EPIDEMIOLOGICAL STATUS OF BRUCELLOSIS AND ITS IMPACT ON ABORTIONS IN HUMANS AND DOMESTIC RUMINANTS IN KAGERA ECOSYSTEM OF TANZANIA.

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EXTENDED ABSTRACT

Brucellosis is a zoonotic disease of both public health and economic importance. Little is known about the local understanding of brucellosis by pastoralists as well as its status and impact in the Kagera region of Tanzania. Extended study was carried out to determine the epidemiological status of brucellosis and estimate its impact on pregnancy outcome in Kagera region, which is a part of the Kagera river basin ecosystem shared by Burundi, Rwanda, Uganda and Tanzania. Specifically, this study aimed to assess the knowledge, perception and practices related to brucellosis among pastoralists in Kagera region; to estimate the prevalence of brucellosis in humans, domestic ruminants in Kagera region; to determine the contribution of brucellosis to abortions in humans and domestic ruminants in Kagera region and to characterize the different *Brucella* species prevalent in humans and domestic ruminants in Kagera region. Firstly, a cross-sectional study was conducted in June 2017 to assess the knowledge, perception and practices on brucellosis among pastoralists of Kagera ecosystem, Tanzania, using qualitative methods. Focus group discussions were conducted with livestock farmers, administration leaders, religious representatives and youth and discussions with key informants were conducted, involving officials of livestock, wildlife and public health departments in each district. Using a content analysis with inductive and deductive methods, this study revealed low knowledge on causes, symptoms and mode of transmission of brucellosis by interviewees. Pastoralists perceived the interactions between humans, livestock and wildlife; also the movement of people and animals crossing borders, to be potential risks for introduction of brucellosis in their communities. Moreover, habits of drinking raw milk, assisting parturitions in animals without protective gears and absence of vaccination programs are practices which could increase the transmission of brucellosis in this area. Secondly, the magnitude of brucellosis and its associated risks factors in humans and domestic ruminants in this region were

estimated. The estimation of brucellosis seroprevalence was important for any action plan regarding the reduction of the socio-economic impact of the disease in the study area. Human sera were analyzed using Rapid Slide agglutination and Fluorescence Polarization Assay (FPA) tests, while animal sera were screened using Rose Bengal Plate (RBPT) and confirmed by the c-ELISA test. Out of 156 patients with malaria-like symptoms screened for brucellosis, 7.7% (95% CI: 3.8-12.2%) had antibodies consisting 1.9 % (95% CI: 0.0-4.5%) and 5.8 % (95% CI: 2.6-12.6%) for *B. abortus* and *B. melitensis*, respectively. At individual animal level, brucellosis was prevalent at 5.9% (95% CI: 4.0-8.6%), 2.5% (95% CI: 0.8-5.7%) and 0.5% (95% CI: 0.01-2.8%) in cattle (n=426), goats (n=206) and sheep (n=197), respectively. At herd level, brucellosis seroprevalence was 18.2% (95% CI: 12.0-25.8%) in cattle and 6.9% (95% CI=2.2-15.3%) in small ruminants. In humans, brucellosis was associated with the assisting in parturition without wearing protective gears (OR= 5.6; p= 0.02). Seropositivity to *Brucella* was associated with bovine species (OR=3.5; p=0.01), specifically in herds of medium size with 50-200 animals (OR= 4.2, p= 0.01). The knowledge of brucellosis among pastoralists (OR=0.1; p=0.007) was a protective factor.

Thirdly, a prospective cohort study involving pregnant women and gravid ruminants was conducted to understand the association of brucellosis with pregnancy outcome. The abortion rate was 11.8% and 12.3% in humans and in ruminants, respectively. Positivity to both RBPT and FPA tests was 21% (95% CI: 12.5-32) in pregnant women (n=76) and 5% (95% CI: 3.1-8) in gravid ruminants (121 cattle, 125 goats and 111 sheep). Among abortive cases, 4 women (out of 9), 2 cows (out of 7), 2 goats (out of 26) and zero sheep (out of 11) were positive to brucellosis. Seropositivity for anti-*Brucella* antibodies was likely similar in aborted and non-aborted cases in humans (p=0.08) and in ruminants (p=0.2). Seropositivity to *Brucella* was associated with a risk of exposure to brucellosis in

pregnant women (OR=19; 95% CI: 1.8-203, p=0.01) also in gravid cow (OR=11; 95% CI: 1.3-18, p=0.02). However, absence of malaria-like symptoms in pregnant women (OR=0.12; 95% CI: 0.0-1.2, p=0.07) and the good disposal of aborted materials in gravid ruminants (OR=0.2; 95% CI: 0.0-1.1, p=0.06) were protective for *Brucella* infections. At population level, brucellosis was associated with abortions (Population attributable risk: PAR) at 3.5% in pregnant women and at 0.5% in gravid ruminants in the study area.

Lastly, *Brucella* species were characterized using genomic DNA which was obtained from samples (of humans and domestic ruminants) positive to serology tests. Molecular-based methods including real-time PCR, PCR amplification of 16S rRNA genes and Sanger sequencing were used. *Brucella* spp. was detected in 47 out of 125 sera and milk samples using real-time PCR. *Brucella* species were detected in raw milk of cows and goats, which could be a possible route of transmission to humans. In addition, presence of *Brucella* in sera from abortive woman supports the suspected contribution of brucellosis to reproductive failures in the study area; despite the limited sample size. Twenty out of 47 samples showed amplification of 16S rRNA gene to PCR. Sequence analysis and blasting confirmed the presence of *Brucella* spp. in pastoral areas of Kagera region. Despite the lack of proof for an epidemiological relationship, in this study, all the clades with Tanzanian sequences connected from clade with sequences of *B. melitensis, B. abortus* and *B. suis* from USA, Sudan and Iran. However, the *Brucella* from Kagera were phylogenetically distinct from other species isolated in USA, New Zealand, Germany and Egypt. This was expected based on the distance between the geographical regions from which the data for the phylogeny reconstruction were obtained. This is the first study to report 16S rRNA gene sequencing of *Brucella* species in East and Central Africa. A livestock vaccination program re-inforced with a high index of *Brucella* diagnosis is needed to eradicate brucellosis in animals and minimize exposure to *Brucella* infections in humans in Kagera river basin

ecosystem. A coordinated One Health approach is recommended and further studies are suggested to reveal further the status of brucellosis in Kagera ecosystem that will guide its control and prevention.

The results from this study have a potential of contributing to the reduction of economic and reproductive burden of brucellosis among pastoral communities, the planning of joint cross-border collaboration in controlling brucellosis in East Africa Community and this promoting safety in animal trade and transactions.

DECLARATION

I, Jean-Bosco Ntirandekura, do hereby declare to the senate of the Sokoine University of Agriculture that this thesis in my own original work and it has neither been submitted for a degree to other institutions.

Jean-Bosco Ntirandekura — 30 /06/ 2020
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The above declaration is confirmed by

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DEDICATION

This thesis is dedicated to my mother Adèle Nzisabira and my late father Simon Ntamasambiro who initiated my education program in the early days of my life. I am who I am because of both of you.

LIST OF PUBLICATIONS

1. Ntirandekura, J. B., Matemba, L. E., Kimera, S. I., Muma, J. B., and Karimuribo, E. D. (2018). Association of brucellosis with abortion prevalence in humans and animals in Africa: a review. ***African journal of reproductive health*** **22(3): 120-136.**
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4. Ntirandekura, J. B., Matemba, L. E., Kimera, S. I., Muma, J. B., and Karimuribo, E. D. (2019). Association of brucellosis to abortions in humans and domestic ruminants in the Kagera ecosystem, Tanzania. ***Transboundary and emerging diseases*** **(DOI: 10.1111/tbed.13516)**
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LIST OF ABBREVIATIONS AND SYMBOLS

CI	Confidence Interval
CRE	Cases at Risk of Exposure
DNA	Deoxyribonucleic acid
DVM	Department of Veterinary Medicine and Public Health, SUA
EAC	East African Community
ELISA	Enzyme-Linked Immunosorbent Assay
FGD	Focus Group Discussion
FMD	Foot and Mouth Disease
FPA	Fluorescence Polarization Assay
IBM®	International Business Machines Corporation
KI	Key Informants
KII	Key Informant Interview
MCL	Maximum Composite Likelihood
MLVA-VNTR	Multiple-Locus Variable Number Tandem Repeat Analysis
Mm	Millimeter
MRT	Milk Ring Test
MSA	Multiple Sequence Alignment
NC	Negative Control
NIMR	National Institute for Medical Research
NR	Not Reported
OR	Odds Ratio
P	p-level
PAR	Population Attributable Risk

PC	Positive Control
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RD	Risk Difference
RR	Relative Risk
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RVF	Rift Valley Fever
SAT	Serum Agglutination Test
SPSS®	Statistical Package for the Social Sciences
SUA	Sokoine University of Agriculture
TTP	Tanzanian Technical Partnership
TVLA	Tanzanian Veterinary Laboratory Agency
UK	United Kingdom
US $	United States Dollar
USA	United States of America
WHO	World Health Organization

CHAPTER ONE

General Introduction

1.1 Background

Brucellosis is a bacterial disease caused by *Brucella spp.* The sources of infection for animals include aborted materials, vaginal discharges, milk and semen from infected animals. Currently, there are recognized 12 *Brucella* species (El-Sayed and Awad, 2018), and six of them, are known to be pathogenic to humans: *B. abortus, B. canis, B. inopinata, B. melitensis, B. pinnipedialis, and B. suis* (Tiller *et al.*, 2010). Brucellosis is transmitted to people through contact with infected animals or consumption of poorly cooked animal products. Brucellosis remains a major problem in the Mediterranean region, Western Asia, parts of sub Saharan Africa and Latin America (Corbel, 1997). The disease is a zoonosis of veterinary and public health importance with economic significance in most developing countries (WHO, 1997). These studies have reported different prevalences in Uganda (Bernard *et al.*, 2005; Makita *et al.*, 2010) and Kenya (Kadohira *et al.*, 1997; Ogola *et al.*, 2014), influenced by the locations, the methods of diagnosis used and according to species of interest. Brucellosis has been recognised as one of the major zoonotic diseases in Tanzania (Swai and Schoonman, 2009; Shirima *et al.*, 2014; Mathew *et al.*, 2015).

1.2 Problem Statement and Justification

In Tanzania, the effectiveness of control measures and prevention of brucellosis in humans and domestic animals has to be supported by a good understanding of the epidemiological status of the disease at national level. However, animal species interacting in the same ecosystems are at risk to get brucellosis infections in pastoral areas (Fyumagwa *et al.*, 2009) including Kagera ecosystem. Brucellosis has been reported as a zoonotic disease in

Tanzania (Swai and Schoonman, 2009; Kunda *et al.*, 2010; Assenga *et al.*, 2015). In Karagwe district, brucellosis was reported in trade stock (Kiputa *et al.*, 2008), however, research studies on brucellosis are scarce in Kagera region which is an ecosystem with strong movements of people and their livestock (United Republic of Tanzania, 2019). It is stated that the increase in animal products demand has the consequences of spreading transboundary animal diseases (Otte *et al.*, 2004).

Furthermore, there is a lack of information on how this disease is contributing to abortions in humans and domestic ruminants in the Kagera ecosystem. It is also unclear how the population and various stakeholders in the ecosystem perceive the brucellosis risk; as well as the socio-economic impact on productivity (reduction of milk production, abortions, infertilities, costs of vaccines and treatments). If the epidemiological factors associated with brucellosis transmission in the ecosystem are well understood, the information will be used to plan for control interventions, thereby improving risk management by policymakers and stakeholders in the area. As the study area is part of Kagera River basin ecosystem shared between Burundi, Tanzania, Rwanda and Uganda, this study looks forward generating information required for planning of joint cross-border collaboration in controlling brucellosis in the East African Community and thus promoting safety in the animal trade. The association of brucellosis with abortions in a farm of dairy cattle was previously reported in Mbeya by Mathew *et al.* (2015), but not in humans. However, there is a gap in understanding how brucellosis could be associated with reproductive failures in humans and their livestock in pastoral settings in Tanzania. Therefore, brucellosis in Kagera ecosystem is calling for researcher's attention with a perspective of extending the knowledge on the magnitude of the disease and its contribution to abortions occurring in humans and domestic ruminants in this area.

1.3 Research Questions

i. What is the knowledge, perception and practices of pastoralists regarding brucellosis in Kagera region?

ii. What is the prevalence of brucellosis in humans and domestic ruminants in the Kagera region?

iii. Does brucellosis contribute to abortions in humans and domestic ruminants in the Kagera region?

iv. What are *Brucella* species prevalent in humans and domestic ruminants in the Kagera region?

1.4 Study Objectives

1.4.1 Overall objective

The overall objective of this study was to establish the epidemiological status of brucellosis and estimate its impact on abortions in humans and livestock in Kagera region, for better understanding the distribution and impact of the disease and possible management strategies.

1.4.2 Specific objectives

i. To assess the knowledge, perception and practices regarding brucellosis in the Kagera region.

ii. To estimate the prevalence of brucellosis in humans and domestic ruminants in the Kagera region.

iii. To determine the association of brucellosis with abortions in humans and domestic ruminants in the Kagera region.

iv. To identify the different *Brucella* species prevalent in humans and domestic ruminants in the Kagera region.

1.5 References

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CHAPTER TWO

Paper One

Association of Brucellosis with Abortion Prevalence in Humans and Animals in Africa: A Review

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REVIEW ARTICLE

Association of Brucellosis with Abortion Prevalence in Humans and Animals in Africa: A Review

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Abstract

Brucellosis is a worldwide zoonotic disease suspected to be the cause of abortions which remain largely undiagnosed in both humans and animals. A review of literature was performed to elucidate the contribution of brucellosis to abortions in humans, livestock and wildlife in Africa. A total number of 18 published articles associated brucellosis to abortions observed in humans and livestock in some parts of Africa. The contribution of brucellosis to abortions in humans was less reported in the literature compared to livestock, and no report was done in wildlife in Africa. The association of brucellosis to abortions in Africa was mostly based on bacteriologic, serologic or molecular techniques and *Brucella abortus biovar* 3 seemed more associated to abortions in cattle. The isolation and molecular characterization of *Brucella* species could advance the assessment of the contribution of brucellosis to abortions in Africa, focusing much in humans. The epidemiologic approach based on case-control comparisons could elucidate more about the contribution of brucellosis to abortions in Africa. The economic impact evaluation of abortions due to brucellosis could justify implementation of eradication programs of this disease in Africa, such as occupational and food hygiene in humans; with a vaccination and culling in animals. *(Afr J Reprod Health 2018; 22[3]: 120-136).*

Keywords: Spontaneous abortions, Brucellosis, mammals, Africa

Résumé

La brucellose est une maladie zoonotique mondiale soupçonnée d'être à l'origine d'avortements qui restent largement non diagnostiqués chez l'homme et chez l'animal. Une revue de la documentation a été réalisée pour élucider la contribution de la brucellose aux avortements chez l'homme, le bétail et la faune en Afrique. Au total, 18 articles publiés ont associé la brucellose aux avortements observés chez l'homme et le bétail dans certaines régions d'Afrique. La contribution de la brucellose aux avortements chez l'homme était moins rapportée dans la documentation par rapport au bétail; et aucun rapport n'a été fait sur la faune sauvage en Afrique. L'association de la brucellose aux avortements en Afrique était principalement basée sur des techniques bactériologiques, sérologiques ou moléculaires et Brucella abortus biovar 3 semblait davantage associés aux avortements chez les bovins. L'isolement et la caractérisation moléculaire des espèces de Brucella pourraient faire progresser l'évaluation de la contribution de la brucellose aux avortements en Afrique, en se concentrant beaucoup sur l'homme. L'approche épidémiologique basée sur des comparaisons cas-témoins pourrait élucider davantage la contribution de la brucellose aux avortements en Afrique. L'évaluation de l'impact économique des avortements dus à la brucellose pourrait justifier la mise en œuvre des programmes d'éradication de cette maladie en Afrique, tels que l'hygiène professionnelle et alimentaire chez l'homme; avec la vaccination et l'élimination chez les animaux. *(Afr J Reprod Health 2018; 22[3]: 120-136).*

Mots-clés: Avortements spontanés, brucellose, mammifères, Afrique

Introduction

Brucellosis is a zoonosis of both veterinary and public health significance with an economic impact on livestock production in most developing countries[1]. This disease, which has a worldwide distribution, is caused by Gram-negative bacteria of the genus *Brucella*. Currently, there are 11 recognized *Brucella* species[2], and six of them, are known to be pathogenic for both animals and to humans, namely: *B. abortus, B. canis, B. inopinata, B. melitensis, B. pinnipedialis, and B. suis*[3]. The sources of infection for animals include aborted materials, vaginal discharges, milk and semen from *Brucella* infected animals. Domestic animals (Cattle, sheep, goats and pigs) are the main reservoirs of *Brucella*. The transmission of brucellosis to humans occurs through occupational or environmental contact with infected animals or their products (cheese, raw milk and unpasteurized milk) including a travel-association to the disease[4]. Person-to-person transmission is extremely rare.

Despite its global distribution, data on the prevalence of brucellosis among humans and animals in Africa is limited. In Africa, the intensive interactions between humans and animals in the ecosystems favor cross-infections in mixed husbandry systems or at the livestock-wildlife interface[5]. Brucellosis remains endemic in most areas of the world[6], even if, some of the developed countries have eradicated it from their domestic animal populations. In some parts of Africa, the disease is underreported due to insufficient awareness, inadequate diagnostic protocols, including lack of laboratory reagents[7]. In addition, limitations in the implementation of blood testing, milk pasteurization, food hygiene measures, slaughter and heifer vaccination programs, are some of factors which negatively affect the surveillance networks of brucellosis in Africa. However, brucellosis infections in humans can be avoided by applying occupational and food hygiene together with the implementation of bio-security measures in laboratories, while the prevention in animals could be based on good herd management and hygiene strengthened with a careful vaccination program[4].

Brucellosis poses diagnostic and confirmation challenges in humans, domestic and wild animals[8]. In humans, brucellosis resembles other febrile diseases such as malaria, and is often misdiagnosed or underreported[9]. Even where good laboratory facilities exist, the disease is still misdiagnosed because of the low diseases suspicion levels among the medical practitioners. In some cases, infections due to brucellosis are not necessarily recognized based on clinical evidences because the disease has no pathognomonic signs, and therefore confirmation must be based only on laboratory tests. In livestock industry, the economic impact of brucellosis is mainly attributed to abortions which mostly occur during the last third of pregnancy. In African countries, abortions are followed in some cases by temporary or definitive infertilities also with a decrease or a total absence of milk production[10]. Unfortunately, in *Brucella* infections, the causes of abortions often remain undiagnosed even after a complete necropsy, histopathologic and microbiological examinations[11]. Furthermore, there are some limitations on how to make a differential diagnosis with other infectious diseases, making it difficult to assess the real contribution of brucellosis to the observed abortions in humans and in animals. Several studies in Africa have shown an association between seropositivity and abortions[11–13]; but in many cases these relationships have been established based on statistical association between prevalence and the history of abortions in herds, not as counter-factual events, which could make an ambiguous interpretation about the role played by brucellosis in the causation of abortions. Furthermore, the presence of organisms does not necessarily indicate a causal association between *Brucella* and abortions in risk groups, because of several other factors that could bring about abortions. Therefore, the aim of this review was to assess the contribution of brucellosis to abortions observed in humans, domestic and wildlife in Africa, considering the above catalogued shortcomings. For this purpose, the objectives of the review study were: to identify different causes of abortions in humans and animals; to evaluate the impact of abortions in humans and animals and

to assess the contribution of brucellosis to abortions observed in humans and animals in Africa.

Methods

This literature review was done to demonstrate from published information the contribution of brucellosis to abortions in livestock, humans and wildlife in Africa. Articles in English and French published between 1997 and 2015 were retrieved using large-scale search engines including the Google, Google Scholar, Pubmed, Gopubmed, Freefullpdf and African journals Online. The inclusion criterion was any article in which the authors attributed the responsibility of *Brucella* infections to abortion occurrence in humans, domestic animals and wildlife in Africa. Articles reporting prevalence of brucellosis in Africa without any association of abortions in humans, domestic and wild animals were excluded. The key words for the search were: [brucellosis, abortion, livestock, Africa]; [brucellosis, abortion, humans, Africa]; [brucellosis, abortion, wildlife, Africa]; [Contribution, brucellosis, abortion, Africa]. For the balanced information, it was necessary to review the *Brucella* and non- *Brucella* causes of abortions in humans and animals, as well as their economic impact evaluation without limit only to Africa continent. In this review, the focus was based only on the causes of spontaneous abortions in humans and animals (not induced abortions).

Results

Different causes of abortions in humans and animals

The general causes of spontaneous abortions are due to infectious and non-infectious causes. There are several causes of spontaneous abortions and some of such common causes in humans and animals are discussed below.

Definition of abortion

Spontaneous abortion, or miscarriage, is defined as a pregnancy that ends spontaneously before the fetus has reached a viable gestational time (20^{th} week of gestation)[14]. Abortion can be defined also as an expulsion of a dead or living fetus of recognizable size at any stage of gestation. Abortion is also defined as a loss of a fetus which occurs from the moment in which the pregnancy diagnosis is usually performed to the point at which the fetus is considered capable of sustaining life outside the uterus[15]. Abortion may be either spontaneous (occurring from natural causes) or induced (artificially or therapeutically). Abortion is most of the time the result of a disturbance in the functioning of the placenta and, it may occur at any stage of pregnancy[16]. In case of brucellosis, the presence in uterus of erythritol (a 4-carbon sugar alcohol) is associated with abortions occurrence because it constitutes the placental tropism for the development of *Brucella* specifically in ruminants[17]. This carbon sugar is not found in human uterus or fetus, a reason which makes it more difficult to understand the contribution of *Brucella* infections to abortions in pregnant women. The pathogenic mechanism for induction of abortion by bacterial and viral infections is not the same depending of the characteristics of each infectious disease which may induce this syndrome[18]. Protozoan parasites are common causes of extensive abortion in livestock and some species, including *Toxoplasma gondii*, *Neospora* and *Sarcocystis*, have a two-host life cycle[19]. In addition, the pathogenesis of fungal abortions is possibly based on the penetration of fungi and their toxins in the uterus and the fetus by hematogenous route[20]. In case of brucellosis, the pathogenesis of abortion is very unclear although, some studies have demonstrated the interactions between brucellosis and the animal trophoblast, which is not the case for the human trophoblast[21].

