaigns are, therefore, needed in Rwanda to raise awareness about the dangers of drinking raw milk in regards to zoonotic brucellosis.

CHAPTER SIX: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1. General discussion

The broad objective of this whole research project was to assess the knowledge of brucellosis among cattle farmers, to determine the prevalence and risk factors of *Brucella* presence in milk produced under the open and zero grazing systems in Rwanda and to assess the risk of exposure to *Brucella* among milk consumers in cattle keeping households. The study was undertaken to contribute new knowledge about brucellosis knowledge among dairy farmers beyond the simple awareness question, *Brucella* prevalence in milk and risk factors of milk contamination and the risk of exposure to *Brucella* for consumers of milk in cattle keeping households where consumed milk products are not necessarily regulated by food safety bodies. Studying brucellosis from such a food safety point of view was expected to not only document the prevalence of *Brucella* in milk, but also and especially evaluate for the public the posed food safety risk, if any, and inform the country's competent authorities to reinforce or adapt brucellosis control strategies.

The study revealed that knowledge beyond having heard about brucellosis was poor; risky practices regarding brucellosis were common and attitudes towards brucellosis were rather encouraging. While many among farmers, and especially farmers from open grazing study areas, would have heard about brucellosis, the study found that very few knew about Brucellosis. Indeed, with selected brucellosis knowledge questions on ways through which brucellosis is contracted by cattle, brucellosis clinical signs and common livestock animals which can contract the disease, very few respondents could state at least two ways through which cattle contact brucellosis, two brucellosis clinical signs and two livestock animals which can contract brucellosis. One could argue that a farmer having heard about brucellosis does not necessarily mean he or she has the basics to prevent, identify or manage brucellosis at the farm. The few respondents who had further knowledge in terms of brucellosis transmission pathways, clinical signs and brucellosis susceptible livestock animals were more from open grazing study areas. This is consistent with their accumulated experience rearing cattle and the likely exposure to cases of brucellosis or suspected brucellosis in their herds.

Risky practices regarding brucellosis were recorded in this study. Only a small percentage of cattle are screened for brucellosis prior to introduction in the herd, only 5% of respondents use PPE while assisting in parturition or handling aborted materials, many among those who experienced abortion in their cattle give or leave the aborted materials to the dogs, all open grazing farms practice natural breeding and, overall, more than half of farms practice natural breeding, only 8 out of 330 study farms had vaccinated their cattle against brucellosis and raw milk is consumed in almost half of all surveyed farms and in up to 95.5 % of surveyed open grazing farms.

Despite poor knowledge and risky practices, farmers' attitudes towards brucellosis were interestingly encouraging with the majority among respondents indicating they would seek help from vets if their animals had or were suspected of brucellosis. Trading or selling to abattoir a brucellosis suspected animal were the bad attitudes stated by some few respondents and would only result in spreading the disease to more herds and even to humans through abattoir's workers.

The current study revealed that anti-*Brucella* antibodies are significantly more prevalent in farm bulk milk from open grazing farms compared to farm bulk milk from zero grazing farms with, notably, more than a third of milk from open grazing farms in Rwanda containing *Brucella* antibodies. Practicing open grazing system and history of abortion and placenta retention are significant risk factors for the presence of anti-*Brucella* antibodies in milk. The transmission and spread of cattle brucellosis in areas practicing open grazing is favored and brucellosis spreading is realized through shared grazing areas, shared bulls (if natural breeding is practiced), shared water sources, contaminated and contaminating aborted materials, vaginal discharges and manure. With regard to findings in this study, however, the high proportion of contaminated herds in open grazing areas may not be due to shared grazing areas as farms are predominantly fenced, but it may be explained by shared water sources and shared bulls.

In the case of Rwanda, in particular, the low sero-prevalence from zero-grazing study sites could be explained by the origin of farm cattle. More among zero grazing farms had sourced their cows from donating governmental ("Girinka") and non-governmental programs which have been distributing cattle to poor families. The distributed heifers are screened by conducting Rose

Bengal brucellosis test for each heifer prior to distribution to farmers. Other factors such as stall feeding with limited cattle movements, preference and use of artificial insemination and additional follow-up veterinary services offered by the cattle donating programs to the benefiting farmers may also explain the low sero-prevalence in zero-grazing farms.

Reproductive disorders of abortion and placenta retention that were significantly associated to high anti-*Brucella* antibodies prevalence in milk in this study, have also been associated to cattle brucellosis at animal and herd levels in several other studies. Reproductive disorders such as abortion are, known symptoms and most frequent clinical signs of brucellosis in animals including cattle.