Causes of abortions in humans

Genetic causes

Abnormal chromosomes (translocations) in either partner can cause miscarriage[22]. Chromosomal aberrations in parents are a major pre-disposing factor and causative of abortion if carried over to

the embryo[23]. Generally in Africa, it is rare to diagnose and get service in such cases[24] due to the difficulties in finding trained medical geneticists, genetic counselors and medical scientists. Other causes of abortions may include genetic factors because of lethal gene combinations[25].

Endocrinologic causes

In humans, an important cause of early and late abortions is due to an insufficient progesterone (disorders of the luteoplacental progesterone) secretion[26].

Immunologic causes

Some studies reported the maternal immunologic aberrations to be the cause of repeated abortions[27,28], and the larger numbers of unexplained abortions may have immunological reasons.

Nutritional deficiency and toxic agents

Abortions may also be due to some deficiencies of vitamins, minerals and energy in the body of pregnant females. In terms of maternal health, clinical deficiency (vitamin B12, E) may be a cause of infertility or recurrent spontaneous abortion[29,30]. Furthermore, poor iodine tenancy in pregnant woman body has been found in West Africa to be associated to reproductive failure including miscarriage[31]. A long exposure to toxic agents such as pesticides may also cause abortions or early embryonic human deaths[32].

Environmental and occupational factors

On rare occasions, an individual may abort after developing a very high fever due to an infection. Spontaneous abortions can be due to environmental factors: for example, the tobacco exposure in some occasions can cause spontaneous abortions[32–34]. Occupations; even the income of people (poverty, lifestyle) can in some cases expose them to risk of abortions[32,35].

Causes of abortions in domestic animals

Genetic causes

In animals, abortions due to genetic abnormalities occur as an individual case problem rather than as a herd problem. Studies reported abortions and neonatal losses in cattle linked to chromosomal aneuploidy[11,23].

Endocrinologic causes

An experimental study reported significant alterations caused by *T. brucei* in the hypothalamus, adenohypophysis, uterus, placenta and fetal liver with infertility in goats[36]. In South Africa, a study reported cases of abortions in Angora goats due to an abnormally low level of adrenal function, coupled with some qualitative changes in adrenal steroid biosynthesis[37].

Immunologic causes

It has been proved with clinical evidence that using some vaccines in pregnant animals can cause abortions. A study reported an outbreak of abortions following the use of intramuscular infectious bovine virus vaccine in a dairy herd in Canada[38]. Abortions which may occur after administration of *Leptospira* vaccines have also been discussed[39]. In case of brucellosis, although the available vaccines RB51 and S19 are effective in controlling brucellosis, studies reported their numerous drawbacks, such as potential to cause abortion in pregnant animals[8,40,41]. For *Brucella* immunization, cattle are vaccinated mostly as heifer calves at 4–12 months of age whereas adult cattle may be vaccinated only in selected high-risk situations.

Nutritional deficiency and toxic agents

Mineral deficiencies were reported in 4% of abortions in goats in California[42]. However, a study conducted in South Africa could not associate the observed abortions in Angora goats

to nutritional deficiencies[43]. Abortions may occur, especially in late gestation if animals are exposed to sufficiently high levels of nitrates in forage (55 % or greater). Experimentally, studies have proved abortions caused by mycotoxins such as zearalenone[44,45] and ergot alkaloids[46].

Environmental factors

Some abortions in animals may be a result of an increase in environmental temperature[47], but, evidences are not sufficient to support heat stress as a common cause of abortions.

Abortions caused by infectious diseases in humans and animals

There are a larger number of infectious agents causing abortions in humans and animals and some of them are zoonotic (Table1). In fact, *Brucella spp.*, are among the important bacterial agents associated with abortion during mid-to late gestation including *Chlamydia spp., Salmonella spp., Campylobacter spp., Listeria monocytogenes* and *Coxiella burnetii*[5,48,49]. In case of *Toxoplasma gondii*, it used to be misidentified while it is the most probably significant cause of repeated abortion in humans, cattle and dogs[50–52]. The evidences are lacking to consider *Neospora caninum* as a cause of abortions in humans; however, it is one the causative agents of abortions in cattle and dogs[53].

Causes of abortions in wildlife

Toxoplasma gondii infections are suspected to be mostly associated to abortions cases in wildlife[54,55]. The *Coxiella burnetii* and *Chlamydiales* species have been associated with abortions in wild ruminants[54–57]. Some infectious diseases are reported in wildlife such a Rift valley fever (RVF) in Kenya[58], Food and Mouth Disease (FMD) in Zimbabwe[59], tuberculosis in African buffalo in South Africa[60], but little is documented about their association with abortion in wild species in Africa. Serological evidence of brucellosis and abortion were reported in wildlife in USA[61] where *B. abortus* was isolated for the first time from an aborted female bison[62]. In addition, *Brucella abortus* biovar 1 was isolated from a bison (*Bison bison*) fetus collected in Yellowstone park[70]. In Africa, different studies are reporting on prevalence of brucellosis in wildlife[61,71–73], but little is known about the association of abortions occurrence with infectious diseases including brucellosis in wild species.

Detection of the causes of abortions

The role of infectious agents could be less important if the presence of organisms does not necessarily indicate a causal association with abortion, although the reports indicate 20-30% of their implication in abortions cases diagnosed in laboratories[74]. The detection of the causes of abortions in the population may be done by serological assay, immunological, bacteriological and molecular techniques, based on clinical evidences. The seroepidemiological approaches can establish a high degree of association between infections and the abortion level in the farm[75]. However, it is difficult to establish that *Brucella* is a cause of abortion based on serological results only. Furthermore, the gold standard for the diagnosis of brucellosis is isolation and identification of the causative bacterium in a biological containment level three[76]. In humans, the history of the patient, the physical examinations, a pelvic ultrasound, the laboratory orientation may be the foundation for a detection of causes of abortions[77].

Management of abortions

In humans, abortions may require expectant management for up to two weeks, and medical therapy which can usually give successful results[78]. In animals, vaccination contributes a lot to protection against infectious diseases of public health importance[79]. Neosporosis, one of the economically most important causes of abortion in cattle, has prompted researchers to invest in the development of measures to prevent infection of cattle by vaccination[80]. However, there are

Table 1: Infectious agents which can cause abortions in humans and animals

Host	Bacteria	Virus	Fungi	Protozoans	References
Humans only	*Staphylococcus aureus, Ureaplasma urealyticum Mycoplasma hominis Treponema pallidum*	*human immunodeficiency Virus Dengue virus Influenza virus Herpes simplex virus*		*Plasmodium*	18,63,64
Humans and Animals	*Brucella Leptospira Salmonella Listeria*			*Toxoplasma gondii Chlamydia Mycoplasma*	48,51,62,65,66
Animals (domestic and wildlife)	*Campylobacter (vibrio) Arcanobactericium (Actinomyces) Escherichia coli Streptococcus Zooepidemicus Rhodococcus equi Leptospiras interrogans*	*Phlebovirus Aphthovirus Bovine herpes virus-1 Equine herpes virus-1 Bovine viral diarrhea Border disease Mycoviruses Bluetongue Parvovirus Suid herpisvirus 1 Equine viral arteritis*	*Aspergillus Mucor Candida*	*Neospora caninum Trichomona fetus Coxiella (Q fever) Coccidia Babesia Trypanosomum equiperdum*	12,44,48–50,52,54,58, 67–69

vaccines which may cause abortions in pregnant animals. In case of abortions due to infectious diseases such as brucellosis, a good disposal of aborted materials and culling are required to avoid the human contaminations and the dissemination of infectious agents in the herd.

The impact of abortions in humans and animals

The economic impact of abortions

The economic impact of abortions in animals can be evaluated based on direct costs (value of fetuses lost) and indirect costs: establishing the diagnosis, re-breeding cows that aborted, possible loss of milk yield, and replacement costs if cows that aborted are culled[74]. Abortions in domestic animals are of great concern to farmers because the fetus that would form replacement stock is lost and a prolonged period of uterine disease and infertility or sterility may follow leading to unproductive females being maintained for long periods[81]. Some loss estimates around US $110.00 for abortions caused by *Neospora caninum* in a pregnant dairy cow in USA[82]. Abortions extend calving interval and increase culling and the economic evaluation from each pregnancy loss was estimated at approximately $2,333 in Korea[83]. In Burkina Faso, a study reported an impact of the spontaneous abortion in women of US $56 (27 668 CFA) and underlined the high expenses with short-term economic repercussions on households' poverty[84]. In sub-Saharan Africa, very few articles focused exclusively on the cost of treating abortion complications in humans, but authors agreed that it consumes a disproportionate amount of hospital resources[85]. In fact, the number of fetus including the milk losses due to abortions in animals are easy to quantify whereas in humans the fetus including the emotion stress associated to abortions are difficult to measure.

Economic impact of abortions due to brucellosis

Higher productivity losses are associated with higher prevalence of brucellosis. *Brucella* seropositive animals have higher rates of abortion, stillbirth, infertility and calf mortality, as well as reduced growth and longer calving intervals. Often, infected females will abort only once, although they may remain infected their entire life. Studies on the economic production losses of bovine brucellosis are reasonably consistent across a range of production systems in Africa and Asia[13].

Table 2: Papers reviewed per region and species on the contribution of brucellosis to abortions in Africa

Region	Countries	Humans	Dom. Animals	Humans & dom. animals	Wildlife	Total	References
North Africa	Morocco, Egypt, Tunisia	1	3	0	0	4	94–97
West Africa	Nigeria, Niger	0	3	0	0	3	12,98,99
Central Africa	Cameroon, Chad	0	1	0	0	1	86
East Africa	Tanzania, Kenya, Rwanda, Ethiopia	1	3	2	0	6	100–105
Southern Africa	Zimbabwe, Zambia, South Africa	1	3	0	0	4	106–109

In African regions where the infection rate is 30% for bovine brucellosis in breeding females (20% of the herd), the result in economic losses approximate to 5.8% of gross income per animal reared[86]. In Mongolia, after estimating the proportion of abortions among brucellosis seropositive animals, a mass vaccination program was implemented with a cost of $8.3 million[87,88]. If the costs of the vaccination were shared between the livestock and public health sectors, the intervention may be cost-saving and cost-effective. In Ethiopia, the economic impact of abortions due to brucellosis in camels was estimated to 21% of the total cost[89] while in Sudan it was representing 8.2% of the total losses due to bovine brucellosis[90].

The public health impact of abortions

Aborting animals shed large quantities of infectious agents and pose considerable risk to humans in contact. In some cases, consumers may also be at risk; for example, *Coxiella burnetti*, responsible for Q-fever, can be excreted in the milk of aborting goats for up to 52 days[91]. The disposal of aborted fetuses might be well managed to avoid humans and animal exposure to the pathogen. The human assistance rendered during parturition in abortive cattle, sheep or goat has been associated in some cases to brucellosis infections in humans[92]. Some case of abortive animals could result in infection of entire households when animals are kept in close proximity to living accommodation, or when they are brought inside of houses, especially in severe weather[4].

The social impact of abortions

Abortion is a tragic loss and can be associated with significant psychological problems for women, their partners and families in general. For women who get an experience of spontaneous abortion, it is a stressful event as well as they are not sure to conceive and arrive at term successfully with the next pregnancies. About 1% of couples will experience recurrent spontaneous abortion[93]. In animals, abortions can lead to nutrition insecurity because of the decrease of milk production and loss of calves. In addition, infectious diseases which lead to abortions can unable livestock producers to meet their social obligations such as the man's position, influence and the respect in the community as well as the payment of children school fees, medication, clothes[110,111].

The contribution of brucellosis to abortions observed in humans and animals in Africa

Some studies in this review (5/18) reported data on the abortion associated with brucellosis in Africa[96,100]. These estimates of abortions reported in this review are high (0.17-16.2%) compared to the normal abortion rate, which is ranged between 2-5% in cattle[86]. Domenech *et al.*[112] demonstrated, using a formula, the existence of correlation between the manifestation of brucellosis symptoms and the increases of abortion cases in African cattle. Some authors have also reported the association between brucellosis and abortions observed in Africa based on the calculated odds ratio[101,103].

Table 3: Data extracted from literature on the contribution of brucellosis to abortions observed in Africa

Studies/ species	Study design	Sample size	Samples type	Diagnostic methods	Prevalence	Ethical issues	Abortion rate	(OR)	References
Humans	Case-control	324 women	Blood	SAT	26.8%	Yes	NR	2.3	[101]
	Cross-sectional	129 women	Blood	SAT	38.8%	Yes	NR	NR	[97]
	Prospective	125 women	Blood, swabs	NR	4%	NR	NR	NR	[106]
Humans and domestic animals	Cross-sectional	60 women, 27 cattle	Blood	RBPT	25%	Yes	NR	NR	[104]
	Cross-sectional	483 cattle, 120 humans	Blood	RBPT, ELISA	0 – 28.95%	Yes	NR	NR	[105]
	Cross-sectional	20 herds (214 cattle)	blood, swabs, milk	RBPT, culture, RT-PCR	31.3%	Yes	NR	NR	[95]
	Cross-sectional	23 sheep	Blood		4.34%	NR	7%	NR	[96]
	Cross-sectional	5192 cattle (681 herds)	Blood, hygromas fluids)	ELISA, MLVA-VNTR	Herd:11.2- 17.2% Individ: 1.3%	Yes	No	3.0	[12]
Domestic animals	Cross-sectional	24 cattle	Blood	RBPT	15.4 - 85%	NR	0.17- 11.8	NR	[86]
	Cross-sectional	700 cattle	Blood, milk	RBPT, culture, ELISA, PCR	6.7 – 9%	Yes	NR	NR	[107]
	Cross-sectional	200 cattle, 50 goats, 35 sheep	Blood, milk, aborted materials	RBPT, ELISA, MRT, culture, PCR, MLVA-VNTR	48%	Yes	NR	NR	[102]
	Cross-sectional	283 cattle, 756camels, 757 goats	Blood,	RBPT, CFT	Cattle : 10.6% camel : 2.2% goats : 1.9%	Yes	Camels: 23.4% cattle: 13.8% goats 12.4%	cattle: 4.7 goats: 6 9 camel: 1	[103]

Cross-sectional	886 cattle	Blood	RBPT, ELISA	23.9%	NR	Herd: 50% Individ: 16.2%	3.4	[108]
Cross-sectional	239 cattle	Blood	RBPT, SAT, CFT	3.8		NR	NR	[109]
Cross-sectional	28 sheep	Milk blood, vaginal swab	RBPT, SAT, MRT, culture	14.3%	NR	NR	NR	[98]
Cross-sectional	22 sheep	Milk	RBPT, SAT, MRT, Culture	14.5%	NR	NR	NR	[99]
Cross-sectional	260 cattle	Blood	ELISA	16.8%	NR	6.5%	0.7 1.1	[100]
Cross-sectional	10 cattle, 5 buffalo, 9 goats, 1 sheep	Blood	RT-PCR	-cattle: 100%; -buffaloes: 50%; -goats:33.3%; -ewe:100%	NR	NR	NR	[94]

CFT: Complement fixation test; **ELISA:** Enzyme-linked immunosorbent assay; **MRT:** Milk ring test; **MLVA-VNTR:** Multiple-locus variable number tandem repeat analysis; **RT-PCR:** reverse transcription-polymerase chain reaction; **RBT:** Rose Bengal plate test; **SAT:** Serum agglutination test; **NR:** not reported

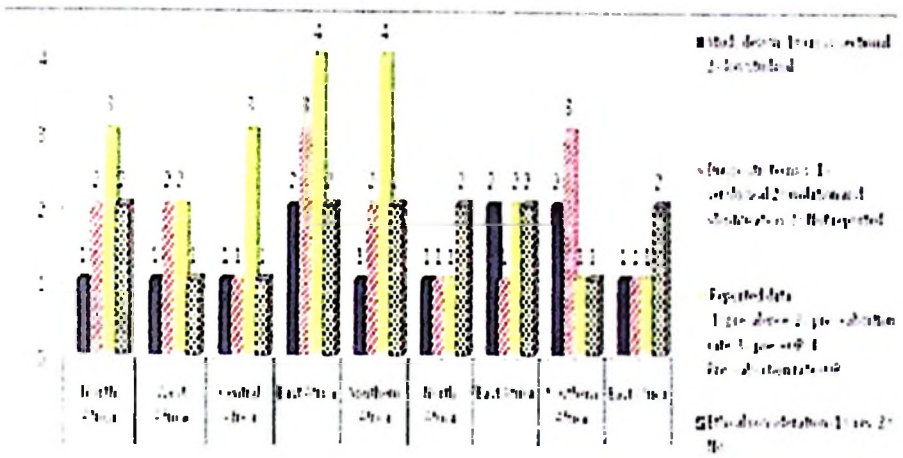

Figure1: The study design, diagnostic methods, reported data, ethical considerations per species and regions to assess the contribution of brucellosis to abortions in Africa

However, some abortions recorded in Africa may be due to co-infections between brucellosis and others infectious diseases[95,100]. Differential diagnosis of brucellosis with others abortive infections prevalent in the study area could reveal the true association between the observed abortions and the prevalence of each disease.

Discussion

Brucellosis is one of the major zoonotic diseases on the African continent and has an economic impact on livestock productivity (abortions, decrease of milk production). The syndrome of abortion affects the household's income and constitutes a risk factor for the dissemination of *Brucella* in humans and animals. A review of online literature has been done with an objective of assessing the contribution of brucellosis to abortions in humans, domestic animals and wildlife in Africa. The association may exist between brucellosis and abortions in animals which manifest clinical symptoms for this disease in Africa[12], and a correlation exists between the manifestation of brucellosis symptoms and the increases of abortion cases in African cattle[86]. Some events at farm or risk group level such as exposure to aborted materials, unpasteurized milk consumption, artificial insemination, could be recorded during follow up for completing the differential diagnosis with other prevalent infectious diseases related to abortions. The high prevalence of brucellosis in cattle reported in Africa is most of time associated with abortions[13]; around one fifth of cows may abort where seroprevalence is higher than 30%.

Actually, studies on the seroepidemiology of brucellosis in Africa are reported with an improvement in the sample size calculation and sampling methods, the diagnostic methods, the ethical considerations[98,101,113]. However, in this review the sample size and type varied from studies, species and regions in Africa (Tables 2 and 3). This could be because brucellosis is a herd level disease: an occurrence of abortion in risk groups is enough to start investigation. Otherwise, the large sample size and appropriate sampling method is very important to determine the association between brucellosis and observed abortions. Methods used for the diagnosis of brucellosis in the reviewed papers include: RBPT, ELISA, MRT, SAT, culture, identification and molecular characterization of *Brucella* species (Figure1). The serologic methods are the most used in Africa to determine the role played by brucellosis in abortions even if these tests are

known to be less specific, and the results can be biased with false positive because of some cross-reactions with other bacteria. However, the contribution of brucellosis to abortions is less highlighted by molecular techniques[114,115], although this disease is considered to be one of the major causes of abortions in cattle in Africa[109,116]. In this review, *Brucella abortus* seemed to be the specie most associated with abortions in Africa[12,99,102,109]. Generally, the cost of molecular techniques could be a challenge to African countries. However, this technique could be a good way to detect the presence of infectious agents in aborted materials and to assess the real contribution of brucellosis in abortions recorded in humans and animals on the continent. Molecular epidemiology could contribute as a tool for identification and characterization of *Brucella* species from aborted animals, determining their origin and possible spillover to other species (especially wildlife). Studies could extend their exploration to the presence of *Brucella* in the human aborted materials or breast milk after abortions, this will complete data on seroprevalence studies and questionnaire surveys in Africa.

In this review, studies reported abortions associated with brucellosis in Africa, focusing much on domestic animals[94,98,103] and less in humans (Tables 2 and 3). Although some authors reported the association between brucellosis and the observed abortions in humans in Africa[97,101,104,106], the pathogenesis of brucellosis in pregnant women still remains to be elucidated. However, the abortion process is well described in animals due to the role played by erythritol (sugar), which may confer the tropism for *Brucella* development in the uterus. Nevertheless, out of Africa, it has been suggested that brucellosis may cause higher rate of abortions, more frequently than do other bacterial infections in pregnant women[115]. Furthermore, in situations where brucellosis is suspected to be a cause of abortions in pregnant women, laboratory analyses are required to confirm the role played by others febrile diseases (such as malaria) or abortive infectious diseases (Rift valley fever, Toxoplasmosis) which are equally prevalent in Africa. The clinical symptoms observed in pregnant women could complete the differential diagnosis with the prevalent others abortive and febrile diseases. Furthermore, this gap can be rectified by applying for the confirmation of the brucellosis in humans based on isolation and molecular techniques.

Generally, no evidence of abortion due to this disease has been documented in wildlife; despite the findings from a single study that reported the impact of brucellosis on abortions in the wildlife-livestock interface[103]. In Africa, interactions are observed between domestic and wild animal species in pastoral farming systems where they may be exposed to aborted materials, when sharing the same pasture or common source of water. Furthermore, smallholder farmers might be affected by the abortions exposure due to brucellosis because of the cut and carry as feeding system[117].

Study Limitations

Eighteen papers were used to extract data because they reported on the role played by *Brucella* infections on abortions observed in animals and humans in Africa (Tables 2 and 3). However, the quality of this review could have been affected by the lack of assessment of bias in the studies, the non-inclusion of statistical management of data, also with the restriction of the study area only to Africa. Nevertheless, these limitations may be avoided by a systematic review (instead of a review) if published data on the contribution of brucellosis to abortions in humans and animals in Africa could have been found with a significant number of papers. Most of the studies reviewed were cross-sectional in design (Figure1). Despite of their time consuming, the loss of subjects (attrition) and the limitations of budgets for research, case control and longitudinal studies could reflect possibly good observations with clinical evidence during the assessment of the contribution of brucellosis to abortions in Africa.

Conclusion

Brucellosis is reported in Africa with high prevalence in humans and animals. However, there is limited published data about the contribution of this zoonotic disease to abortions in humans and animals. The literature reviewed stated little about the estimation of the abortion rate and the calculated odds ratio which are strong indications of association between brucellosis and abortions in humans and animals in Africa. More data are reported by eastern and southern parts of Africa on the assessments of the contribution of brucellosis to abortions, but generally in Africa, there is a lack of a rigorous sample size calculation, an inadequate study design planning, and ethical clearance considerations are also required. The identification of the causes of abortions in Africa, specifically the role played by brucellosis, is based on routine test (RBT) and immunological diagnosis (ELISA); but, the detection should be based on more definitive methods such as isolation and molecular characterization from blood, milk and aborted materials. Furthermore, little is reported about the association of brucellosis to abortions in humans (five papers out of 18). As the causes of abortions are multiple, the clinical observations in humans and animals could complete the differential diagnosis with the prevalent abortive diseases. In addition, the contribution of brucellosis to abortions in wildlife in Africa is not elucidated in the literature, and, the economic impact evaluation of abortions in the herds due to this disease remains to be completed. The epidemiologic approach based on collecting core data concerning both aborted and non-aborted individuals (humans and animals) could determine the contribution of brucellosis to abortions in populations and could help to monitor the prophylaxis in humans and the progress of vaccination programs in animals. Due to the strong interactions in the human-livestock-wildlife interface in Africa, the contribution of brucellosis to abortions calls for the interdisciplinary collaboration for its understanding and controlling.

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Contribution of Authors

The first and the fourth authors prepared the manuscript for publication. All the authors mentioned in the article reviewed the final version and they approved the manuscript.

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Paper Two

Knowledge, perceptions and practices regarding brucellosis in pastoral communities of Kagera Region in Tanzania

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Original Article

Knowledge, perceptions and practices regarding brucellosis in pastoral communities of Kagera Region in Tanzania

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ABSTRACT

Objective: A cross-sectional study was conducted in June 2017 to assess the knowledge, perception and practices on brucellosis by pastoralists of Kagera ecosystem in Tanzania, using qualitative methods.

Materials and methods: Five focus group discussions of six participants each were conducted with livestock farmers, administration leaders, religious representatives and youth. In addition, discussions with three key informants were conducted, involving officials of livestock, wildlife and public health departments in each district. Data were analyzed using content analysis with inductive and deductive methods.

Results: The study revealed low knowledge regarding brucellosis among respondents. Although participants recognized brucellosis as a zoonotic disease, they consider it of less importance. In addition, participants had low knowledge on causes, symptoms and mode of transmission of this disease. However, they perceived the interactions between humans, livestock and wildlife together with movements between borders to be potential risks for introduction of brucellosis in their communities. Moreover, their habit of drinking unpasteurized milk, the lack of protective gears during assisting animals giving birth and poor vaccination program need to be improved by community health education.

Conclusion: A coordinated One Health approach is needed and further studies are suggested to reveal the status of brucellosis in Kagera ecosystem to guide its control and prevention.

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KEYWORDS

Brucellosis; Knowledge; Pastoral communities; Practices

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INTRODUCTION

Brucellosis is a worldwide zoonotic disease for both public health and economic importance, affecting humans, livestock and wildlife. This zoonotic disease has a worldwide distribution where Africa is one of the endemic areas (Corbel, 2006). Different *Brucella* species are identified as causative agents of brucellosis and some of them are known to be pathogenic to humans which include *B. abortus, B. canis, B. inopinata, B. melitensis, B. pinnipedialis, and B. suis* (Tiller et al., 2010; Zheludkov and Tsirelson, 2010; Whatmore et al., 2014). In sub-Saharan Africa, the presence of various *Brucella* species (*B. abortus, B. melitensis, B. suis, B. canis, and B. ovis*) is reported, specifically in domestic animals (Ducrotoy et al. 2017). However, there is a scarcity of knowledge for *Brucella* species in humans and those associated to marine animals in Africa. *B. pinnipedia* and *B. cetaceae* mostly affect marine animals, but they are also responsible of threats in humans (typically neurobrucellosis) (Godfroid et al., 2005). It is known that brucellosis is endemic in several areas in East African region (Chota et al., 2016) where it reduces animal productivity through abortions and weak offsprings; causing a major threat in national and international livestock trade.

In Tanzania, previous studies have reported existence of risk factors for brucellosis transmission in pastoral communities including occurrence of abortions in herds, poor hygiene practices in assisting animals during parturitions, individuals living in close proximity with livestock and animal slaughtering occupation (Swai and Schoonman, 2009; Kunda et al., 2010; Assenga et al., 2016). In some communities, brucellosis transmission in humans was associated with people who drink raw-milk/animal blood, consume raw meat or share a bed or utensils with brucellosis patients (Mubyazi et al., 2013). Previous studies in Tanzania demonstrated higher understanding by pastoralist of the existences of diseases transmitted between humans and animals (Swai et al., 2010; Mangesho et al., 2017). Moreover, livestock keepers could recognize abortions, emaciation, a drop in milk production and fever as clinical signs associated with brucellosis (Shirima, 2005). Despite the good perception and knowledge of common diseases circulating in their area, livestock farmers needs to improve their practices to control those diseases, which most of the times leads to failure at individual and national levels (Chengula et al., 2013). Activities undertaken for controlling brucellosis, may involve capacities for detection of the disease, the participation of stakeholders in mass vaccination or culling, the epidemiosurveillance system based on the perception of the risk for humans, livestock and wildlife in the ecosystem. Despite their knowledge and perception of the threat caused by certain diseases in their communities, pastoralists adopt some cultural behaviors which could favor the transmission of infectious disease in the localities (Musallam et al., 2016). The understanding and the eradication of brucellosis, needs a characterization of the disease, the multidisciplinary actions from different stakeholders in the exposed areas (Zinsstag et al., 2005). Also, the transboundary transmission of zoonotic diseases may be considered and be evaluated from the local understanding of communities. Little is known about the local understanding of brucellosis by pastoralists in Kagera, Tanzania. This study was conducted to assess the knowledge, perception, and practices regarding brucellosis of different stakeholders in the pastoral communities of Kagera Region; an ecosystem located on borders between Tanzania, Burundi, Rwanda and Uganda.

MATERIALS AND METHODS

Study area: This study was conducted in two districts namely Karagwe and Ngara, of Kagera Region, in north-western part of Tanzania (**Figure1**). Livestock contributes significantly to the economy of Kagera region, and animals are exported to neighboring countries (United Republic of Tanzania, 2013). Kagera ecosystem is subdivided into three agro-ecological zones (Lake Shore and Islands, Plateau Area and Lowland) in which crops grown are mainly bananas, cassava, beans, maize, coffee and tea. The area has game reserves such as Kimisi and Burigi in which zebras, impalas, buffalos, elephants, giraffes, leopards, hippos and crocodiles can be found. Health facilities are distributed in all districts and various transport means link Kagera to other regions and neighboring countries particularly Burundi, Rwanda and Uganda. The climate is equatorial with temperatures ranging between 20°C and 28°C. Kagera Region, in general has rainfall ranging between 900 - 2,000 mm per annum.

Study design: A cross-sectional study design was used to assess the knowledge, perception and practices of brucellosis in pastoral communities of Kagera in June 2017, using a qualitative research method.

Participants selection and data collection procedure: Two focus group discussions (FGDs) and one Key Informants Interview (KIIs) were conducted in Ngara district, while three FGDs and one KII were done in

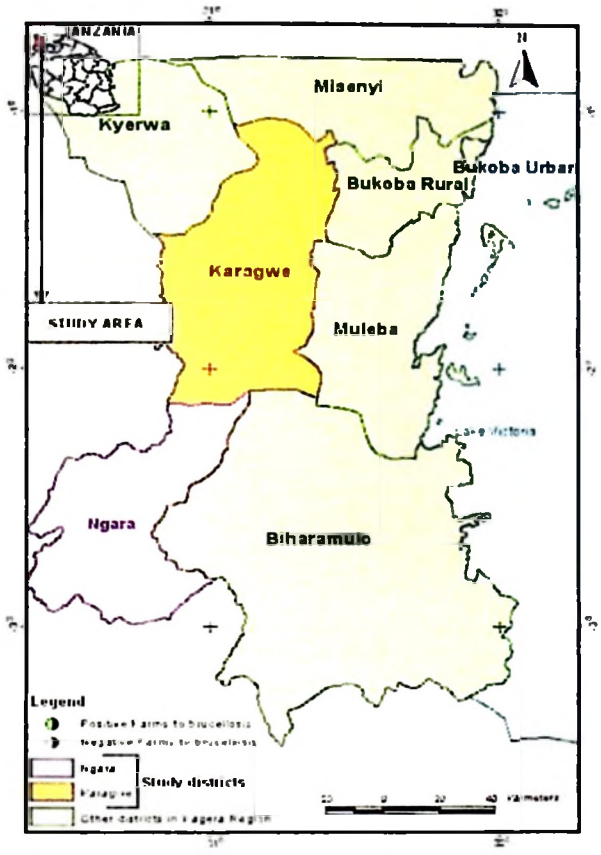

Figure 1. Map of Kagera Region

Karagwe district. Each FGD involved a minimum of six persons selected purposively: three farmers, one representative of local government, one religious leader and youth representative (15 to 24 years old). Discussions with KIs involved three government officials from the animal health, public health and wildlife departments in each district. Participants in this study originated from five villages selected purposively (urban, peri-urban and rural areas) to get a variation of insights on brucellosis from different people according to their location (**Table 1**). FGDs and KIIs approaches were combined to get coverage of information from experts and non-experts regarding brucellosis.

The FGDs and KIIs were conducted in the respective communities of the participants; i.e, ward executive and district official's offices (or hospitals). Digital recording by mobiles phones was used to record discussions and interviews. FGDs were conducted in Swahili language by a facilitator, while interviews with KI were conducted in English by the researcher. The interview guide was structured around four main themes as follows:

(i) Perception of brucellosis by the population in Kagera ecosystem: Participants were asked about the local name of brucellosis, existence of the disease in their locality. The knowledge on the causes, main symptoms, and the mode of transmission of brucellosis were also assessed. Furthermore, the socio economic impact and the prophylactic approach of this zoonotic disease in the ecosystem were discussed.

(ii) Risk factors for brucellosis prevalence in Kagera ecosystem.

(iii) Potential for transmission of brucellosis in Kagera ecosystem due to neighboring with other countries.

(iv) Roles of different stakeholders in the ecosystem in the control of brucellosis.

The facilitator introduced the aim of the study, explaining each theme clearly to participants. The discussions lasted approximately 45 minutes. For the KIIs, interviews were conducted in English by the researcher and both FGDs and KIIs groups were asked the same questions.

Data analysis: Data recorded from FGDs were transcribed verbatim to Microsoft Word and later translated from Swahili to English. The coding of the categories was done manually using Microsoft Excel since the data was small and themes and sub-themes were easily identifiable. Later, the content analysis was done with inductive and deductive methods based on the categories grouped in different themes and subthemes as well as emerging themes. Themes and sub-themes were analyzed in their chronological order of inquiry.

Ethical considerations: This study was approved by institutional review board of Sokoine University of Agriculture , and ethics clearance was also obtained from the Medical Research Coordinating Committee of the National Institute for Medical Research (ref: NIMR/HQ/R.8a/Vol.IX/2456). Informed verbal consent was obtained before conducting each FGD from all the team members. For confidentiality matter, participants were ensured for none use of their names during analysis, report or publication. Approval by participants for recording was requested prior to this activity.

RESULTS

Socio-demographic description of the participants

Thirty seven participants from six villages of Karagwe and Ngara districts were recruited to participate into Focus group discussions (**Table 1**). The mean age of the participants was 49 years (standard deviation = 10.55) and 30.55% of participants were females. People interviewed were from different tribes specially:

Table 1. FGDs per district and locations.

District	Village	Characteristic	Participants		FGDs conducted
			Females	Males	
Karagwe	Bweranyange	Rural area participants	1	6	1
	Nyagasimbi	Rural area participants	1	5	1
	Nyakahanga	Urban and peri-urban area participants	2	4	1
	Kayanga	Officials from Livestock, Public health and wildlife department	1	2	1
Ngara	Benaco	Rural area participants	3	3	1
	Ngara mjini	Urban and peri-urban area participants	2	4	1
	Ngara	Officials from Livestock, Public health and wildlife department	1	2	1
Total			11	26	7

Wanyambo, Wahaya, and Wahangaza. The focus group discussions involved farmers, youth, religious leaders and local government representatives. Four of the participants had no formal education, 21 had primary education, and 12 had secondary school or higher education. Key informants interviews were conducted in group of three individuals from public health, livestock and wildlife departments at district level. All the key informants were degree holders.

Knowledge and perceptions on brucellosis in pastoral communities of Kagera ecosystem

The understanding of brucellosis among the study participants in Kagera Region was not direct because some of them confused it with the "abortion process". In Tanzania, brucellosis is normally known in Kiswahili as "ugonjwa wa kutupa mimba" meaning the "disease of abortion". Describing the disease to participants, the term abortion was used as a prominent symptom; but it wasn't enough to differentiate brucellosis from other diseases associated with abortion which people are accustomed to encounter or report in humans and livestock. Thorough explanations were needed to make participants distinguish the phenomena of abortions from brucellosis, as this confusing heavily influenced their responses during the focus group discussions.

Participants gave different local names of brucellosis: Amakole, Omwizi, Entandago, Kuramburura, and Kururumura. However, the most common local name of brucellosis used in the two districts was "Kutoroga". The existence of brucellosis in their locality, as well as the zoonotic nature of the disease were acknowledged by all the groups who participated in this study. However, participants provided different causes of brucellosis. Five groups out of seven believed that brucellosis is caused by seasons (three groups mentioned dry season and two groups mentioned rainy season), while three groups said that brucellosis is caused by other diseases (malaria, Foot and Mouth Disease). One group mentioned vectors (tsetse flies, mosquitoes), age, contaminated water, drought and famine as causes of this disease.

> "….. *Few days ago, this disease could occur when cattle were drinking contaminated water with bacteria. Also, dry season causes abortions because of high temperature. There are so many causes including different diseases. That's what I know.*" (Farmer1- FGD Bweranyange- Karagwe District).

Six out of seven groups mentioned abortion as a clinical sign of brucellosis in humans and livestock. Other signs in humans were fever, tiredness, skin discoloration. The two key informants groups insisted on the fact that brucellosis may have a resemblance of symptoms with other febrile diseases such as malaria.

In livestock, participants mentioned additional symptoms of brucellosis such as fever, hygroma, vaginal discharges, skin changes, lack of appetite, orchitis, tiredness, general weakness and coughing. Only one group of key informants mentioned hygroma as symptom of brucellosis observed in wildlife.

> *"The signs are the same, cattle can get high fever, then hair rise up and blood start to come out, and abortion can occur almost within two days. We as farmers, we are accustomed to the problems of cows than those riches (cattle owners) who give us the cattle to graze for them."*(Farmer2- FGD Nyakasimbi-Karagwe District -).

The mode of brucellosis transmission involved different ways in humans: consumption of uncooked meat and unpasteurized milk, sexual intercourse and unprotected assistance of their animals during parturition. According to Key informants, milk is mostly consumed directly from animal and locally collected by farmers for informal commercialization. In addition, they mentioned the poor disposal of aborted materials and placentas. In livestock,

participants centered the transmission of brucellosis on the sharing of pasture and water between domestic animals and wildlife, the physical and sexual contact between animals, vectors (mosquitoes and tsetse flies) and contact with vaginal discharges of infected animals. Two groups mentioned the interactions between animals and the dissemination of vaginal discharges as source of contamination of brucellosis in wildlife.

> *"In animals, the transmission can be due to the increase of the number of cattle in the same area where contamination by contact can occur. In addition, the disease can be transmitted during the sharing of pastures with non0-vaccinated animals. It may happen that you perform vaccination very well but the problem arises when sharing pastures with infected herds. This may result in the transmission of some diseases which you cannot recognize"* (Farmer1- FGD - Nyakahanga-Karagwe District).

Throughout the discussions, the participants talked about the social impact of brucellosis in their localities. Participants in three groups believed that brucellosis could affect their willingness of raising animals and could reduce their faith in marriage. In addition, the economic impact of brucellosis was pointed out as a consequence of the loss of milk production, unnecessary expenditure to cover the treatments (incomes decrease), which could also contribute to the inability to pay school fees for their children. All the groups agreed that brucellosis decreases the total number of livestock. Furthermore, participants highlighted the negative impact of brucellosis on their health through the abortion, the deaths and the nutrition problems due to the decrease of milk production.

> *"On medical aspect, first of all, if you fail to diagnose brucellosis timely, you will not treat correctly and result into an avoidable death, if you treat wrongly the patient, thinking that maybe it is malaria or typhoid while it wasn't t, the outcome of improper treatment has bad consequences to the patient, like death; and misuse of medicines."* (KII1-Ngara District).
>
> *"... but this problem can cause the failure of production for both animals and humans."* (Cheikh - FGD Ngara District).

Regarding the prophylactic approach for brucellosis, focus group participants agreed that women are more prone to seeking medical care in health centers and hospitals. Key informants specified the use of antibacterial drugs such as doxycycline and rifampicin as treatment options in case of suspicion of brucellosis, even if according to them, some of these drugs particularly rifampicin were commonly used to treat tuberculosis. For livestock sector, farmers in all groups attested to call for veterinary services; also they confirmed buying drugs themselves and rarely get vaccinations. The use of traditional medicine to treat brucellosis in livestock and humans in case of abortions was mentioned by participants in two groups.

> *"Ah no, when you suspect something even if it is not yet confirmed, but if you see that it is likely to be, you start to treat. So alternatively, we use doxycycline; even if it is not available in the hospital, it is available in the pharmacies."*(KII1-Ngara District).
>
> *"Here the government has never provided such vaccine or medicine but ourselves when the problem occur, we go to the pharmacy to buy some medicines for treating our animals. But about prevention cases from the government; we didn't receive any."* (Farmer2- FGD Bweranyange- Karagwe District).

Risk factors for brucellosis in humans, livestock and wildlife

The important risk factors for brucellosis mentioned by participants (five groups) were: a movement of livestock and wildlife in the ecosystem, the sharing of pastures and drinking points shared between wildlife and livestock.

> *"....because most of the people who are living here close to this Kimisi game reserve are involved in movement of animals inside the game reserve. They take their livestock to graze inside the game reserve. So, the interaction with wildlife can increase the magnitude of the disease. "*(KII-Karagwe District-wildlife official).

KII groups recognized the habits of drinking unpasteurized milk, poaching and the poor disposal of aborted material (placentas and aborted materials are thrown in the environment or given to dogs) as major risk factors for brucellosis transmission in humans in their communities. Climate change, consumption of uncooked meat and sexual intercourse (favored by the movement of people in the ecosystem) were also reported in two groups as risk of introduction of brucellosis in the study area.

The risk for transmission of brucellosis in Kagera ecosystem due to neighboring with other countries

Six groups stated that the interactions observed on borders between livestock and wildlife and the existence of games reserves on borders constitute a risk for transmission of brucellosis from others countries. Furthermore, the movements of people crossing borders for pastoral and commercial activities, the migration of people including refugees' camps were proposed by

different groups as potential risks for the introduction of brucellosis from neighboring countries.

> *"During the conflicts in Rwanda and Burundi I was here keeping goats but this disease was already there before the refugees came here. At the time, there were some refugees who brought some cattle and used to sell them to indigenous people. However, there were no any benefit from it, because all of the purchased animals died. We are not sure if those animals died because of this disease or if the problem was the change of environment. But, I think the problem was the environment, they were not supporting the weather here.* (Pastor- FGD Benaco- Ngara District).

During the discussions, five groups mentioned also the uncontrolled movement of wildlife on borders (wild animals don't know borders) to be a risk of introduction of brucellosis from a country to another.

The role of different stakeholders in the ecosystem in the brucellosis control

Brucellosis is not controlled in the pastoral communities of Kagera. Little is being done for the effective surveillance of this zoonotic disease. All the groups confirmed that few farmers were vaccinating their animals. Otherwise, participants from all groups requested the government to apply for the community health education (trainings and seminars) and they shared the opinion about the necessity of mass vaccination program against brucellosis as it is done for others diseases (Foot and Mouth Disease, East Coast Fever). Two groups implored the improvement of the equipment in health facilities, also solicited the reinforcement of livestock service in the local communities (increase the number of field livestock officers).

> *"....so, it's better if the government can bring the service near and if possible every village should have an animal health center."*(Farmer2- FGD Bweranyange-Karagwe District).

Key informants proposed to build a laboratory for the diagnosis of brucellosis, to conduct research for mapping brucellosis in the area and they advocated for multisectoral collaboration (sharing information between livestock, wildlife and public health department) about brucellosis.

"I think there is a need of conducting research to be sure if really brucellosis is existing or not. We are assuming and assumption can be possible, but from what is happening, it is likely that brucellosis exists. To be sure of that, we need to have a research to confirm, to see the magnitude of the problem." (KII- Ngara District).

DISCUSSION

This study revealed low knowledge, poor perception and practices regarding brucellosis in pastoral communities of Kagera Region, northern Tanzania. Previous studies in Tanzania informed on the magnitude on brucellosis in some areas of the country (Kunda et al., 2005; Kiputa et al., 2008; Roug et al., 2014; Assenga et al., 2016), indicating the disease being one of important threats to both veterinary and public health in the country. Qualitative research studies like the current study are limited but provide better understanding of the problem, and hence, contribute to improving surveillance and management of brucellosis (Mangesho et al., 2017) in affected communities.

All participants described brucellosis as a zoonotic disease and most admitted the presence of the disease in their areas. Nevertheless, the presence of a disease can't be confirmed from mere perceptions of people. For example, some local names like "Okutoroga" didn't mean exclusively brucellosis as a disease, but they were indicating the syndrome of abortion in general, which could be attributed to the existence of other abortive diseases in the area. Respondents in this study perceive brucellosis as a zoonotic disease. On the other hand, a study conducted in Tanga and Arusha revealed that rabies, tuberculosis and anthrax were considered to be the most common zoonotic diseases (Swai et al., 2010). It comes out that farmers understand the possibility of transmission of infectious diseases from animals to humans without much consideration for their threat (Mangesho et al., 2017).

A study conducted in Kenya showed a high level of knowledge of brucellosis in pastoral communities where respondents reported brucellosis to be a zoonotic disease and abortion as its common symptom (Obonyo and Gufu, 2015). But, in Ethiopia, none of the respondents to a study reported the zoonotic importance of brucellosis (Tesfaye et al., 2013). The zoonotic aspect of brucellosis is mostly favored by the lack of awareness of the disease among pastoralists, the scarce collaboration between different sectors and the small investment in the control of the disease by governments in developing countries. In addition, experts stated that the approach used in developed countries (animal slaughter and milk pasteurization) is not suitable for the control of *Brucella* species infections in humans in Africa (Marcotty et al., 2009). Furthermore, diagnostics tools need to be reinforced by rapid and reliable diagnostic tests for effective detection of brucellosis at different stages in human.