This study revealed that 14.2 % of all surveyed households were exposed to *Brucella* by having at least one household member consuming raw milk while the farm bulk milk sample had turned *Brucella* seropositive and 4.8 % of all surveyed individual household members were exposed to *Brucella* by consuming raw milk in a household for which the farm bulk milk sample had turned *Brucella* seropositive. Practicing open grazing cattle production system and having the role or job of being a cattle keeper in the household were significantly associated with being exposed to *Brucella* by consuming raw milk in a household with a *Brucella* seropositive farm bulk milk. The high exposure to *Brucella* through milk consumption established in open grazing areas in this study, is consistent with data on high *Brucella* prevalence in open grazing farm bulk milks and previously reported data on brucellosis prevalence in Rwandan cattle.

Cattle keepers were the household members most exposed to *Brucella* through milk consumption. This finding is consistent with the study’s observation that in households where raw milk was consumed, it was the cattle keeper who was the household member most likely to be consuming the raw milk. Not all surveyed households/farms had dedicated paid cattle keepers. The finding about the cattle keeper being the most exposed to *Brucella* contaminated milk is, also, in line with the observation that farms which had cattle keepers were open grazing farms in which anti-*Brucella* antibodies prevalence was high in farm bulk milk. Furthermore none of the zero grazing households/farms in which anti-*Brucella* antibodies prevalence was low had a dedicated cattle keeper. The high risk of raw milk consumption if the household member is the cattle keeper is, also, consistent with how, among milk products, raw milk is the most available and most accessible for the cattle keeper who is usually in charge of milking the cows. Raw milk, also, requires no other energy and time consuming preparations like boiling or fermentation. The easy accessibility and no preparation could, therefore, explain why the cattle keepers were found to have the highest risk of raw milk consumption compared to other household members.

6.2. General conclusions

Beyond having heard about brucellosis, it was established that brucellosis knowledge among farmers in Rwanda is generally poor across both zero grazing and open grazing study areas. *Brucella* is prevalent in farm bulk milk in Rwanda and the prevalence of anti-*Brucella* antibodies is notably high in farm bulk milk from open grazing farms. The risk of exposure to *Brucella* through milk consumption exists in cattle keeping households in Rwanda and open grazing households/farms are significantly more exposed to *Brucella* through the consumption of raw milk.

6.3. Recommendations

To intervene against poor brucellosis knowledge and the risk of exposure to *Brucella* through milk consumption, it is recommended that, through the Ministry of agriculture (MINAGRI) and Districts' Animal Resources Officers (DAROs), educational programs be designed and delivered to farmers to raise awareness and knowledge about brucellosis.

To intervene against *Brucella* prevalence in farm bulk milk in Rwanda, it is recommended that brucellosis prevalence in livestock be controlled by intensifying and scaling up brucellosis vaccination starting with open grazing areas in which brucellosis prevalence is high. It is, also, recommended that MINAGRI reviews the brucellosis vaccination provisions in the animal health law published in 2009 and improves into a systematic, continual and consistent brucellosis control strategy

To ensure further research to culturally and molecularly identify the prevalent *Brucella* species and devise more informed control strategies, it is recommended that research institutions including RAB and the University of Rwanda (UR) work in collaboration to put in place an appropriate and dedicated laboratory facility to study zoonotic bacteria including *Brucella*.

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Appendix: Questionnaire on brucellosis knowledge, attitudes, practices and risk factors and milk consumption habits

A. General information

1. Household head (HHH)
    a) Name:
    b) Gender:
    c) Age:
    d) Education level:
2. Respondent's relationship to HHH:
    a) HHH
    b) Wife
    c) Child
    d) Relative/Friend
    e) Worker/Cattle keeper
3. Respondent's gender
    a) Male
    b) Female
4. Respondent's age (years):
5. Respondent's level of education:
    a) No education
    b) Primary school

c) Secondary school

d) TVET

e) IPRC

f) University

6. Location of the household/farm:

Village:	
Cell:	
Sector:	
District:	

7. How many cows do you own/have at the farm?

8. Cattle herd composition

Category	Number
Calves (< 6 months)	
Heifers (6 to 18 months)	
Bulls	
Lactating cows	
Dry cows	
Cows in gestation	