In the study area, brucellosis was perceived to be caused by others diseases such as malaria in humans, Foot and Mouth Disease in livestock; which indicates that the disease could be less considered among the principal threats in the study area. In addition, if brucellosis is one of the causes of losses in pastoral communities, this situation could lead to the negligence of its real burden. Although abortion was mentioned as common symptom of brucellosis in humans and livestock, women who participated in this study affirmed not to observe a big number of abortions in humans nowadays and, according to them, the rare cases which can occur could not be associated to brucellosis. Studies also documented that *Brucella* species occasionally are causing spontaneous human abortions, but the contribution of brucellosis to abortions in women is still controversial (Khan et al., 2001; Kurdoglu et al., 2015).

Participants talked mostly about cattle, and this could indicate the major importance attributed to cattle compare to small ruminants as far as brucellosis in concerned. In fact, brucellosis can be transmitted to humans from small ruminants by assisting goats or sheep births in Tanzania (Cash-Goldwasser et al., 2018). However, little is known about the transmission from goat or sheep milk, which could also demonstrate the low awareness of brucellosis and its zoonotic health implication in the study area. Other studies in Tanzania reported findings in which pastoralists did not perceive the products from animal origin to be dangerous (Swai et al., 2010; Mangesho et al., 2017).

Respondents had also knowledge of the impact of brucellosis on their social, maternal, nutritional health and economic situation. Zoonotic diseases like brucellosis can cause losses with far-reaching social impacts (Ducrotoy et al., 2014). Losses particularly due to brucellosis remain to be quantified through epidemiological studies, because abortions due to brucellosis in humans and livestock are not well understood. Furthermore, studies on the economic impact of brucellosis in livestock are reasonably consistent in different production systems in Africa and Asia (McDermott et al., 2013). Economic burden in pastoral areas are also due to other infectious diseases, but generally in Africa, in areas where the infection rate can reach 30% for bovine brucellosis, the economic losses are estimated at 5.8% of gross income per animal reared (Domenech et al., 1982).

Poor prophylactic practices regarding brucellosis were observed in this study. If domestic animals are infected with brucellosis, a direct consumption of milk locally collected by farmers and informally commercialized could increase the risk of brucellosis infections in humans. In Uganda, a study that confirmed the presence of *Brucella* in cattle reported a high risk of human brucellosis associated with informally marketed milk and (Hoffman et al., 2016). A systematic review on treatment of brucellosis in human for the last twenty years, concluded that doxycycline-aminoglycoside combination was the first choice with doxycycline- rifampin and the study recommended doxycycline-cotrimoxazole to be the alternative regimens (Alavi and Alavi, 2013). However, treating suspected cases combined with self-medication by people suggests that population of Kagera Region could be exposed to an antimicrobial resistance threat in humans and their livestock. Tanzania is placed among countries which are in need of standard surveillance of antimicrobial resistance in human and livestock pathogens (Mshana et al., 2013).

Diseases can be misdiagnosed in the population because of the absence of diagnostic tools. Furthermore, sound control of diseases require relevant skills and information about their causes, symptoms and mode of transmission (Lindahl et al., 2015). Animal health strategy for diseases control is well established in Tanzania. However, limitations exist in sensitization campaigns and mass vaccination programs for brucellosis (Matthew et al., 2016). Efforts are needed to sensitize people for mass vaccination against brucellosis which could lead to the control of it zoonotic transmission (Olsen and Stoffregen, 2005). Some participants reported to use local medicines to treat brucellosis in humans and animals. This practice is shared by smallholder dairy farmers in Pakistan (Arif et al., 2017). About 193 plants are documented in the East African region to be used by farmers for treating diseases of their livestock including brucellosis (Katerere and Luseba, 2010). However, these practices are sometimes kept as secrets by farmers and are transmitted from generation to generation. Moreover, traditional medicines are valuable resources for new agents against antibiotic-resistant strains, and studies have been conducted in this aspect (Motamedi et al., 2010; Noudk et al., 2017).

Key informants reported drinking unpasteurized milk and eating non-inspected meat to be among possible factors which could contribute to transmission of brucellosis in humans in the study area. Possible risk factors for brucellosis infections in humans were practices of assisting animals during parturition without any protection and the disposal in the nature of placentas and aborted materials which could be attributed to the lack of

community health education. Protective gears used during assistance of parturition could not be available in pastoral areas; and the limited incomes of small farmers could perpetuate such poor practices. In addition, this behavior can be related to the low risk perception of brucellosis in the communities. Small scale farmers in Tajikistan didn't use any protection when handling cows getting an abortion or when dealing with aborted materials (Lindahl et al., 2015). Other studies in Tanzania revealed a knowledge of pastoralists of the risk for brucellosis infections in humans due to the occurrence of abortions in herds, individuals living in close proximity with livestock and animal slaughtering occupation (Swai and Schoonman, 2009; Kunda et al., 2010; Assenga et al., 2016). The interactions between wildlife and livestock were reported as potential risk for brucellosis transmission to humans and livestock. Scholars have documented the presence of brucellosis in wildlife (Eyumajwa et al., 2009; Godfroid et al., 2010; Muma et al., 2010). However, the role played by wild species in spillover of brucellosis to humans and livestock remains to be clarified. Little was discussed in this study, by participants about the mode of transmission, the risk factors or the impact of brucellosis in wildlife in their communities. In the other hand, respondents in a study conducted in Uganda believed that the proximity of livestock to wildlife contributes to the emergence of brucellosis (Kansiime et al., 2015). Moreover, experts from wildlife sector could increase the diagnosis and surveillance of prevalent diseases and share the information with the rest of stakeholders in the communities.

In the Kagera ecosystem, there are games reserves like Burigi, Kimisi on the Tanzania side; Ruvubu National Park in Burundi, and Akagera National Park in Rwanda where an uncontrolled movement of wildlife species can be observed. These interactions may be controlled to minimize the risk as long as the reservoirs of brucellosis in the ecosystem are domestic and wild animals which may carry Brucella regardless of infection prevalence in the main hosts (Zheludkov and Tsirelson, 2010). Even though the introduction of brucellosis in Kagera region is not documented, observations from a study stated that the potential impact of a disease outbreak can be amplified by interactions of drivers (Suk et al., 2014). Participants to this study mentioned also the movement of refugees with their livestock in the area, together with an increase of sexual intercourses, consequent to cross border exchanges as potential drivers of brucellosis in humans and livestock in their communities. Moreover, the increase in animal product demand can favor the spread of transboundary animal diseases (Otte et al., 2004), including brucellosis.

Participants converged to solicit community education on integrated health management of zoonotic diseases, brucellosis included. Even though, some recommendations were addressed specifically to the Government to control brucellosis in their communities, farmers should act through associations or in their cooperatives where mass vaccination programs can be implemented. Studies suggested the increased knowledge in local communities as a strategy for prevention and control of brucellosis (Obonyo and Gufu, 2015). A reinforcement of livestock personnel skills at community level was proposed. In Uganda, the training and recruitment of more health personnel, the education of the communities about brucellosis diagnosis and vaccination were underlined as important gaps for the prevention of brucellosis in the communities (Kansiime et al., 2015). The exchange of information between neighboring countries at multidisciplinary level could also increase the risk management and control of brucellosis in the ecosystem. A collaboration between veterinary and public health services could also improve human and animal health sectors(Kahn et al., 2007).

Study limitations: During discussions, there were confusions in understanding the differences between brucellosis and other abortive diseases in the area, because in Swahili, brucellosis is called "Ugonjwa wa kutupa mimba"= "Disease of abortions". Participants were requesting for more clarifications to understand differences between abortions as symptom and brucellosis as disease. Discussions with key informants were made in groups of three persons instead of independent interviews due to their limited time. With such approach, participants could influence each other's response during the discussion. However, the information collected from the Key Informants complemented the knowledge from the rest of participants of this study. This research was conducted in pastoral communities, where there are strong interactions between humans, livestock and wildlife in an ecosystem located on borders between four countries (Tanzania, Burundi, Rwanda and Uganda), which is the strength for this study.

CONCLUSION

This study assessed the knowledge and perception regarding brucellosis in pastoral communities of Kagera Region, Tanzania. Focus group discussions and

interviews with keys informants revealed a low knowledge, perception and practices of brucellosis in the study area. Participants possessed low knowledge on causes, symptoms and mode of transmission of brucellosis. However, people from these pastoral communities attributed different local names to brucellosis and they were aware that it is pertaining to zoonotic diseases. Despite their knowledge on the existence of strong interactions between humans, domestic animals and wildlife in the bordering ecosystem, their risk perception of brucellosis is poor due to the neglected and cultural behavior of people in their communities. The improvement of the knowledge and practices regarding brucellosis request a clear community health educaiion program and should involve cross border collaboration with stakeholders in neighboring countries. More research is needed to elucidate the status of this transboundary disease in the pastoral areas of Kagera Region.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

This work was a result of the contribution of all authors. JBN designed the study and conducted the interviews and edited the manuscript; JBN and HN coded and analyzed the data; HN, LEM, SIK, EK reviewed the manuscript; EK assisted for funding the research. The authors approved the final manuscript.

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Paper Three

Brucellosis and its associated risk factors to humans and domestic ruminants in Kagera ecosystem, Tanzania

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3.1 Abstract

Background: Brucellosis is one of the important zoonotic diseases for both veterinary and public health. A study was conducted to estimate brucellosis seroprevalence and identify its associated risks factors in humans and in domestic ruminants in pastoral areas of Kagera region, Tanzania.

Methods: Sera from 156 humans with malaria-like symptoms were collected in Ngara and Karagwe districts and analyzed using the Rapid Slide and Fluorescence Polarization Assay (FPA). Sera from cattle (426), goats (206) and sheep (197) were also collected in Ngara and Karagwe districts and analyzed using Rose Bengal Plate (RBPT) and c-ELISA tests.

Results: In humans, seroprevalence of brucellosis was 7.7% (95% CI: 3.8-12.2%): 1.9 % (95% CI: 0.0-4.5%) and 5.8 % (95% CI: 2.6-12.6%) for *B. abortus* and *B. melitensis*, respectively. At individual animal level, the seroprevalence of brucellosis was 5.9% (95% CI: 4.0-8.6%), 2.5% (95% CI: 0.8-5.7%) and 0.5% (95% CI: 0.01-2.8%) in cattle, goats and sheep, respectively. At herd level, brucellosis seroprevalence was 18.2% (95% CI: 12.0-25.8%) in cattle and 6.9% (95% CI=2.2-15.3%) in small ruminants. In humans, brucellosis was associated with the assisting in parturition of pregnant animals without wearing protective gears (OR= 5.6; p= 0.02). Seropositivity to *Brucella* was associated with bovine species (OR=3.5; p=0.01) specifically in herds of medium size with 50-200 animals (OR= 4.2, p= 0.01). The knowledge of brucellosis among pastoralists (OR=0.1; p=0.007) was a protective factor.

Conclusion: Suspected *Brucella* infections could be occurring in pastoralists and their domestic ruminants in Kagera. A community health education and passive surveillance of brucellosis are necessary for the control of this zoonotic disease in Tanzania.

Key-words: Brucellosis; Pastoralists; Risk-factors; Tanzania.

3.2 Introduction

Brucellosis is a zoonotic disease that affects humans and animals globally. Human brucellosis is acquired by direct or indirect contact with infected animals or their products. In humans, the disease is under-diagnosed worldwide (Corbel, 1997) and the symptoms are often vague but may include: undulating fever (the most common symptom), body-wide aches and pains, headache and night sweats. Domestic animals are infected through direct contact with aborted materials, vaginal discharges, milk and semen from *Brucella* infected animals. In East African countries, efforts are made to understand the epidemiology of the disease. In Uganda (Bernard, 2005) and Kenya (Kadohira *et al.*, 1997) studies reported different prevalence levels, depending upon the locations, the methods of diagnosis used and according to species of interest. In animals, the main risk factors reported for *Brucella* infections are: sharing common pasture with wildlife and using designated calving areas (Kadohira *et al.*, 1997). Previous studies on brucellosis in Tanzania have demonstrated the importance of the disease as zoonosis (Kunda *et al.*, 2007; Assenga *et al.*, 2015; Shirima and Kunda, 2016; Cash-Goldwasser *et al.*, 2018). Other studies conducted on brucellosis in some ecosystems in Tanzania reported prevalence of this disease in humans, livestock and wildlife interface. For instance, anti-*Brucella* antibodies were detected in humans (0.6 %); in cattle (6.8 %), in goats (1.6 %) and in buffaloes (7.9 %) (Assenga *et al.*, 2015). In addition, a 10.5% prevalence of brucellosis in trade stock from Karagwe district has been reported, which poses a risk of its transmission through livestock trade (Kiputa *et al.*, 2008). Due to its economic importance and social impact in the population (abortions, infertilities and reduction of milk production), brucellosis in Kagera ecosystem calls for researchers' attention. This region is part of the Kagera River basin ecosystem shared between Burundi, Tanzania, Rwanda and Uganda where, domestic animals, wildlife and human populations are constantly interacting. Epidemiological studies on brucellosis in this shared ecosystem between bordering countries are scarce. However, such studies could underscore the

understanding of the transboundary issues associated with the disease transmission and the movement of people, their livestock and animal products within ecosystems in East African Community (EAC). Therefore, the objective of this study was to estimate the magnitude of *Brucella* infection and identify associated risk factors among pastoralists and their domestic ruminants in Kagera ecosystem, Tanzania.

3.3 Methodology

3.3.1 Study design

A cross-sectional study was conducted in June 2017 to identify risk factors associated with brucellosis in humans and domestic ruminants in pastoral areas of Kagera region with two districts involved in the study namely Ngara and Karagwe. Eighteen villages were purposively selected from peri-urban and rural areas (Figure 3.1). Ten herds were also purposively selected in each village and all the health facilities (dispensaries and health centres) located in the selected villages were included in the study for blood sampling of patients with malaria-like symptoms (fever, joint pain, headache, back pain, fatigue and nausea). In addition, two district hospitals were included for human sampling as they served the majority of patients from the study villages. Participants were recruited in health facilities (15 health centres and 5 hospitals) at the moment they came for malaria screening. Majority of patients that were sampled lived in close proximity with domestic animals with 94% keeping animals. Assisted by local phlebotomists, plain vacutainer tubes were used to collect 5mL of venous blood from participating patients. Prior to this, consent was obtained after explaining the study objectives to the participants. Using a formula: Z^{2*} Pexp (1 –Pexp) /D^2 (Martin *et al.*, 1987), a sample size of 234 humans, 492 cows, 200 goats and 200 sheep was estimated.

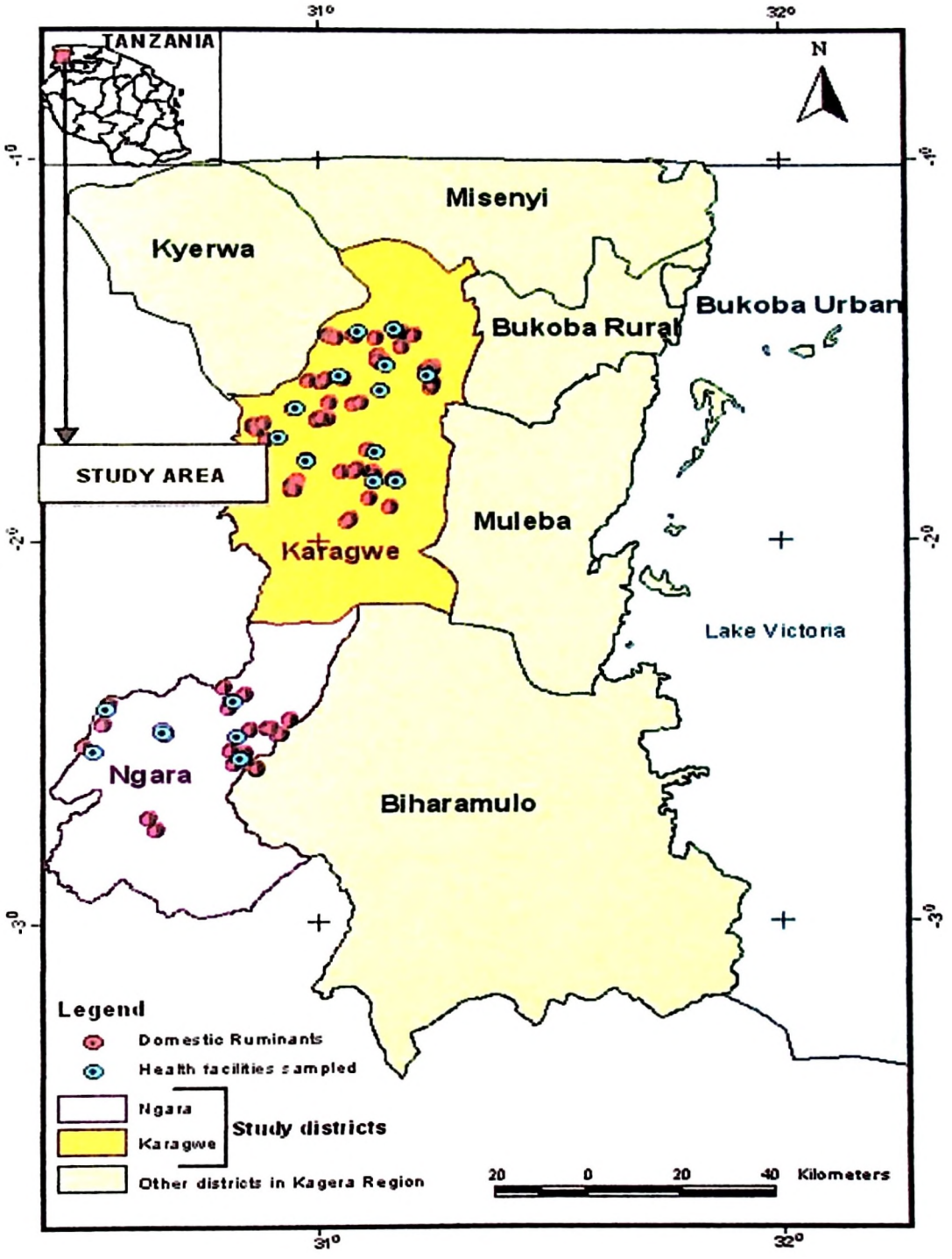

Figure 3.1: Study districts and origin of samples used in this study

Households were selected based on risk factors criteria among herds such as sharing source of water, communal grazing, sharing bulls and history of abortion. Domestic ruminants were selected using a simple random sampling in households and plain vacutainer tubes were used to collect 5mL of venous blood assisted by local veterinary technicians. Interviews were conducted on patients and owners of animals in Kiswahili (national language). Structured questionnaires digitalized by *AfyaData* software (Karimuribo *et al.*, 2017) were filled using smart mobile phones to collect data on risk factors with main variables in patients (symptoms, consumption of unboiled milk, assisting parturition without wearing protective gears, living in close proximity with domestic animals, livestock keeping). In case of domestic ruminants, factors evaluated included: sharing source of water, communal grazing, sharing bulls and history of abortion.

3.3.2 Laboratory analysis

Human sera were double-checked for anti-*Brucella* antibodies of *B. abortus* and *B. melitensis* or for cross-reactions using the commercial rapid agglutination test according to the manufacturer's instructions (ARKRAY Healthcare Pvt.ltd-INDIA, lots 15SA402-05 and 15SA403-05). Only samples with agglutinations on the slides were subjected to the confirmation using Fluorescent Polarization Assay according to the manufacturer's instructions (Ellie LLC, USA, *Brucella* FPA, code B1001). For domestic animals, sera were screened firstly using RBPT. Samples only with a positive reaction were then subjected to confirmation using the c-ELISA test according to manufacturers' instructions (APHA Scientific, UK. Code RAI2006). Patients (humans) reacting to Rapid slide and FPA tests were considered to be seropositive to brucellosis. Domestic ruminants were considered to be seropositive if they reacted to both RBP and c-ELISA tests.

3.3.3 Ethical consideration

This study was also approved by the Institutional Review Board of Sokoine University of Agriculture and the Medical Research Coordinating Committee of the National Institute for Medical Research (Ref: NIMR/HQ/R.8a/Vol.IX/2456).

3.3.4 Data analysis

Questionnaires were uploaded in Excel® sheet for analysis. Seroprevalence in humans and domestic ruminants were determined based on criteria of seropositivity for an individual during laboratory analysis. The overall seroprevalence was determined for each species and the herd prevalence was also computed by species. All independent variables were screened by univariable logistic regression analysis for their association with the positivity of brucellosis in animals and humans in Kagera using IBM® SPSS® Statistics 21. Variables with p-value less than 0.2 (univariable logistic regression) were included in the risk factors assessment by a multivariable logistic regression model (enter method), reporting odds ratio with 95% confidence intervals. A *P*-value less than 0.05 was considered as significant.

3.4 Results

3.4.1 Demographic characteristics of humans and ruminants sampled in Kagera region

A total of 192 households were visited and 156, 426, 206 and 197 sera were sampled from humans, cattle, goats and sheep, respectively. Patients were aged between 5 and 77 years (mean =35±16.6) with a total of 55 males and 101 females (sex ratio of 1:2) in the two districts. Livestock owners were between 24 and 84 years of age (mean = 47.5 ± 11.7), and the age of animals sampled was between 18 months and 8 years for cattle, and 8 months to 5 years for small ruminants.

3.4.2 Seroprevalence of brucellosis in humans and domestic ruminants in Kagera region

In humans, brucellosis was 7.7% (95% CI: 3.8-12.2%) with antibodies of 1.9 % (95% CI: 0.0-4.5%) and 5.8 % (95% CI: 2.6-12.6%) for *B. abortus* and *B. melitensis*, respectively. The individual prevalence in domestic ruminants is presented in Table 3.1. The overall seroprevalence of brucellosis in domestic ruminants was 3.7% (95% CI: 4 -8.6) while the herd prevalence was 13.5% (95% CI: 9 -19.2%). At the herd level, the prevalence of brucellosis was 18.2% (95% CI: 12-25.8%) and 6.8% (95% CI=2.2-15.3%) in bovine and small ruminants, respectively.

Table 3.1: Serological results of brucellosis in humans and domestic ruminants in Kagera region

Variable	Serological test used	No. Samples tested	Positive	Seroprevalence (%) and 95% CI
Human	Rapid slide and FPA	156	12	7.7(3.8-12.2%)
Cattle	RBPT and c-Elisa	426	25	5.9(4-8.7)
Goats	RBPT and c-Elisa	206	5	2.5(0.8-5.7)
Sheep	RBPT and c-Elisa	197	1	0.5(0.01-2.8)

3.4.3 Logistic regressions

The univariable logistic regression was done to assess the association between each variable and *Brucella* seropositivity in Kagera region (Table 3.2 and Table 3.3). Assisting pregnant animals without wearing protective gears (OR=5.6, p=0.02) was associated with increased brucellosis in humans. Also, the multivariable logistic regression identified two factors namely species (OR=3.5, p=0.01) and herd size (OR=4.2, p=0.01) to be risks for *Brucella* infections in domestic ruminants in the study area. However, knowledge of the disease was found to be a protective factor (OR: 0.1, P<0.01) (Table 3.4).

Table 3.2: Association of variables with seropositivity in humans during Univariable Logistic regression analysis

Variable	Extent	Positive (95% CI)	OR (95% CI)	p-value
District	Karagwe	6.8(2.7-13.5)	0.7 (0.2-2.3)	0.56
	Ngara	9.4(3.1-20.6)	Ref	
Health facilities	Local dispensaries	8(3.7-14.5)	0.8(0.2-3.4)	0.8
	District hospitals	7(1.4-19)	Ref	
Sex	Female	8.2(3.6-15.6)	1.2 (0.3-4.3)	0.7
	Male	6.8 (1.8-16.4)	Ref	
Age	5-30 years	6.7(2.3-15)	1.2(0.5-2.8)	0.6
	31-60 years	8(2.6-17)	Ref	
	>60 years	10(1.2-31)	Ref	
Marital status	Single	2.8(0.07-14.5)	1.1(0.4-2.7)	0.6
	Married	10(5.1-17.1)	Ref	
	Divorced	0	Ref	
	Widower	0	Ref	
	No	7.3(3.7-12.6)	Ref	
Dinking unboiled milk	Yes	11.4(5-21.2)	0.4 (0.1-1.3)	0.1
	No	4.7(1.2-11.4)	Ref	
Consuming cheese	Yes	12.1(3.4-28)	0.5 (0.1-1.8)	0.3
	No	6.5(2.8-12.4)	Ref	
Living with domestic animals	Yes	7.5(3.4-13.7)	1.1 (0.3-4.4)	0.9
	No	8.3(1.75-22.4)	Ref	
Assisting parturition without protective gears	Yes	21.1(6-45)	0.2 (0.06-0.9)	0.03
	No	5.8(2.5-11)	Ref	
Abortion history (herd)	Yes	7.8(3.6-14.2)	0.1 (0.2-3.7)	0.95
	No	7.5(1.5-20.3)	Ref	

Table 3.3: Association of variables with seropositivity in animals during Univariable Logistic regression analysis

Variable	Extent	Positive (95% CI)	OR (95% CI)	p-value
District	Karagwe	4.7(3.1-6.7)	3 (1-8.6)	0.04
	Ngara	1.6(0.4-4)	Ref	
Species	Cattle	1.5(0.6-3.4)	0.2(0.0-0.6)	<0.01
	Small ruminant	5.9(3.9-8.7)	Ref	
Age cattle	>36 months	5.8(4-8.7)	1.6(1.1-2.3)	0.09
	12-36 months	1.5(0.6-3.4)	Ref	
	< 12 months	0	Ref	
Herd management	Pastoralism	4.1(2.8-5.8)	0.5 (1.1-1.6)	0.2
	Zero grazing	0	Ref	
	Mixing	2.3(0.06-12)	Ref	
Herd size	Medium (50-200)	5(3.5-7.6)	3.2 (1.2-8.4)	0.02
	Small (<50)	1.6(0.5-3.8)	Ref	
Herd location	Rural	3.9(2.7-5.6)	1.6 (0.4-6.9)	0.5
	Peri-urban	2(0.3-8.5)	Ref	
Communal grazing	Yes	5.3(2.5-9.8)	0.6 (0.3-1.3)	0.3
	No	3.3(2.1-5)	Ref	
Sharing pasture with wildlife	Yes	5.3(2.8-8.8)	1.7 (0.8-3.6)	0.1
	No	3.1(1.9-4.9)	Ref	
Sharing water sources among herds	Yes	4.0(2.6-6)	1.2 (0.6-3)	0.5
	No	3.1(1.3-6)	Ref	
Sharing bulls among herds	Yes	2.1(0.4-4.7)	0.5 (0.2-1.2)	0.1
	No	4.4(1.2-6.5)	Ref	
Abortion history (in herds)	Yes	4.3(2.9-6.4)	2 (0.8-5.3)	0.1
	No	2.2(0.7-5)	Ref	
Knowledge on brucellosis among pastoralists	Yes	7.8 (2.3-26)	0.8(0.2-2.5)	<0.01
	No	6.1(4-8.7)	Ref	
Vaccination of brucellosis (in herds)	Yes	1.6(0.04-8)	0.4(0.0-3)	0.4
	No	3.9(2.7-5.6)	Ref	

Table 3.4: Final model of risk factors associated with brucellosis seropositivity in humans and domestic ruminants in Kagera

Variables	Extent	OR	95% IC	p-value
Risk factors in humans				
Drinking unboiled milk	Yes	3.2	0.8-12.4	0.09
	No	Ref		
Assisting parturition without protective gears	Yes	5.6	1.3-23.5	0.02
	No	Ref		
Risk factors in domestic ruminants (animal herd level)				
District	Karagwe	0.5	0.07-2.5	0.3
	Ngara	Ref		
Species	Bovine	3.5	1.3-9.8	0.01
	Small ruminants	Ref		
Herd size	Small (<50)	Ref		
	Medium (50-200)	4.2	1.4-12.7	0.01
Abortion history in herds	Yes	4.4	0.8-22.9	0.07
	No	Ref		
Knowledge on brucellosis among pastoralists	Yes	0.1	0.0-0.5	<0.01
	No	Ref		

3.5 Discussion

Out of 156 samples from malaria-like symptoms patients, 7.7% were confirmed to have *B.abortus* and *B.melitensis*. this is similar to the findings reported by Cash-Goldwaser *et al.* (2018) in Northen Tanzania but lower compared to other studies from Sengerema and Mwanza (Mngumi *et al.*, 2016; Mirambo *et al.*, 2018). However, there was no difference between sexes of people or between sampled districts, suggesting that people had a similar level of seropositivity to *Brucella* regardless of their gender or district of habitation in Kagera region. Nevertheless, there was no difference regarding positivity to brucellosis among febrile and non-febrile participants. In Mikumi ecosystem, the assessment of brucellosis prevalence in febrile group revealed higher positivity of 23.9% than in non-febrile group 3.7% (James, 2013). In another report in Tanzania, no prevalence of

brucellosis was found in humans in agro-pastoral communities of Serengeti district (Shirima and Kunda, 2016). Elsewhere, seropositivity of brucellosis in humans was also reported in Uganda (17%) (Tumwine *et al.*, 2015); in Kenya (2.2-14.1%) (Ogola *et al.*, 2014) and in Ethiopia (3-34.9%) (Genene *et al.*, 2009).

Assisting parturitions without wearing protective gears (OR= 5.6, p=0.02) increased the risk of exposure to *Brucella* infections in humans in Kagera. Similar results were reported in the northern part of Tanzania (John *et al.*, 2010; Cash-Goldwasser *et al.*, 2018). In addition, the habit of drinking unboiled milk (OR=3.2, p=0.09) and throwing aborted materials to dogs (OR=2.6, p=0.17) seemed to be factors of risk for humans even if they were less significantly associated with brucellosis infection in Kagera. Similar findings by Ntirandekura *et al.* (2018) reported drinking milk and disposal of aborted materials as risks factors for human brucellosis. In this study, the symptoms related to malaria were not statistically associated with the seroprevalence of brucellosis in humans. This may be due to the small number of persons who were seropositive to brucellosis in this group. Positive patients of brucellosis in the study conducted in Arusha and Manyara regions were observed at least to present two common symptoms of malaria (John *et al.*, 2010).

The overall *Brucella* seroposivitiy (5.9%) reported in this study is similar to the results reported in indigenous cattle (animal level 5.6% and herd-level 21.7%) in western part of Tanzania (Chitupila, 2015) and closer to those reported in Lushoto and Rungwe districts (Mfune, 2015) in smallholder dairy cattle. Despite the geographical and farming system variations; sampling and sample size, screening tests and species tested may also influence the seroprevalence. However, the seroprevalence varied across the country with some herds having high *Brucella* seropositivity (Karimuribo *et al.*, 2007; Assenga *et al.*, 2015; Sagamiko *et al.*, 2018). The prevalence of brucellosis at individual level in Kagera region

may be due to the high density of animals, which prompt them to trek long distances in search of pastures and water sources during prolonged dry seasons. In this study, the prevalence of bovine brucellosis at herd level was within the range (16.2%, 95% CI: 10.2 - 25.7%) reported in Sub-Saharan Africa (Mangen *et al.*, 2002).

In this study, cattle seemed to be at higher risk of getting *Brucella* infections compared to small ruminants (OR=3.5; p=0.01). This could be due to the abundance of this species in households and frequent movement to search for pastures and water where they mixed with other herds and wild animals and predispose them to infection. Also, the herds size influenced *Brucella* transmission with herds with 50-200 cattle being high risk of acquiring *Brucella* infection (OR= 4.2, p= 0.01). These findings were confirmed by other studies elsewhere (Megersa *et al.*, 2011; Awah-Ndukum *et al.*, 2018; Sagamiko *et al.*, 2018). In fact, animals in large herds of cattle are condemned to long mobility and migration for pasture and water sources which increase the risk of intermingling between domestic ruminants and favour the *Brucella* transmission (Kadohira *et al.*, 1997). Herds with history of abortion were 4.4 times more likely to be *Brucella* seropositive than those without abortion. This could be due to the proximity of animals within and in between the flocks maximizing the risk of contact with their aborted materials. However, bovine brucellosis is persistent in livestock with low level and relative stability of transmission in pastoral areas (Racloz *et al.*, 2013). In this study, the knowledge of brucellosis among pastoralists (OR= 0.1, p<0.01) was a protective factor for the disease in domestic ruminants. Also, vaccination against brucellosis was a protective factor under the current study (OR=0.4) where coupled with improved knowledge, hygiene and bio-security may control the disease. Similar findings were reported by Shirima *et al.* (2014). Therefore, the current study indicated that the knowledge of the disease among pastoralists should be complemented with the vaccination program and practices of good hygiene for controlling

brucellosis in their herds as reported before in the study area (Ntirandekura *et al.*, 2018). Risk factors like sharing pasture with wildlife and sharing bulls were not statistically associated with brucellosis positivity in Kagera region. Therefore, herds with constant abortion history; could be suspected to be a potential source of brucellosis transmission within and between herds.

3.5.1 Study limitations

Due to financial limitations, this study couldn't extend the human sample size to the community in the non-febrile group which could have generated additional information for the understanding of seroprevalence of brucellosis in the study area. However, sampled patients lived in close proximity with domestic animals that were also sampled in this study and majority of them (94%) were animal keepers. This contributed to understanding the consistent of known animal husbandry and associated brucellosis risk factors in the study area.

3.6 Conclusion

Brucellosis is prevalent in Ngara and Karagwe districts of Kagera region affecting both domestic ruminants and humans. The major factor contributing to human exposure was handling animals during parturition without protective gears whereas in animals, herd size and species influenced the *Brucella* infection. Furthermore, knowledge of brucellosis among pastoralists, vaccinating cattle against brucellosis were protective factors for *Brucella* seropositivity hence advocated to be included in the brucellosis control strategy.

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Paper Four

Association of brucellosis with abortions in humans and domestic ruminants in the Kagera ecosystem, Tanzania

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Association of brucellosis to abortions in humans and domestic ruminants in Kagera ecosystem, Tanzania

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Abstract

Brucellosis is a worldwide zoonotic disease of socio-economic importance. Understanding the association of this disease with pregnancy outcome has the potential of contributing to the reduction of its reproductive burden in humans and animals among pastoral communities in Tanzania. A prospective cohort study was conducted in Kagera Region on pregnant women (n = 76) and gravid ruminants (121 cattle, 125 goats and 111 sheep). Exposed and non-exposed groups to brucellosis were followed for 6 months (from 15 November 2017 to 15 April 2018). Sera were collected and analysed using Rose Bengal Test (RBT) and Fluorescence polarization assay (FPA) test. Measures of effect, univariable and multivariable logistic regression analyses were computed. Positivity to both RBT and FPA tests was 21% (95% CI: 12.5–32) in pregnant women and 5% (95% CI: 3.1–8) in gravid ruminants. Among aborted cases, four women (out of nine), two cows (out of seven), two goats (out of 26) and zero sheep (out of 11) were positive to brucellosis. The abortion rate in humans and ruminants was 11.8% and 12.3%, respectively. Seropositivity to brucellosis was similar in aborted and non-aborted cases in humans (p = .08) and in ruminants (p = .2). At the population level, brucellosis was associated with abortions (population attributable risk: PAR) at 3.5% in pregnant women and at 0.5% in gravid ruminants in the study area. Infections to brucellosis were increased in exposed pregnant women (OR = 19; 95% CI: 1.8–203, p = .01) and in cattle (OR = 11; 95% CI: 1.3–88, p = .02). There is an indication that brucellosis could be contributing to abortions in pregnant women and domestic ruminants Kagera Region. Molecular tools could support more the results from serological tests to avoid cross-reaction with other pathogen agents. Control of brucellosis in animals is likely to reduce the threat of abortions in humans.

KEYWORDS
abortions, association, brucellosis, cattle, human, Tanzania

1 | INTRODUCTION

Brucellosis is a zoonotic disease which remains a major problem in the Mediterranean region, Western Asia, parts of Africa and Latin America (Corbel, 1997). Human infections are acquired through contact, ingestion or inhalation of organisms from infected animals, principally cattle, goats and sheep. The sources of infection for animals include aborted materials, vaginal discharges, milk and semen from infected animals (Sammartino, Gil, & Elzer, 2005). In livestock, brucellosis results in reduced productivity, abortions and weak offsprings.

Moreover, *Brucella* species occasionally cause spontaneous human abortions, but theories regarding whether they do so more frequently than do other infectious pathogens remain controversial (Khan, Mah, & Memish, 2001; Kurdoglu, Cetin, Kurdoglu, & Akdeniz, 2015).

In the livestock industry, the economic impact of brucellosis is mainly attributed to abortions which mostly occur during the last third of pregnancy. In humans, abortions due to brucellosis are mostly recorded in the first- and second-trimester periods of pregnancy (Khan et al., 2001). The causes of abortions in humans and domestic animals include infectious disease agents such as *Brucella* spp., Toxoplasma and Neospora, and others are non-infectious causes which include genetic, environmental and immunologic causes.

Due to the socio-economic importance of brucellosis (abortions, infertilities and reduction of milk production), this zoonotic disease, in Kagera ecosystem, calls for research attention. There are some reports of abortions in domestic animals in Tanzania: 11.3% in Njombe and Mbeya Regions (Mathew, 2017) and 35% in wildlife–livestock interface and non-interface of Tanzania (Mdetele, Kasanga, Seth, & Kayunze, 2015). In addition, non-negligible pregnancy outcomes in humans (15% of miscarriage at national level) were reported in Tanzania (Keogh et al., 2015).

Brucellosis has been reported in different areas of Tanzania (Asakura, Makingi, Kazwala, & Makita, 2018; Assenga, Matemba, Muller, Malakalinga, & Kazwala, 2015; Bouley et al., 2012; Kassuku, 2017; Kunda et al., 2007; Sagamiko et al., 2018; Swai & Schoonman, 2009); however, the contribution of this disease to the recorded abortions in diverse species remains to be appraised. The disease was also reported previously in Kagera (Kiputa, Kimera, & Wambura, 2008), and its prevalence seemed to have enhanced the transmission risk of the disease in traditional herds. Furthermore, it is unclear how the population and various stakeholders in the ecosystem perceive the impact of brucellosis prevalence on women reproduction and livestock productivity.

In addition, there is limited information about the contribution of brucellosis to abortions in humans and livestock in Africa in general (Ntirandekura, Matemba, Kimera, Muma, & Karimuribo, 2018) and particularly in Tanzania. Therefore, this study was conducted to appraise the association of brucellosis with abortions recorded in pregnant women and gravid domestic ruminants in Kagera ecosystem, Tanzania. Serological methods are more used for diagnosis brucellosis since isolation and molecular technics are expensive and time-consuming. For rapid screening of brucellosis, Rose Bengal Plate test is widely used and FPA test is also used for antibodies detection in humans and animals (OIE, 2012); the reason to choose these two tests in this study.

2 | METHODOLOGY

2.1 | Study design

A prospective cohort study was conducted for 6 months (from November 2017 to April 2018) to appraise the contribution of brucellosis to abortions in humans and domestic ruminants in pastoral areas of Kagera Region (Ngara and Karagwe districts). Due to strong interactions between domestic ruminants and people in the same pastoral settings, pregnant women and gravid ruminants were selected together to understand the contribution of brucellosis in observed abortions in this ecosystem. Four hospitals (Nyakahanga, Nyaishozi, Nyamiaga and Rukole) were included in this study on selected pregnant women attending pre-natal medical care during that period (Figure 1). A pregnant woman was considered to be at risk of exposure to *Brucella* if she was in any of the following circumstances: being in contact with domestic animals (living with domestic animals; assisting animals during parturition without wearing protective gears); drinking raw milk (from cow in lactating period); living with brucellosis suspected infected herds (herds with repetitive and unexplained abortions, herds with clinical symptoms of brucellosis); and being a livestock keeper. Non-exposed pregnant women to brucellosis were selected based on criteria opposite to that set for the exposed group. Assisted by local phlebotomists, plain vacutainer tubes were used to collect 5 ml of venous blood from every woman after delivering normally or after miscarriage (in case of abortion occurrence). Prior to this, consent was obtained after explaining the study objectives to the participants. The participants were interviewed for assessment of potential risk factors for brucellosis on following variables: consumption of raw milk, assisting parturition without wearing protective gears, living in close proximity with domestic animals and livestock keeping activity.

Domestic gravid ruminants were selected from eight villages (five villages in Karagwe district and three villages in Ngara district) in peri-urban and rural areas (Figure 1). A gravid domestic ruminant was considered to be a risk of exposure to *Brucella* if it was from a herd with a history of repetitive and unexplained abortions; a herd with unhygienic handling of aborted materials; a herd in which hygromas were observed; or a herd that interacted with wild animals which can act as reservoirs hosts for brucellosis (buffalo, zebra and antelope). Non-exposed cases were pregnant animals which did not fulfil any of these previous criteria. Assisted by local veterinary technicians, plain vacutainer tubes were used to collect 5 ml of venous blood from each gravid animal after normal delivery or in case of abortion occurrence. Questionnaires (close-ended with multiple choices) were administered to the owners of animals and factors evaluated included the following: herd size and location, sharing the source of water with other herds, communal grazing, sharing bulls and history of abortion. Sampling and interviews were done after getting the participant's consent.

Assuming that the anticipated incidences of brucellosis in the non-exposed group were 0.43 in humans (Khan et al., 2001) and 0.35 in domestic ruminants (Shirima, 2005), and using a confidence level of 95%, and anticipated relative risk of 3 and applying the formula: $n=\frac{(p_1+q_1-p_2+q_2)K}{(p_1-p_2)^2}$ (Lwanga, Lemeshow, & World Health Organization, 1991), the minimum sample size of 36 women, 48 cows, 48 goats and 48 sheep was estimated in each group (the exposed and non-exposed subjects) for the follow-up in this study. In the formula, n = number required in each cohort; $K = (Z\alpha + Z\beta)^2$; $Z\alpha$ = the value of Student's *t* at the specified confidence level; $Z\beta$ = the value of Student's t (2-tailed) at the specified power; p_1 = anticipated incidence in unexposed animals; $q_1 = 1 - p_1$; p_2 = minimum incidence to be detected in exposed

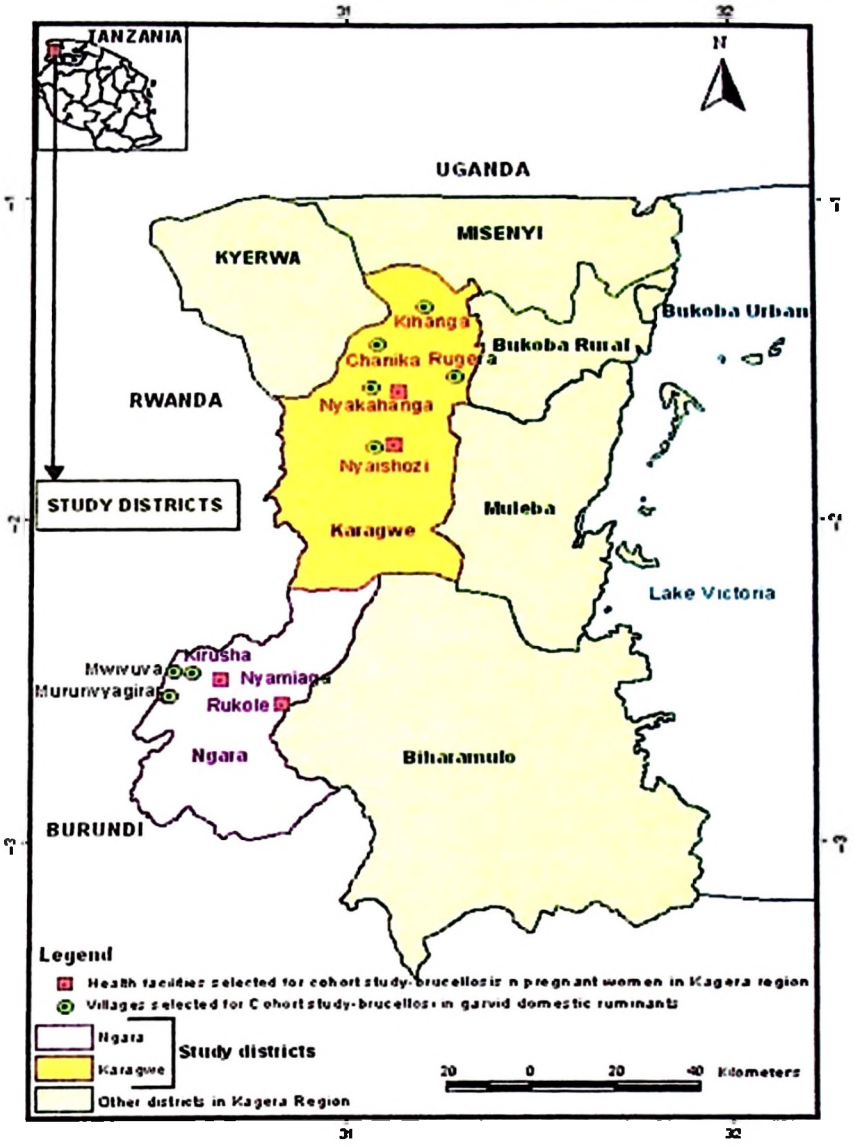

FIGURE 1 Map showing study area (humans and domestic ruminants sampling)

animals (based on the RR to be detected = minimal Relative Risk that is considered as important) and $q_2 = 1 - p_2$.