9. What grazing system do you practice?

   a) Zero/intensive grazing system

   b) Open/Extensive grazing system

B. Knowledge/awareness and attitudes towards brucellosis

10. Have you heard about brucellosis in farm animals?

    a) Yes

    b) No

11. From where did you hear about brucellosis

    a) News (Radio, newspaper, TV)

    b) Fellow farmer/Neighbour/family member/Friend

    c) VET/Animal health worker

    d) Training

    e) Education/Schooling

12. How do farm animals contract brucellosis

    a) From feed/watering

    b) From faeces/dung

    c) From contact with infected animals

    d) From bulls (natural insemination)

    e) From other sources

    f) Don't know

13. What are brucellosis clinical signs in farm animals

a) Abortion

b) Abortion during late gestation

c) Weak calves

d) Still birth

e) Low calving rate

f) Retained placenta

g) Other (Specify)

14. Which farm animals can become infected with brucellosis?

a) Cattle

b) Sheep

c) Goats

d) Other (Specify)

e) Don't know

15. How can you prevent brucellosis in farm animals

a) Hygiene in animal housing

b) Separation from other animals and practicing 0-grazing

c) Isolation of infected animals

d) Treatment of infected animals

e) Slaughter of infected animals

f) Vaccination of healthy animals

g) Regular animal testing

h) Don't know

16. What would you do if your animal had or was suspected of having brucellosis?

a) Selling

b) Slaughtering

c) Seek help from vets

d) Separate animal

e) Leave animal to heal itself

f) Treat traditionally

g) Don't know

17. Are you aware that brucellosis can be transmitted from farm animals to humans?

a) Yes

b) No

18. What are the transmission routes of brucellosis from farm animals to humans?

a) Drinking milk from infected animal

b) Drinking raw milk from infected animal

c) Contact with infected animal

d) Air from infected animal

e) Handling faeces from infected animal

f) Touching or drinking blood from infected animal

g) Eating meat from infected animal

h) Eating undercooked meat from infected animal

i) Helping in parturition

j) Other modes of transmission (Specify)

k) Don't know

C. Farm management practices

19. What is the origin of your cattle?

a) Farm bred

b) Purchased

c) Girinka (govt' cow donation project)

d) Cow donating NGOs

e) Gift from friends/family

f) Kept for others "Indagizo"

20. Did any of your farm animals abort?

a) Yes

b) No

21. If one of your farm animals aborted, indicate the following

Animal species	Stage of abortion
Cattle	
Goat	
Sheep	

Stages of abortion:

a) Early gestation

b) Mid gestation

c) Late gestation

d) Not sure

22. If one your farm animals aborted, what did you do with the aborted materials

a) I buried the aborted materials

b) I gave the aborted materials to dogs

c) Aborted materials were taken/stolen by dogs

d) Other (Specify)

23. Are PPE used when assisting parturition or when handling aborted materials?

a) Yes

b) No

24. Have you observed any of the following in your farm animals?

a) Placenta retention

b) Longer calving interval (>1 year)

c) Still births

d) Weak calves at birth

e) Arthritis

f) Hygromas

25. Which breeding methods do you use?

a) Artificial insemination

b) Bull (natural breeding)

c) Artificial insemination and bull

26. Are your cattle vaccinated against brucellosis

a) Yes

b) No

c) Don’t know/Don’t remember

D. Milk consumption habits

27. How many household members (residing in the household for at least 6 months)?

28. Milk production estimations

Litres of milk produced per day	Litres of milk sold/given away	Litres of milk consumed at home

29. In which form (s) is milk consumed in the household?

a) Fresh-raw

b) Fresh-boiled

c) Tea milk

d) Porridge milk and milk products

e) Sour/fermented milk (from fresh-raw milk)

f) Sour/fermented milk (from fresh-boiled milk)

g) Processed

h) Other (Specify)

i) Cheese (from fresh-boiled milk)

j) Processed (from dairy industry)

k) Other form (specify)

30. Milk consumption habits for individual household members

Household member [A]	Household member relationship to the HHH[B]	Gender	Age	Milk product consumed [C]	Frequency of consumption [D]	Volume/consumption [D]

[A] HH members are those leaving in the household for at least 6 months a year and will be categorized/qualified as: HHH, wife, child, relative, friend, worker or cattle keeper

[B] Household member's relationship to the HHH will be categorized/qualified as: HHH, wife, child, relative, friend, worker or cattle keeper

[C] Milk product consumed is one or more of the following: Fresh raw milk, fresh boiled milk, tea milk, porridge milk, sour/fermented milk (from fresh-raw milk), sour/fermented milk (from fresh-boiled milk), processed milk and milk products, other milk product (to be specified)

[D] Frequency and volumes can be estimations