2.2 | Laboratory analysis

Human and domestic ruminant sera were screened using Rose Bengal Plate test and were subjected for confirmation using FPA test (Ellie LLC, *Brucella* FPA, code B1001). Samples reacting to RBT were then subjected to FPA test for confirmation.

2.3 | Data analysis

Answers from questionnaires and serological data were filled using Excel sheet (version 2010) for analysis; then, the proportion of positives among pregnant women and animals tested was determined. The relative and absolute measures of effect were computed. Relative risk (RR) = Ra/Rna (Ra: risk of abortion(s) in exposed group; Rna: risk of abortion(s) in non-exposed group). The risk difference (RD) is the difference between the incidence proportion of abortions in exposed cases to brucellosis and the incidence proportion of abortions in non-exposed cases to brucellosis. The population attributable risk (PAR) estimated the excess risk among the exposed that can be attributed to the risk factor in terms of the whole population. In addition, all variables were screened by univariable logistic regression analysis for their association with the positivity of brucellosis in Kagera. Using IBM® SPSS® Statistics 21, all variables were included in the risk factors assessment by a multivariable logistic regression model (backward conditional), reporting odds ratio. A p-value < .05 was considered as significant.

3 | RESULTS

3.1 | Demographic characteristics of pregnant women and ruminants sampled in Kagera Region

A total of 76 pregnant women (38 exposed and 38 non-exposed) were followed up in this study and were aged between 17 and 43 years with a mean age of 25 (±6.3). They were between 1 and 6 months of gestation period, and majority of them (80.2%) were from families living in pastoral areas. A total of 121 cow (50 exposed and 71 non-exposed), 125 goats (68 exposed and 57 non-exposed) and 111 sheep (56 exposed and 55 non-exposed) were selected for a follow-up and were between 1 and 6 months of gravid period (according to species). Their age was between 3–8 years for cattle, 2–7 years for goats and 2–6 years for sheep.

3.2 | Seroprevalence of brucellosis and measures of effect of abortion in pregnant women and ruminants in Kagera Region

The seroprevalence of brucellosis in pregnant women and ruminants in Kagera is presented in Figure 2. In aborted cases, the seroprevalence of brucellosis is reported in all species except in sheep. Abortion and positive cases to brucellosis in all species are recorded in Table 1, and measures of association and effect are presented in Table 2. The abortion rate in exposed women was 11.8%, and the OR for abortions in exposed women was 4.1 (95% CI: 0.8–21). The OR for abortions in women positive to brucellosis was 3.7 (95% CI: 0.9–15.7). The abortion rate in exposed gravid ruminant was 12.3%; the OR for abortions in exposed pregnant animals was 7.8 (95% CI: 3.4–17.8). At the species level, the abortion rates were 5.8% in cattle, 20.6% in goat and 10% in sheep.

3.3 | Logistic regressions

In pregnant women, none of the variables was associated with brucellosis positivity (Table 3), while in gravid domestic ruminants, cattle (OR = 10; 95% CI: 1.2–78; $p = .03$) seemed to be associated with brucellosis positivity by univariable logistic regression (Table 4). Multivariable regression model revealed odds in pregnant women for exposure to brucellosis (OR = 19; 95% CI: 1.8–203; $p = .01$) and exposure to brucellosis in cattle (OR = 11; 95% CI: 1.3–88; $p = .02$) in gravid ruminants (Table 5).

4 | DISCUSSION

The association of brucellosis prevalence to the occurrence of abortions in Africa could be a bit biased at the moment since most of the relationships established are based on odds in history of abortion in the herds, temporal or definitive infertilities with a decrease or a total absence of milk production (Mangen, Otte, Pfeiffer, & Chilonda, 2002). In this study, the abortion rate in pregnant women (11.8%) was lower compared to the previous report on miscarriage's distribution (15%) at the national level including in Lake Zone (Keogh et al., 2015). This may be due to the increased of antenatal medical care in the health facilities in the study area. However, the prevalence of brucellosis in pregnant women (21%) was lower compared to the previous reports in Tanzania (Chota et al., 2016), but was higher to that reported from Moshi hospital (Cash-Goldwasser et al., 2018).

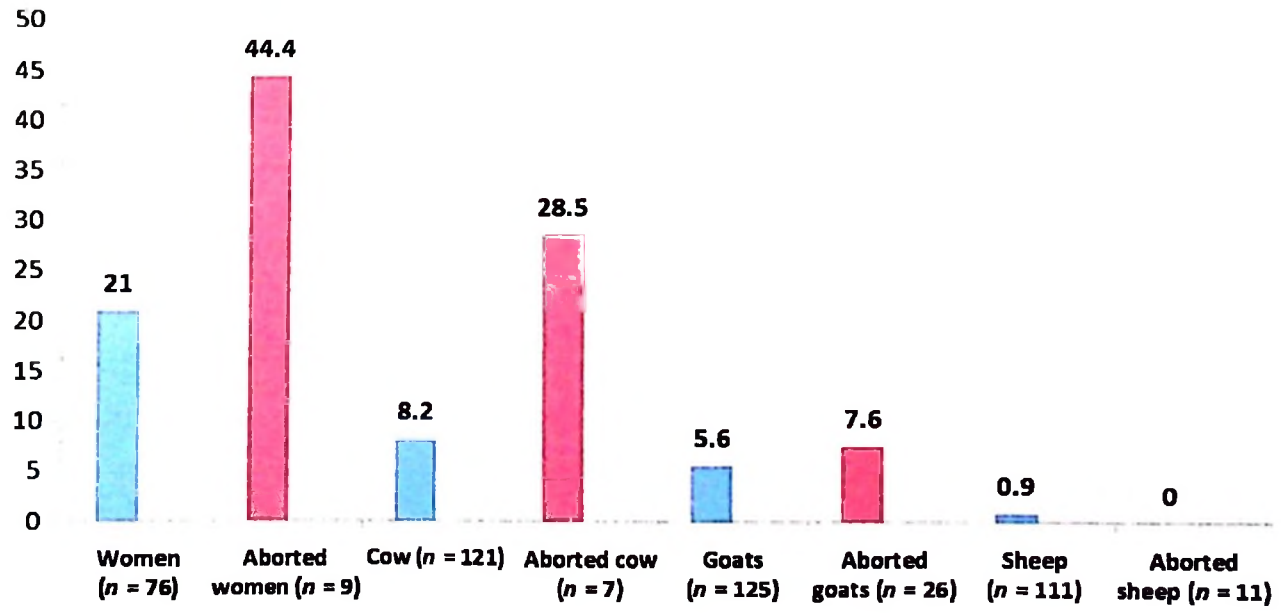

FIGURE 2 Proportion of positivity to brucellosis in pregnant women and domestic ruminants Kagera Region

TABLE 1 Exposure and positivity to brucellosis according to species in Kagera Region

Variables	Species			
	Humans (%)	Cattle (%)	Goats (%)	Sheep (%)
Exposed group				
Abortion cases	7 (18.4)	5 (10)	23 (34.3)	9 (16)
Non-abortion cases	31 (81.6)	45 (90)	44 (65.7)	47 (84)
Non-exposed group				
Abortion cases	2 (5.2)	2 (2.8)	3 (5.2)	2 (3.6)
Non-abortion cases	36 (94.8)	69 (97.2)	55 (94.8)	53 (96.4)
Positive to FPA test				
Abortion cases	4 (44.4)	2 (28.5)	2 (7.6)	0 (0)
Non-abortion cases	12 (17.9)	8 (7)	5 (5)	1 (1)
Negative to FPA test				
Abortion cases	5 (55.5)	5 (71.5)	24 (92.4)	11 (100)
Non-abortion cases	55 (82.1)	106 (79.3)	94 (95)	99 (99)

TABLE 2 Measures of effect on abortions in humans and domestic ruminants in Kagera

Indicators	Species (Pregnant)				
	Women (95% CI)	Ruminants (95% CI)	Cattle (95% CI)	Goats (95% CI)	Sheep (95% CI)
RR of abortion (Exposed cases)	3.5 (0.8–15)	6.2 (2.8–13.7)	3.5 (0.7–17.5)	6.6 (2.1–21)	6.2 (1.4–27.6)
RR of abortion (Positives cases)	3 (0.9–9.8)	1.8 (0.7–4.7)	4.4 (0.1–20)	4.4 (0.1–20)	0
RD of abortion (Exposed cases)	0.1 (0–0.3)	0.2 (0.1–0.2)	0.1 (0–0.1)	0.3 (0–0.1)	0.2 (0–0.3)
RD of abortion (Positive cases)	0.1 (0–0.3)	0.1 (0–0.3)	0.1 (0–0.3)	0.1 (0–0.3)	−0.1 (−0.7–0.5)
PAR of abortion (Exposed cases)	0.06	0.08	0.03	0.03	0.06
PAR of abortion (Positives cases)	0.035	0.005	0.012	0.012	−0.0009

This situation could be explained by the persistence of exposure to *Brucella* infections in pregnant women. In this study, the exposure to brucellosis (RR = 3.5; 95% CI: 0.8–15) and the positivity to the disease (RR = 3; 95% CI: 0.9–9.8) did not increase the risk of abortions in pregnant women. In fact, the risk of abortions in pregnant women could not be readily associated with the exposure (OR = 4; 95% CI: 0.78–21) nor to the positivity to brucellosis (OR = 3.7; 95% CI: 0.9–15.7) in the study area. Abortions might occur due to the effects of other contributing factors. Moreover, there was no statistical difference between positivity to brucellosis in aborted and non-aborted cases (OR = 3.6; 95% CI: 0.8–15.7). Our results are similar to those reported in Jordan (Abo-shehada & Abu-Halaweh, 2011). However, a study reported differences between brucellosis prevalence in miscarriage and non-miscarriage cases in Mwanza-Tanzania (Mujuni et al., 2018). In this study, there was a high association between the exposure and the positivity to brucellosis (OR = 19; 95% CI = 1.8–203). Or, the case definition for exposed women was those who lived in close contact with domestic animals, had a habit of drinking raw milk and assisted animals during parturition without wearing protective gears. These elements could increase significantly the risk of *Brucella* infections among pregnant women. The results could have been influenced by the small sample size. Nevertheless, seropositivity does not necessarily mean that the women were infected. It, however, shows that there were exposed to the *Brucella* antigen, which is common in areas where *Brucella abortus* is endemic (Arenas-Gamboa et al., 2016).

For gravid domestic ruminants, the prevalence (5%; 95% CI: 3.1–8) was within the range previously reported in Tanzania (Sagamiko et al., 2018; Shirima, 2005). In this study, the abortion rate in cattle (5.8%) was lower compared to the previous reports in Tanzania (Mathew, 2017; Mdetele et al., 2015). In Zambia, the abortion rate (16.2%) in exposed cattle to brucellosis (history of abortion) was higher compared to our investigation (Muma, Godfroid, Samui, & Skjerve, 2007). In this study, there was not a statistical difference between brucellosis prevalence in aborted and non-aborted domestic ruminants (OR = 2.1; 95% CI: 0.7–6.8). In general, gravid domestic ruminants were six times at risk of aborting due to the exposure to brucellosis in this study. This could be explained by the persistence of exposure in animals to traditional risk factors to which there are subjected in different seasons in pastoral areas as reported in Morogoro (Asakura et al., 2018). The outcome of infection in animals can be influenced by age, immunologic conditions and virulence of pathogens. In addition, where a high prevalence of brucellosis can be found in Africa, there is an increased probability of recording abortions in domestic ruminants (Domenech, Coulomb, & Lucet, 1982; Mangen et al., 2002). Moreover, there was no risk of abortions in positive cases to brucellosis among gravid ruminants (RR = 1.8; 95% CI = 0.7–4.7). The presence of organisms could not necessarily indicate a causal association

TABLE 3 Univariable association between positivity to brucellosis in pregnant women and different variables in Kagera Region

Variable	Extent	Positive (%)	OR (95% CI)	Wald stat.	*p*-value
District	Karagwe	26.7	2.4 (0.7–8.4)	2.0	.16
	Ngara	13	Reference		
Exposure to brucellosis	Yes	26.32	1.9 (0.6–5.9)	1.2	.26
	No	15.8	Reference		
Fatigue	Yes	12.5	0.4 (0.1–1.3)	2.3	.12
	No	27.2	Reference		
Back pain	Yes	9.6	2 (0.5–8)	0.6	.4
	No	4.8	Reference		
Joint pain	Yes	13.3	0.5 (0.1–2.5)	0.4	.5
	No	22.3	Reference		
Other symptoms (not malaria)	Yes	10.53	0.3 (0.1–1.7)	1.6	.2
	No	24.6	Reference		
Abortion occurrence	Yes	44.4	3.6 (0.8–15.7)	3	.08
	No	18	Reference		
	No	19.6	Reference		
Consuming fresh blood	Yes	23.1	1.6 (0.2–4.8)	0.0	.8
	No	20.6	Reference		
Livestock keeping activity	Yes	23	1.9 (0.4–9.6)	0.6	.4
	No	13.3	Reference		

TABLE 4 Univariable association between positivity to brucellosis in gravid domestic ruminants and different variables in Kagera Region

Variable	Extent	Positive (%)	OR (95% CI)	Wald stat.	*p*-value
District	Karagwe	6	2.3 (0.6–8)	1.6	.2
	Ngara	2.8	Reference		
Specie	Cattle	8.3	10 (1.2–8)	4.7	.03
	Goat	5.6	6.5 (0.8–53)	3	.08
	Sheep	0.9	Reference		
Exposure to brucellosis	Yes	6.7	2 (0.7–5)	1.7	.2
	No	3.6	Reference		
Abortion occurrence	Yes	9	2.1 (0.7–6.8)	1.6	.2
	No	4.5			
Herd location	Rural	12.2	0.0 (0.1–0.9)	4.5	.03
	Peri-urban	4.1	Reference		
Good disposal of aborted materials	Yes	2.1	0.3 (0.1–1.4)	2.2	.1
	No	6.1			
Communal grazing	Yes	6	2.7 (0.6–12.2)	1.8	.2
	No	2.2	Reference		
Sharing bulls	Yes	6.7	3 (0.8–10)	3	.08
	No	2.3	Reference		

between *Brucella* infections and abortions in risk groups. Based on the biology of the disease, the risk of abortion should be higher in the exposed animals. In this study, the abortion rate was higher in goats (n = 26) compared to other species. These abortions could be attributed also to the susceptibility of this species to other infectious abortive pathogens (Rift Valley Fever and Peste des Petits Ruminants) reported in the study area. The proportion of abortion occurrence was less associated with exposure in pregnant women (PAR = 6.5%) compared to the exposure in domestic ruminants (PAR = 8%) in the study area. It is believed that brucellosis can cause less spontaneous

TABLE 5 Risk factors for brucellosis in different species in Kagera

Variables	Extent	Wald statistics	OR	95% CI	*p*-value
Risk factors in pregnant women					
Exposure to brucellosis	Yes	6	19	1.8–203	.01
	No		Reference		
Manifesting other symptoms different from malaria	Yes	3.1	0.12	0.0–1.2	.07
	No		Reference		
Living with domestic animals	No	5.2	0.1	0.0–0.7	.02
	Yes		Reference		
Risk factors in gravid domestic ruminants (animal level)					
Species	Cattle	5.1	11	1.3–88	.02
	Goat	3.7	8	0.9–66	.05
Communal grazing	Yes	3.4	4.1	0.9–18	.06
	No		Reference		
Good disposal of aborted materials	Yes	3.3	0.2	0.0–1.1	.06
	No		Reference		

abortions in women than it occurs in animals due to the controversial presence of erythritol (sugar) in the placenta (Al-tawfiq & Memish, 2013; Petersen et al., 2013). In addition, at the population level, abortions were less associated with positivity to brucellosis in gravid ruminant (PAR = 0.5%) compared to the proportion of abortions associated with *Brucella* infections in pregnant women (PAR = 3.5%). This situation could be explained by the endemic prevalence of the disease in domestic ruminants which could expose pregnant women to a high risk of infections in pastoral areas. It is also noted that animals may abort during the first pregnancy, but the subsequent one may be normal birds (Nicoletti, 1980). Furthermore, women handling livestock in pastoral areas are likely to get elevated abortion rate due to brucellosis (Boschiroli, Foulongne, & O'Callaghan, 2001), although the disease outcome is associated with exposure and occupation, rather than gender (Khan & Zahoor, 2018).

4.1 | Limitations of this study

This study gave important information for *Brucella* infections in pregnant women and gravid ruminant in Kagera ecosystem. If it was not due to financial limitations, it could have been of importance to confirm *Brucella* infections through isolation (culture) and molecular characterization of the infecting *Brucella* species.

5 | CONCLUSION

Brucellosis is prevalent in pregnant women and gravid domestic ruminants in Kagera Region. In this study, the abortion rate was lower compared to some previous reports in the country. Despite the statistical similarities of positivity to brucellosis in aborted and non-aborted cases, a proportion of 0.5% of the abortions was attributable to *Brucella* infections in gravid ruminant, while 3.5% of abortions were attributed to positivity of the disease in pregnant women at the population level. Furthermore, positivity to brucellosis was highly associated with the exposure of the disease in pregnant women, while exposed cattle seemed to be at higher risk of contracting *Brucella* infections compared to other species. In Kagera Region, pregnant women and ruminants are at risk of *Brucella* infections which endemic prevalence could contribute to the reproductive failures recorded in these species. Results from the serological tests used in this study are important to describe the risk for brucellosis infection in pregnant women and gravid ruminants. However, molecular tools could support more the results from serological tests to avoid interpretation from cross-reaction with other pathogen agents. Differential diagnosis of brucellosis with other infectious and febrile diseases is recommended for spontaneous abortions in humans and domestic ruminants. More effort is needed using a multidisciplinary approach for the prevention and control of brucellosis in humans and animals.

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ETHICAL APPROVAL

This study was approved by the institutional review board of Sokoine University of Agriculture and the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (ref: NIMR/HQ/R.8a/Vol.IX/2456).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Paper Five

Molecular characterization of *Brucella* species detected in humans and domestic ruminants of pastoral areas in Kagera ecosystem, Tanzania

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ORIGINAL ARTICLE

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Association of brucellosis to abortions in humans and domestic ruminants in Kagera ecosystem, Tanzania

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Abstract

Brucellosis is a worldwide zoonotic disease of socio-economic importance. Understanding the association of this disease with pregnancy outcome has the potential of contributing to the reduction of its reproductive burden in humans and animals among pastoral communities in Tanzania. A prospective cohort study was conducted in Kagera Region on pregnant women (n = 76) and gravid ruminants (121 cattle, 125 goats and 111 sheep). Exposed and non-exposed groups to brucellosis were followed for 6 months (from 15 November 2017 to 15 April 2018). Sera were collected and analysed using Rose Bengal Test (RBT) and Fluorescence polarization assay (FPA) test. Measures of effect, univariable and multivariable logistic regression analyses were computed. Positivity to both RBT and FPA tests was 21% (95% CI: 12.5–32) in pregnant women and 5% (95% CI: 3.1–8) in gravid ruminants. Among aborted cases, four women (out of nine), two cows (out of seven), two goats (out of 26) and zero sheep (out of 11) were positive to brucellosis. The abortion rate in humans and ruminants was 11.8% and 12.3%, respectively. Seropositivity to brucellosis was similar in aborted and non-aborted cases in humans (p = .08) and in ruminants (p = .2). At the population level, brucellosis was associated with abortions (population attributable risk: PAR) at 3.5% in pregnant women and at 0.5% in gravid ruminants in the study area. Infections to brucellosis were increased in exposed pregnant women (OR = 19; 95% CI: 1.8–203, p = .01) and in cattle (OR = 11; 95% CI: 1.3–88, p = .02). There is an indication that brucellosis could be contributing to abortions in pregnant women and domestic ruminants Kagera Region. Molecular tools could support more the results from serological tests to avoid cross-reaction with other pathogen agents. Control of brucellosis in animals is likely to reduce the threat of abortions in humans.

KEYWORDS
abortions, association, brucellosis, cattle, human, Tanzania

1 | INTRODUCTION

Brucellosis is an important disease among livestock and humans in Sub-Saharan Africa (Ducrotoy et al., 2017). In rural Africa, livestock is used as a primary source of household food as well as income from the sale of animals and their products; representing an important asset to many families (World Bank, 2013). Brucellosis occurs primarily in cattle, bison and swine, although cervids, goats, sheep and horses are also susceptible. Despite the efforts made to control the disease in many countries (Corbel, 1997), *Brucella* transmissions are persisting in domestic animals and, consequently, infections frequently occur to humans. Currently, there are 12 recognized *Brucella* species causing brucellosis (El-Sayed & Awad, 2018) which six of them, are known to be pathogenic to humans: *B. abortus*, *B. canis*, *B. inopinata*, *B. melitensis*, *B. pinnipedialis and B. suis* (Tiller et al., 2010). Globally, *Brucella* species infecting humans and animals are often found in the human-animal ecosystem interface (Godfroid et al., 2011; Assenga, Matemba, Muller, Malakalinga, & Kazwala, 2015) where interactions are strong between humans, livestock and wildlife in the same environment. There are several studies that have reported on the presence of *B. abortus* in Uganda (Hoffman et al., 2016; Mugizi et al., 2015) and *B. abortus*, *B.melitensis* and *B. suis* species in Kenya (Njeru et al., 2016). These species seem to have the highest impact on domestic livestock productivity and human health in Africa (Ducrotoy et al., 2017). In Tanzania, brucellosis has mainly been documented in humans-livestock and wildlife interfaces (Assenga et al., 2015; James, 2013; Shirima et al., 2010). However, one study reported on the isolation and characterization of *B. abortus biovar* 3 in a dairy farm in Mbeya region following abortion (Mathew et al., 2015). Consequently, there are scarce reports on molecular characterization of *Brucella* spp. which are infecting humans and livestock in pastoral settings. Various approaches have been used worldwide for identification and characterization of *Brucella* species; and for determination of origin and possible spillover to other species including humans. Actually, molecular and bioinformatics tools are giving to the knowledge an advance in understanding in single differences between species of *Brucella* evolutionary history, specificity and pathogenicity in different hosts (Vidal, Ortiz, & Olivera, 2018). Exploration of phylogenetic studies such as MLVA (Multilocus variable number of tandem repeats analysis), variable number of tandem repeats (VNTR) (Menshawy et al., 2014), single nucleotide polymorphisms (SNPs) (Wattam et al., 2014) and Multilocus sequence typing (MLST) (Shome et al., 2016) are useful for establishing relationship and grouping of *Brucella* species. Among other technics, one of the most used approach is 16S rRNA gene sequencing, which is a rapid way to confirm identity of *Brucella* species, and therefore allow implementation of needed public health responses (Gee et al., 2004; Khan et al., 2018). However, due to the variations which can be exhibited in the conserved regions of the 16S rRNA gene, it is important to design reliably of the primer for this diagnostic tool (Martinez-Porchas, Villalpando-Canchola, Ortiz Suarez, & Vargas-Albores, 2017). This study aimed at molecular characterization of *Brucella* species in humans and livestock in pastoral areas of Kagera in Tanzania. Real-time PCR was used on sera, aborted materials (from women, cattle, goats and sheep) and milk taken from cattle and goats. The 16S rRNA gene was amplified on positive samples from Real-time PCR: sera (from women, cattle and goats) and milk (from cattle and goats) while Sanger sequencing was done using the fragment amplified from the 16S rRNA gene.

2 | METHODOLOGY

2.1 | Study design

The samples were collected during previous cross-sectional and prospective cohort studies conducted in Ngara and Karagwe districts (Figure 1) in June 2017 (personal communication) and November 2017- April 2018 in Kagera region, Tanzania (Ntirandekura, Matemba, Kimera, Muma, & Karimuribo, 2020). Five millilitres of venous blood were firstly taken from humans going for malaria checking (July 2017) (personal communication) and from cattle, goats and sheep in the same villages. Secondly, 5ml of venous blood were taken from pregnant women attending antenatal care (November 2017-April 2018) and gravid ruminants in the same pastoral area (Ntirandekura et al., 2020). Each blood samples reacting to the rose Bengal, C-Elisa and FPA tests was aliquoted and kept at −20°C for molecular analysis. For the prospective study, aborted materials (from women and ruminants) and milk (from cattle, goat and sheep) were collected and stored at −20°C until the DNA extraction. During the two studies, structured questionnaires were used to complete the information on brucellosis status in the study area. Domestic ruminants were apparently healthy.

This study used sera which were positive for *Brucella* by the previous serological tests (RBPT, c-Elisa and FPA), milk samples and aborted materials (Table 1).

The objective for this study was to characterize *Brucella spp.* using 16S rRNA gene sequencing. However, 16S rRNA PCR would have given too many samples for sequencing which is rather too costly. Therefore, after the DNA extraction, a real-Time PCR (targeting genus *Brucella*) was first used to screen for *Brucella* positive samples only, then continued with a 16S rRNA PCR and sequencing.

2.2 | DNA extraction

Genomic DNA from serum samples and aborted materials were extracted using the QIAamp DNA Mini Kit (Qiagen kit Germany) according to the manufacturer's instructions. To obtain genomic DNA from milk, the samples were centrifuged for 10 min at 10,000 × g, following which the supernatant was discarded. The GeneJET Genomic DNA Purification Kit (Thermofisher Scientific-K0721) was used to extract DNA from the pellet, according to the manufacturer's instructions.

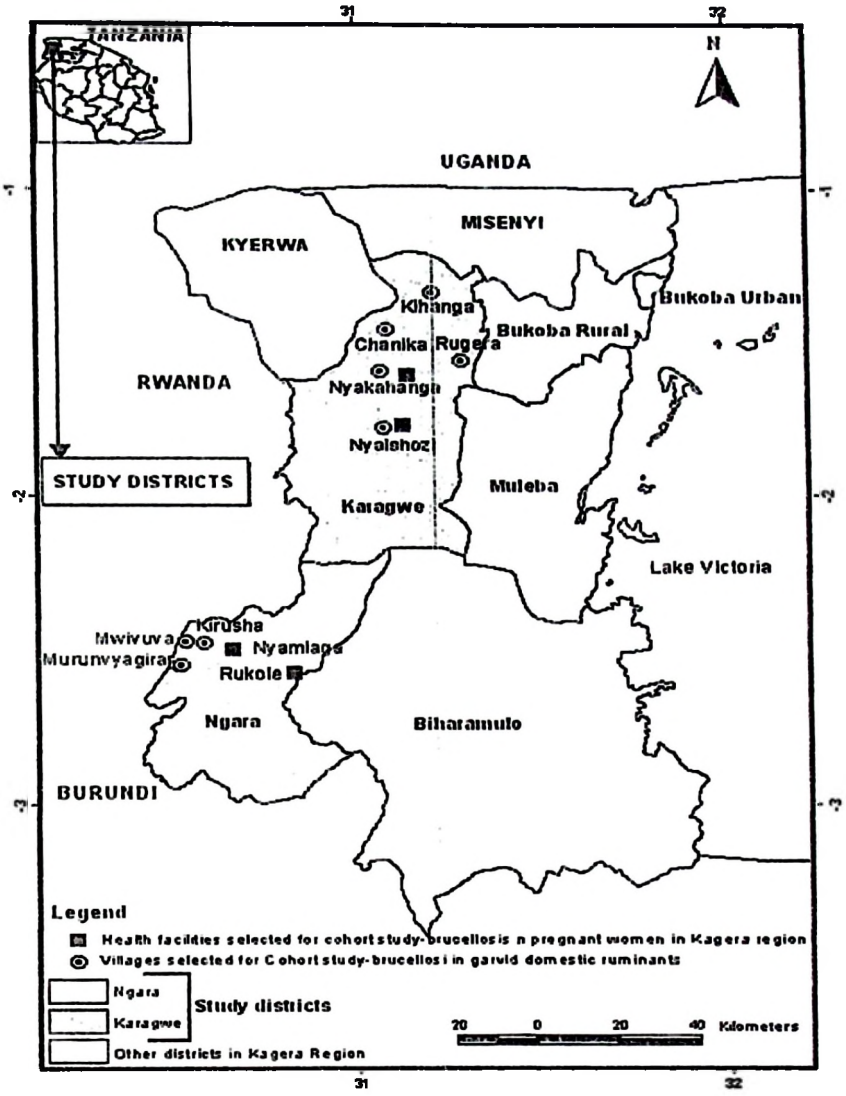


FIGURE 1 Map showing the origin of the samples used in this study (Kagera region)

2.3 | Real-time PCR for *Brucella* spp

One hundred twenty-five samples (Table 1) were subjected to real-time PCR which was performed on the PikoReal machine (Thermo Fisher Scientific) for *Brucella* spp. detection. Targeting *Vdcc* gene, the forward GTGGCGATCTTGTCCG and the reverse ACGGCGATGGATTTCCG *Brucella* spp. specific primers were used (Winchell, Wolff, Tiller, Bowen, & Hoffmaster, 2010). A vaccine *Brucella* strain S19 was used as a positive control. The final reaction volume was 25 µl consisting of 2.5 µl DNA template, 1x of RealQ Plus 2x Master Mix Green (Low Rox), 10 µm of each primer, 10 µm of probe (5' FAM- AAATCTTCCACCTTGCCCTTGCCATCA-BHQ 3') and 5.5 µl of PCR grade water. The PCR reaction started with initial heating at 50°C for 2 min, then at 95°C for 7 min, followed by 35 cycles at 95°C for 5 s and 60°C for 30 s. Data were acquired at 60°C (the extension step).

2.4 | PCR Amplification Of 16S rRNA genes

PCR amplification of 16S rRNA genes was run on genomic DNA from 47 samples positive on real-time PCR. Primers used for amplification were the universal 16S rRNA forward (5-GTG-CCA-GCA-GCC-GCC-GTA-ATA-C-3) and reverse (5-TGG-TGT-GAC-GGG-CGG-TGT-GTA-CAA-G-3) primers according to Bricker, Ewalt, Olsen, & Jensen, (2003). The expected PCR product was 800 base pairs. DNA template (5 µl) was added to a final reaction volume of 25 µl consisting of 12.5 µl of one Taq2x master mix, 1 µl of each primer and 5.5 µl of PCR grade water. PCR reaction was run at an initial denaturation of 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s; annealing at 52 °C for 30 s; extension at 75 °C for 90 s; and a final extension at 75 °C for 5–60 min. The PCR products were visualized in a 2% agarose gel after electrophoresis at 110 volts for 60 min.

TABLE 1 Description of samples used in this study

Sample/type	Species				
	Humans	Bovine	Goat	Sheep	Total
Serum (reacted to Rapid slide test, RBT, c-Elisa and FPA tests)	28	35	12	2	77
Milk	–	23	12	–	35
Aborted materials	1	7	5	–	13
Total	29	65	29	2	125

2.5 | 16S rRNA gene sequencing

Samples with a PCR amplified 16S rRNA gene were subjected to Sanger sequencing (at Sokoine University of Agriculture-Tanzania). First PCR products were purified using GFX™ PCR DNA and Gel Band Purification Kit (UK Limited Little Chalfont, Lot 16,919,854) according to the manufacturer's instructions. Then sequencing was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies). The sequencing reaction had a final volume of 10 µl. This consisted of 2 µl of 5 × sequencing buffer (mixed with 0.5 µl BigDye® Terminator v3.1) and 3 µl of appropriate sequencing primer (1.6 pmol). Sequencing primers were the forward: 5-GTG-CCA-GCA-GCC-GCC-GTA-ATA-C-3 and reverse: 5-TGG-TGT-GAC-GGG-CGG-TGT-GTA-CAA-G-3 16S rRNA primers. Sequencing was done in duplicate for each primer. The sequencing conditions included incubation at 96°C for 1 min, followed by 25 cycles of denaturation at 96°C for 10 s, 50°C for 5 s and 60°C for 4 min (Bio-Rad) on a heated lid. Following the cycle-sequencing protocol, the reactions were cleaned up by ethanol precipitation. In this procedure, 5 µl of freshly prepared 125 mM EDTA and 60 µl of 100% ethanol were added to each reaction tube containing the sequencing products. After vortexing, the mixture was incubated in the dark for 15 min at room temperature to precipitate the extension products. As the BigDye® reagent is light-sensitive, the precipitation was carried out in the dark. Following precipitation, the tubes were centrifuged at 13,000 × g for 30 min and the supernatant was discarded without disturbing the pellet. Subsequently, the pellets were washed with 60 µl of 70% ethanol and centrifuged at 13,000 × g for 30 min. After the supernatant had been removed, the pellets were shaded from direct light and dried in a vacuum drier until no ethanol was present. Before loading onto the ABI 3,730 DNA Analyser the samples were re-suspended in 20 µl of HiDi Formamide (Life Technologies) and analysis was done according to the manufacturer's instructions.

2.6 | Data analysis

Similarity searches for the 16S rRNA gene sequences obtained using Applied Biosystems 3,500 genetic analyzer (Thermo Fisher Scientific) were done using the blastn (NCBI) in GenBank databases. *Brucella spp.* gene sequences from USA and New Zealand (Gee et al., 2004), Germany (Scholz et al., 2006), Iran (Kazemi et al., 2008), Sudan (personal communication) and Egypt (Bakhiet, Mohamed, Montasser, & Abdul-Raouf, 2013) were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/) and together with Tanzanian sequences, they were used for reconstruction of phylogenetic relationship. Multiple sequence alignment (MSA) for the sequences was done using the CLUSTAL W program and phylogenetic analysis was performed using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). All analyses were done in MEGA X (Kumar *et al.*, 2018).

3 | RESULTS

3.1 | Real-time PCR for *Brucella* spp

Forty-seven out of 125samples were *Brucella* positive in real-time PCR (Table 2). The SYBR green master mix was picking well the positive control (Figure 2) in contrast to the samples under study (Figure 3). Positive control and samples were picked by the probe and were considered to be positive when numbers of cycles were enough for the fluorescence to cross the thresholds (data were acquired only in show chanel1 with thresholds of 200 RFU). All aborted materials (13 samples) were negative to real-time PCR.

It shows a strong positive reaction indicating abundance of target nucleic acid in the positive control used (A vaccine *Brucella* strain S19).

It shows positive reaction indicating moderate amount of target nucleic acid in our samples.

3.2 | PCR amplification of 16S rRNA genes

The DNA fragment of *Brucella* species amplified from 16S rRNA gene is of different size according to the primers used during the amplification. In this study, we used primers (according to Bricker et al., 2003) targeting a band size of 800 base pairs. An expected 800 bp PCR product was amplified in 20 out of 47 (Table 2) real-time PCR positive samples. Of the positive samples, five were sera from cattle (Figure 4), three sera from human (Figure 5), two sera from goats, eight milk from cow and two were milk from goats.

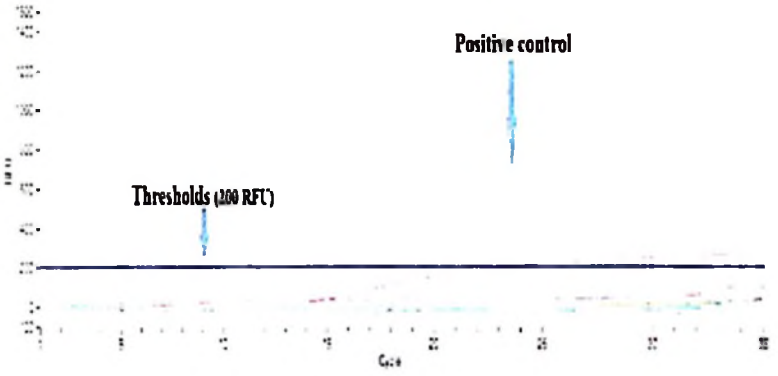
(L: A 100-bp DNA ladder, NC: Negative Control; PC: Positive Control. Numbers 3, 5, 8, 9 and 12: Samples with 800 bp amplification

TABLE 2 Distribution of samples for molecular analysis according species

Tests	Samples type	Results according species				
		Humans	Bovine	Goat	Sheep	Total
Real-time PCR	Serum	9 (28)	12(35)	7 (12)	0(2)	28 (77)
	Milk	–	11(23)	8(12)	–	19 (35)
	Aborted materials	0 (1)	0(7)	0(5)	–	0(13)
	Total Real time PCR	9 (29)	23(65)	15(29)	0(2)	47(125)
16S rRNA amplification	Serum	3	5	2	0	10
	Milk	–	8	2	–	10
	Aborted materials	0	0	0	–	0
	Total	3	13	4	0	20(45)
16S rRNA sequencing	Serum	2	4	1	0	7
	Milk	–	2	1	–	3
	Aborted materials	0	0	0	–	0
	Total	2	6	2	0	10
Sequences used for phylogenetic reconstruction	Serum	2	0	0		2
	Milk	0	2	1		3
	Total	2	2	1		5

Bold values are explaining the total number of samples used per each test and each specie (human, bovine, caprine ans ovine).

FIGURE 2 Positive control picked by the probe (with Ct value of 20.7)



of 16S rRNA gene. Numbers 1, 2, 4, 6, 7, 10, 11 and 13: Samples without amplification of 16S rRNA gene).

(L: A 100-bp DNA ladder; Numbers 8, 12 and 14: Samples with 800 bp amplification of 16S rRNA gene. Numbers 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13 and 15: Samples without amplification of 16S rRNA gene).

3.3 | 16S rRNA gene sequencing and phylogeny reconstruction

Sanger sequencing was successful for 10 out of the 20 samples (Table 2) with an expected PCR product size by 16S rRNA primers. The sequences were identified as belonging to *Brucella* spp. following similarity search by blastn (sequence identity of 85%). In addition, each sequence on blastn search showed same similarity with different *Brucella* spp. (*B. abortus, B. melitensis and B. suis*) deposited in Genbank considering the percent identity, the query cover and the E value. On cleaning, five sequences were used for multiple sequence alignment (MSA) and phylogeny reconstruction. The sequences were deposed in GenBank with accession numbers: MN396774, MN396775, MN396777, MN39679 and MN396782. Phylogenetic analysis of the 16S rRNA gene sequences (Figure 6) indicated that *Brucella* spp. in Kagera fall in clade closer from *B. melitensis, B. abortus* and B. suis reported in United States, Sudan and Iran. However, they fall also in clade away from *Brucella* spp. reported in USA, Germany, Iran and Egypt. The tree was well rooted by *Ochrobactrum*

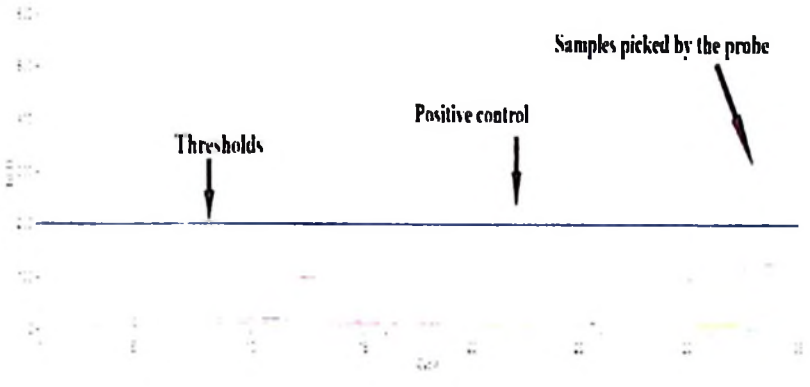


FIGURE 3 Samples picked by the probe (with Ct values ranging between 32.42 and 34.46)

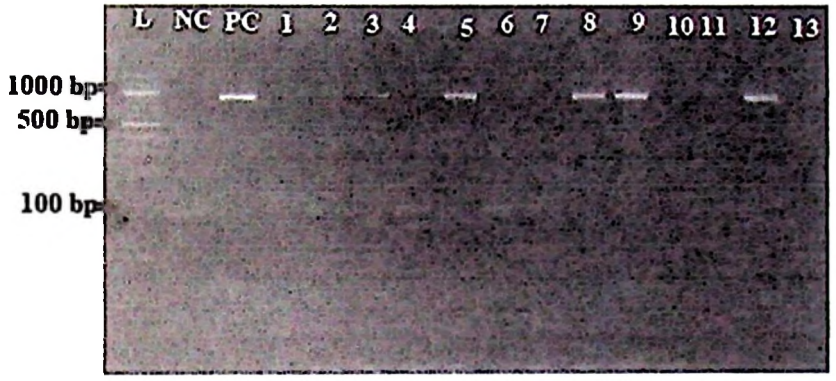


FIGURE 4 PCR amplification of 16S rRNA gene in sera from cattle

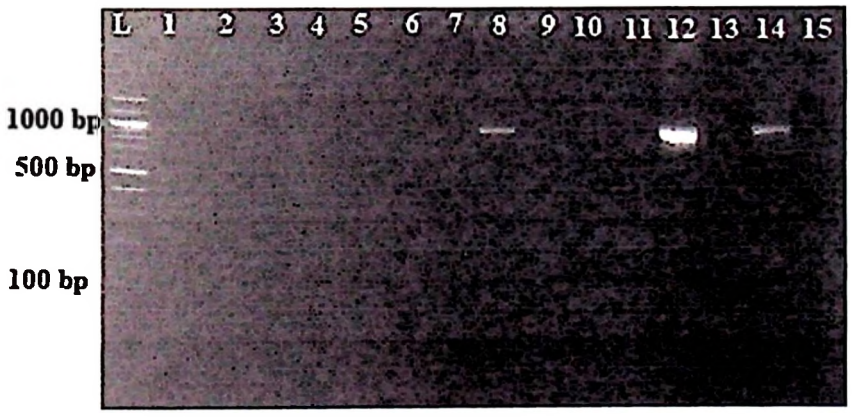


FIGURE 5 PCR amplification of 16S rRNA gene in sera from humans

intermedium (AJ867325.1) deposited in GenBank by Lebhun *et al.* (2000) from Germany.

The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura, 1980). Initial tree(s) for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 16 nucleotide sequences. All positions with less than 95% site coverage were eliminated and there were a total of 377 positions in the final dataset.

4 | DISCUSSION

For identifying and characterizing *Brucella* spp. in humans and domestic ruminants in Kagera region, we are reporting results of real-time PCR, PCR amplification of 16S rRNA genes and Sanger sequencing for phylogeny reconstruction. Previously to this study, sera were pre-screened using serological methods (Table 1), but culture of all samples (sera, milk and aborted materials) could have been used as a standard diagnostic method for isolation of *Brucella* species. However, this method is exigent, hazardous and time consuming (Bounaadja et al., 2009). Moreover the requirements in given medium for blood culture (level of CO_2, anticoagulants,

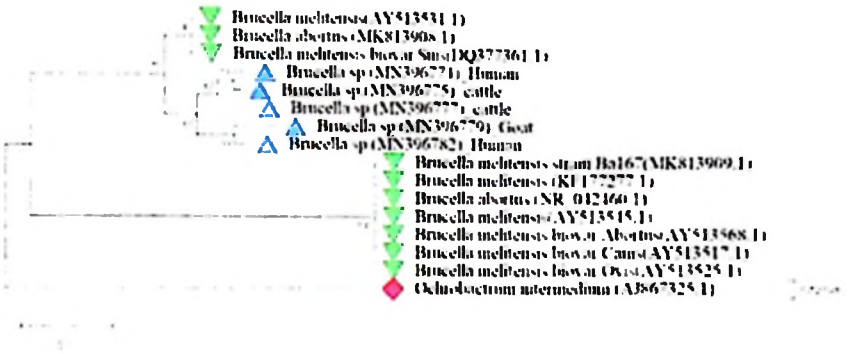


FIGURE 6 Evolutionary analysis of 16S rRNA gene sequences of samples from Kagera region in relation with other Brucella spp. by Maximum Likelihood method. ▽ *Brucella spp* downoaloded from GenBank. ▲ *Brucella spp* from Kagera Tanzania: MN396774: serum from aborted women. MN396782: serum from a human complaining with malaria-like symptoms. MN396775, MN396777: milk from cattle. MN39679: milk from aborted goat. ◊ *Ochrobactrum intermedium* used in this analysis as an outgroup organism.

inoculum) are determining factors which could make long and fastidious process for *Brucella* isolation (Ruiz, Lorente, Perez, Simarro, & Martinez-Campos, 1997).

This study used real-time PCR with specific primers for *Brucella* spp. as a screening tool for *Brucella* positive samples, before embarking on 16S rRNA gene sequencing. This is because, the target region for sequencing is highly conserved among bacteria and the primers used were universal bacterial primers available to us at the time of the study. Real time PCR narrowed down our samples to 47 and has indicated the presence of *Brucella spp.* in pastoral areas of Tanzania as reported previously (Assenga et al., 2015; Mathew et al., 2015; Kassuku, 2017).

We detected *Brucella* species in cow milk, which is similar to previous reports in Egypt (Wareth, Melzer, Elschner, Neubauer, & Roesler, 2014), in Uganda (Hoffman et al., 2016) and in Tanzania (Assenga et al., 2015; Mathew et al., 2015). This calls for public health attention since some residents in the study area drink unboiled milk as it was reported previously (Ntirandekura, Matemba, Ngowi, Kimera, & Karimuribo, 2018a). We are also reporting the presence of *Brucella* in goat milk. This is in contrast to a previous study in Katavi region which could not detect any *Brucella* spp. in milk from goat (Assenga et al., 2015). However, *Brucella abortus* was detected in serum from goats in Morogoro region (Kassuku, 2017). Even though people from the study area do not consume goat milk, brucellosis in goats still poses a risk of spill over to cattle and humans in a livestock pastoral setting.

Brucellosis is reported to be associated with abortions in humans and animals (Khan, Mah, & Memish, 2001; Kurdoglu, Cetin, Kurdoglu, & Akdeniz, 2015; Mathew, 2017; Megersa et al., 2011; Muma, Godfroid, Samui, & Skjerve, 2007; Nigro et al., 2011). This study detected *Brucella* in sera from abortive woman, cow and goat. Although there was a failure to detect *Brucella* from a limited sample size of aborted materials, the detection of *Brucella* from sera of aborted individuals could raise the same suspicions regarding the contribution of brucellosis to reproductive failure in this pastoral area as reported earlier (Ntirandekura et al., 2020). The failure to detect *Brucella* species in aborted materials could have been associated to the transport medium used (liquid nitrogen), long time for conservation of samples before analysis (9 months) and the low DNA concentration among other factors. However, other factors associated with reproductive failures need to be ruled out for a causal relationship between brucellosis and abortions to hold (Ntirandekura, Matemba, Ngowi, et al., 2018).

NtirandekurReal-time PCR positive samples were used for PCR amplification of 16S rRNA genes to get an 800 bp fragment as reported earlier (Gee et al., 2004; Khan et al., 2018). Nevertheless, a fraction of these samples showed an expected product by conventional PCR and this could be due to several optimization failures for conventional PCR assay (Martinez-Porchas et al., 2017). The same factors which could have affected the conventional PCR might have also affected the sequencing reaction, since a fraction of samples were successfully sequenced. Despite these limitations, we were able to get five sequences from 16S rRNA sequencing which were used in the identity search and phylogenetic reconstruction. On blastn search, each sequence was similar with different species of *Brucella* spp. deposited in Genbank, which indicated that they shared homology from a common ancestry and similar structure as stated by Pearson (2013). In addition, study sequences exhibited level of sequence similarity (with E values, percent identity and query cover) equally to *B. abortus, B. melitensis and B. suis*. It is known that the 12 recognized *Brucella* species have a genetic similarity although they differ according to their host predilection (Corbel, 2006). Hence, for phylogeny reconstruction we retrieved 16S rRNA gene sequences for *Brucella* species from GenBank. These sequences belonged to *Brucella* spp. reported from USA, Germany, Iran, Sudan and Egypt. We could not find 16S rRNA gene sequences for *Brucella* spp. isolated from East and central African regions in the DNA databases. Studies that identified *Brucella* in the region by sequencing targeted other genes (Hoffman et al., 2016; Mathew, 2017; Mugizi et al., 2015). To the best of our knowledge, this is the first study to report *Brucella* species identified by 16S rRNA gene sequencing in the region. *Brucella* spp. reported in Kagera were grouped in two clades and three branches, indicating genetic heterogeneity among

the species circulating in the same region. However, all clades with Tanzanian sequences connected from clade with sequences of *B. melitensis, B. abortus and B. suis* from USA, Sudan and Iran, although there is not likely an epidemiological linkage between them.

5 | CONCLUSION

This study showed that *Brucella* spp. are circulating in humans and their livestock in Kagera ecosystem. *Brucella* species were detected in raw milk of cow and goat, which could be a possible route of transmission to humans. In addition, the presence of *Brucella* in sera from abortive woman, cow and goat, raises the suspicion of the contribution of brucellosis to reproductive failures in the study area; despite the limited sample size. Real-time PCR narrows down the number of samples to be sequenced and hence save cost in a limited resource setting like ours. *Brucella* spp. detected in Kagera ecosystem are phylogenetically closer to *B. melitensis, B. abortus and B. suis* reported from USA, Sudan and Iran. However they were distant from other *Brucella* spp. reported from USA, Germany, New Zealand and Egypt. There is a need to conduct more epidemiologic studies using these advanced molecular tools and contribute to the body of knowledge on the genetic and phylogenetic characteristics of this *Brucella* spp. in Kagera.

6 | ETHICAL CONSIDERATION

This study was approved by institutional review boards of Sokoine University of Agriculture and the Medical Research Coordinating Committee of the National Institute for Medical Research (ref: NIMR/HQ/R.8a/Vol.IX/2456).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest for this study.

AUTHOR CONTRIBUTIONS

JBN and VAM conceived and designed the study. NJB analyzed the samples. JBN VAM and JBM analyzed the data. All authors (JBN, VAM, LEM, SIK, CJK, JBM and EDK) revised and approved the final version of this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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CHAPTER THREE

General Conclusions and Recommendations

6.1 Contribution of the Study to the Knowledge

This study was conducted to determine the magnitude of brucellosis and to assess the association of *Brucella* infections with abortions in humans and their domestic ruminants in Kagera ecosystem. The research questions were answered through the four objectives. The study has therefore contributed to existing scientific knowledge by describing the epidemiology and impact of brucellosis in the North- western part of Tanzania. From our research findings, it was evidenced that:

- There is low knowledge of brucellosis among pastoralists of Kagera region. Poor practices and behaviour such as drinking unboiled milk, assisting animals during parturition without wearing protective gears; together with cross-border interactions between humans and animals are some of the factors considered to increase the risk for brucellosis transmission in this ecosystem.
- Prevalence of brucellosis in humans (7.7%) and domestic ruminants (13.5%) in Kagera is associated with the assistance to parturitions without protective gears in humans and herd size in domestic animals. Cattle are at higher risk of getting *Brucella* infections compared to other livestock species in Kagera.
- The abortion rate in women (11.8%) and in ruminants (12.3%) was within the range of previous reports from Tanzania. Prevalence of brucellosis in pregnant women was associated with the risk of exposure of the disease. Pregnant cow were at risk to get brucellosis compared to other species. Despite the slight contribution of brucellosis to abortions in humans and domestic ruminants in Kagera, there was similarity in brucellosis positivity between aborted and non-aborted cases.

- *Brucella* species were confirmed in raw milk from cow and goat which could constitute a threat for *Brucella* infections especially in humans with a habit of drinking unboiled milk. Despite the small sample size, the detection of *Brucella* spp. in sera from aborted woman increased the suspicion of the contribution of the disease to the abortions in this pastoral area.
- The nucleotides sequences of *Brucella* spp. detected in Kagera were deposited in GenBank and will serve as a baseline of others *Brucella* spp. in Tanzania. The *Brucella* spp. from Kagera was phylogenetically grouped in clades closer to *B. melitensis*, *B. abortus* and *B. suis* from USA, Sudan and Iran, although there was no epidemiological relationship between them. It was also shown that *Brucella* spp. from Kagera are grouped in two different clades and three branches, suggesting a possibility of genetic variability among them.

6.2 Conclusions

These studies aimed to determine the epidemiological status of brucellosis and its contribution to abortions in humans and domestic ruminants in Kagera ecosystem Tanzania. Although, pastoralists from Kagera region possessed in general low knowledge on causes, symptoms and mode of transmission of brucellosis, they were aware of the zoonotic nature of this disease. The participants were also conscientious of strong interactions existing between humans, domestic animals and wildlife in the bordering ecosystem. However, their risk perception of brucellosis was poor and this was likely associated to their neglected and cultural practices including drinking unboiled milk and assisting animals in parturitions without protecting gears. The prevalence of brucellosis in Kagera region seemed to be distributed in species interacting in this ecosystem, but, cattle were at a higher risk of getting the disease, especially in herds with considerable number of animals (medium herds). *Brucella* infections were likely to be distributed equally in febrile and non-febrile patients

visiting the local health facilities regardless of their sex, or district of origin. Levels of exposure to the drivers of the disease were likely to be the same in humans in Kagera ecosystem. Poor practices such as drinking raw milk and assisting animals during parturition without protecting gears were identified as main risk factors for risk exposure to *Brucella* infections in humans (especially in pregnant women). Possessing basic knowledge of brucellosis among pastoralists seemed to be a protective factor for their domestic ruminants in Kagera region.

Infections to brucellosis were increased in pregnant women and domestic ruminants at risk of exposure to the disease, however, in these studies; the positivity to brucellosis was the same in aborted and non-aborted cases. Although the abortion rate in women was 11.8% and 12.3% in ruminants, these abortions were slightly associated with brucellosis infections in Kagera ecosystem. A proportion of 0.5% of the abortions was associated to *Brucella* infections in gravid ruminants while 3.5% of abortions were associated to positivity of the disease in pregnant women at population level. Molecular analysis confirmed the presence of *Brucella* in sera from abortive woman, supporting the suspected contribution of brucellosis to reproductive failures in the study area; despite the limited sample size. *Brucella* species detected in raw milk could constitute a privileged pathway for *Brucella* infections in humans. Real-time PCR narrowed down the number of samples to be sequenced and hence saved cost in a limited resource setting like ours and this is the first study to report *Brucella* species identified using 16S rRNA gene sequencing in the region. Phylogenetic analysis showed that *Brucella* species detected in Kagera region are distant from species detected elsewhere (USA, Germany, New Zealand and Egypt). However, the phylogenetic reconstruction grouped them in two clades and branches closer to *B. melitensis*, *B. abortus* and *B. suis* reported from USA, Sudan and Iran.

6.3 Recommendations

Based on the results obtained from these studies, recommendations can be addressed as follows:

a) Community health education programs are needed to enhance the knowledge, perception and practices regarding brucellosis in Kagera ecosystem and should involve cross border collaboration with stakeholders in neighbouring countries.

b) A coordinated One Health approach is recommended using a multidisciplinary initiative for prevention and control of brucellosis in humans and animals.

c) Public health programs should adopt a screening routine of brucellosis to all patients complaining of malaria like-symptoms, including pregnant women, in health centres and dispensaries; this could reinforce the control of the disease in humans especially in pastoral settings by Tanzania.

d) These results are proposed for use in the understanding of brucellosis in Tanzania and also for the design, implementation of control and surveillance of this disease in East Africa.

6.4 Areas for Further Researches

Due to limitations of time and finances, this study did not cover themes which could have brought a global vision for the magnitude of brucellosis and its impact on reproductive failure in humans and their livestock. For this reason:

a) Studies on economic impact evaluation of abortions due to brucellosis could justify the implementation of elimination programs of this disease in Tanzania, such as occupational and food hygiene in humans; with vaccination and culling in animals.

b) Differential diagnosis of brucellosis with other infectious diseases and febrile conditions should be investigated to establish the possible reasons for spontaneous abortions in humans and domestic ruminants in Tanzania.

c) Research on genomic characterization of *Brucella* spp. could contribute to know about their genetic variability and come up with a vaccine for the strains circulating in Tanzania.

APPENDICES

Appendix 1: Questionnaire for assessment of knowledge, perceptions and practices on brucellosis by stakeholders in Kagera region

Guide for Interviews

Themes for focus group discussion and key informant interviews

(i) The perception of brucellosis by the population in Kagera ecosystem (symptoms, mode of transmission, preventive approach, impact).

- Participants will talk about how they know brucellosis (it cause, if it zoonotic, if it exists in their locality, and how they call it in the local language).
- Different symptoms in animals and humans.
- Mode of transmission for domestic animals, for humans, for wild species (which species can infect others).
- A vaccination program, treatment protocols, in domestic animals, humans and wild species.
- Surveillance system for brucellosis in the study area.
- The social and economic impact of brucellosis in their community.

(ii) The assessment of risk factors for brucellosis by the population.

- What are the main risk factors in general (in domestic animals, in humans, wild species) for brucellosis in the area?
- What are the most important risk factors (in domestic animals and humans) for brucellosis in the area?

(iii) The risk for brucellosis transmission in Kagera ecosystem for bordering with other countries.

- Is to be on border constitute a risk for transmission for brucellosis?
- If yes, in which way?
- How can be the magnitude of transmission for brucellosis in domestic ruminants and humans in bordering area? (Importations, trade, exchanges, movement of people and livestock).

(iv) The role of different stakeholders in the ecosystem to control brucellosis.

- How to control brucellosis in domestic ruminants?
- How to control brucellosis in humans?
- How to control brucellosis on borders shared with Kagera region?
- What are the real activities done in herds, health facilities to control brucellosis in domestic animals and humans?
- What are the responsibilities of stakeholders in the region for controlling Brucellosis?

Thank you for your cooperation

Appendix 2: Questionnaire for a Cross-Sectional Study to Estimate the Prevalence of Brucellosis in Humans in Kagera Region

1. **Interviewee coordinates**

Date	
Code number (instead of name)	
age	
Sex	
Marital status	
Health facility	
Village	
District	

2. **Geographic coordinates**

3. **Data on symptoms**
- Fever Yes /No
- Fatigue Yes /No
- Joint pain Yes /No
- Back pain Yes /No
- Headache Yes /No
- Sleep disturbances Yes /No
- Others symptoms Yes /No

4. **Data on risk factors**
- Consumptions of the product of animal origin
  Do you drink unboiled milk? Yes /No
  Do you consume cheese? Yes /No
  Do you eat raw meat? Yes /No
  Do you consume fresh blood? Yes /No

- Animal contact
  Do you share your house with your domestic animals? Yes /No
  Do you assist animals during parturition without protective gears? Yes /No
  How do you dispose of the aborted materials?
  - Feeding dogs Yes /No
  - Burying in the pit Yes /No
  - Throwing anywhere Yes /No

- Occupational job
  Are you a livestock keeper? Yes /No
  Are you working in a slaughterhouse? Yes /No
  Are you a veterinarian? Yes /No
  Are you working in a laboratory? Yes /No

Thank you for your cooperation

Appendix 3: Questionnaire for a Cross-Sectional Study to Estimate the Prevalence of Brucellosis in Domestic Ruminants in Kagera Region

1. Interviewee coordinates (owners of animals)

Date	
Code number (instead of name)	
age	
Sex	
Marital status	
Village	
District	
The person responsible for the herd	

2. Geographical coordinates

3. Data on livestock system

a. The species in the herd: cattle (1) ____ small ruminants (2) ____ others (3)_____
b. Race in the herd:

Cattle: Ankole (1) _____ cross breed (2) _____ Fricsian (3) _____,

Sahiwal (4), _____ Jersey (5) _____

Goats: local (1) _____ boer (2) _____ mixed (3) _____

c. The age of animals (months):

Cattle: 0-12 (1) _____ 12-36 (2) _____ > 36 (3) _____,

Goats: 0-8 (1) _____ 8-24 (2) _____ > 24(3) _____

d. The animal management: Pastoralism (1) ___ Mixed(2)_____ Zero grazing (3) ____

e. The herd size: Small (1)_____, Medium (2)_____ Large (3)_____
f. The herd location: urban (1)_____, peri-urban (2)_____, and rural (3)_____

4. Animal movement and contact

Are you grazing your animals with other herds? Yes (1)_____ No (2) _____
Are your animals pasturing with wildlife? Yes (1)_____ No (2) _____
Are your animals sharing sources of water with other herds? Yes (1)_____ No (2) _____
Are your animals sharing bulls with other herds? Yes (1)_____ No (2) _____
Are you restocking your herd from importations? Yes (1)_____ No (2) _____
Are you restocking your herd from markets? Yes (1)_____ No (2) _____

5. Reproduction and production management

a. What is the reproduction method used in your herd?
Natural service _____ artificial insemination_____
b. Are you assisting your animals during parturitions? Yes (1)_____ No (2) _____
If yes, what are the protection measures? _____
c. Are abortions (expulsion of a dead fetus) occurring in your herd?
Yes (1) _____ No (2) _____ If yes,
- In which specie? Cattle (1) _____ goats (2)_____

- At what stage of pregnancy? Cattle (1) _____ goats (2)_____
- What was (were) the suspicion cause (s)? _____

d. Is placenta retention with abortion/stillbirth occurring in your herd?
Yes (1) _____ No (2) _____

e. Is placenta retention with normal calving occurring in your herd?
Yes (1) _____ No (2) _____

f. How are you disposing of the placenta, aborted fetus and dead calves in your herd?
- Feeding dogs Yes /No
- Burying the pit Yes /No
- Throwing anywhere Yes /No

g. Milk production per day:
Up to 10 liters (1) _____ >10-20lts (2) _____ >20lts (3) _____

6. Knowledge on brucellosis

a. What is the brucellosis local name? _____

b. Can you recognize brucellosis in animals?
Yes (1) _____ No (2) _____
If yes, what are the symptoms in females? _____
If yes, what are the symptoms in males? _____

c. Can brucellosis be transmitted to humans? Yes (1) _____ No (2) _____

7. Prevention and control measures

a. Are you reporting animal diseases to the veterinary services?
Yes (1) _____ No (2) _____

b. Are you reporting abortions in domestic animal to the veterinary services? Yes (1) _____ No (2) _____
If yes,
- Are sampling and laboratory analysis done by veterinarians?
  Yes (1) _____ No (2) _____
- Are feedback and epidemiological investigation done?
  Yes (1) _____ No (2) _____
- Nothing is done _____

c. Is a vaccination program done in herd? Yes (1)_____ No (2) _____
If yes, which disease? _____

d. Is a vaccination program for brucellosis done in the herd?
Yes (1) _____ No (2) _____
If yes,
Who is implementing the vaccination program? _____

e. Veterinary services
Public service (extension service) Yes (1) _____ No (2) _____

Private services Yes (1) _____ No (2) _____

Thank you for your cooperation

Appendix 4: Form for the follow up of pregnant women (Six Months)

Form for following abortions cases in Kagera		
Name of research assistant:		
Date		
Health centre		
Village		
District		
1. Code number		
2. Age		
3. Symptoms	Fever	
	Joint pain	
	Fatigue	
	Others	
4. Risk factors	a. Animal contact:	
	Living with domestic animals	
	Assistance to animal parturitions	
	Disposition of aborted materials	
	b. Consumption of products of animal origin:	
	Unboiled milk	
	Cheese	
	Blood	
	c. Occupational job:	
	Farmer	
	Slaughterhouse worker	
	Veterinarian	
	Laboratory staff	
5. Parturition	Normal	
	Placenta retention	
6. Abortion	Period	
	Placenta retention	
7. Sample taken	Blood	
	Aborted materials	

Signature of research assistant_____

Appendix 5: Form for the follow up of gravid ruminants (Six Months)

Form for following abortions cases in Kagera		
Name of research assistant:		
Date		
Herd (code number)		
Village		
District		
1. Age		
2. Symptoms	Fever	
	Joint pain	
	Fatigue	
	Others	
3. Risk factors	Herd size	
	Herd location	
	Living with domestic animals	
	Assistance to animal parturitions in the herd	
	Disposition of aborted materials in the herd	
	Sharing common pasture with other herds	
	sharing common pasture with wildlife	
	Sharing source of water	
	Sharing the same bull	
	Animal restocking from importations	
	Animal restocking from market	
4. Parturition	Normal	
	Placenta retention	
5. Abortion	Period	
	Placenta retention with stillbirth	
6. Sample taken	Blood	
	Aborted materials	

Signature of research assistant_____

Appendix 6: Characteristics of owners of animals and the herds sampled in Kagera region

District	Village	Herds	Age of owners (average)	Sex		Marital status		Number of animals sampled		
Karagwe				F	M	Single	Married	Cattle	Goat	Sheep
	Ruhita	9	37.44	0	9	4	5	23	0	0
	Kiruruma	11	43.91	2	9	8	3	27	6	1
	Nyakasimbi	14	41.79	2	12	2	12	36	13	6
	Rugera	13	48.83	0	13	5	8	24	25	11
	Bweranyange	10	47.00	1	9	2	8	27	13	4
	Nyaishozi	10	52.10	1	9	0	10	44	5	17
	Kihanga	11	48.14	0	11	1	10	44	18	. 8
	Chanika	8	54.88	0	8	2	6	22	21	11
	Nyakahanga	10	42.90	0	10	2	8	25	5	19
	Chonyonyo	8	43.67	2	6	1	7	11	12	27
	Kayanga	13	47.62	7	6	0	13	13	11	12
	Nyakabanga	10	44.10	1	9	1	9	19	7	12
Ngara	Goyagoya	11	50.08	0	11	1	10	24	10	10
	Kasange	13	43.92	6	7	0	13	21	10	10
	Rwakalemela	12	50.00	0	12	0	12	17	12	12
	Nyakaliba	13	50.30	0	13	0	13	20	12	13
	Rusumo	12	53.00	1	11	0	12	29	22	4
	Muruvyagila	4	51.50	0	4	0	4	0	4	20
Total		192	47.2	29	163	29	163	426	206	197

Appendix 7: Persons interviewed and sampled in health facilities in Kagera region

District	Village	Health facilities	Number of patients sampled	Age (mean)	Sex		Marital status	
Karagwe					F	M	Single	Married
	Ruhita	Ruhita	10	35.10	5	5	2	8
	Kiruruma	Kiruruma	9	36.00	6	3	4	5
	Nyakasimbi	Nyakasimbi	8	35.00	0	8	1	7
	Rugera	Buhamira	8	43.50	3	5	2	6
	Bweranyange	Kijumbura	8	37.13	6	2	1	7
	Nyaishozi	Nyaishozi hospital	8	47.71	6	2	1	7
	Kihanga	Kishojo	10	38.00	8	2	0	10
	Chanika	Chanika	7	48.57	5	2	4	3
	Bugene	Nyakahanga Hospital	10	26.30	5	5	3	7
	Chonyonyo	Chonyonyo	8	25.13	5	3	0	8
	Kayanga	Kayanga	8	37.88	8	0	2	6
	Nyakabanga	Nyakabanga	6	45.83	4	2	0	6
Ngara	Goyagoya	Rulenge Hospital	8	27.00	4	4	0	8
	Mshikamano	Mshikamano	7	31.57	4	3	2	5
	Kasulo	Kasulo	9	19.67	8	1	5	4
	Nyakaliba	Lukole Hospital	8	47.25	6	2	0	8
	Muruvyagira	Muruvyagira	7	32.43	6	1	1	6
	Kasange	Kasange	7	31.00	6	1	2	5
	Mubinyange	Nyamiaga Hospital	10	31.30	4	6	2	8
Total			156	34	99	57	32	124