Exploration of sustainable and affordable control options of bovine brucellosis in the endemic zone in Tanzania

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Thesis submitted for the degree of Doctor of Philosophy

Graduate School of Veterinary Medicine

Rakuno Gakuen University

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（タンザニア国牛ブルセラ病常在地域における持続的かつ支払可能な疾病制御法の検討）

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ABBREVIATIONS

C-ELISA	Competitive Enzyme Linked Immunosorbent Assay
CFT	Compliment Fixation Test
CI	Confidence Interval
DNA	Deoxyribose Nucleic Acid
FAO	Food and Agriculture Organization
GPS	Global Positioning System
I-ELISA	Indirect Enzyme Linked Immunosorbent Assay
MRT	Milk Ring Test
OIE	World Organisation for Animal Health (Office International des Epizooties)
OD	Optical Density
OR	Odds Ratio
PCR	Polymerase Chain Reaction
R-LPS	Rough Lipopolysaccharide
RBPT	Rose Bengal Plate Test
RB51	*Brucella abortus* rough strain 51
S-LPS	Smooth Lipopolysaccharide
S19	*Brucella abortus* strain 19
SE	Standard Error
SUA	Sokoine University of Agriculture
Tsh	Tanzania shilling
WHO	World Health Organization
χ^2	Chi square

Chapter 1 General introduction

1.1. Background information

Brucellosis is one of the most-widespread bacterial zoonotic diseases in the world [127]. The genus *Brucella* consists of ten distinct species, *Brucella abortus*, *B. melitensis, B. suis, B. canis, B. neotomae, B. ovis, B. pinnipedialis, B. ceti, B. microti* and *B. inopinata* [15]. Among them, *B. abortus*, *B. melitensis* and *B. suis*, which mainly affect cattle, goats and swine, respectively, are considered to be serious pathogens of human brucellosis [46]. The disease causes reproductive losses in animals such as abortion, weak offspring, weight loss and reduced milk production, which inflict economical damage on farmers [86]. The major routes of human brucellosis infection are the consumption of contaminated raw dairy products, contact with infected animals, uterine secretions or aborted foetuses [27]. Brucellosis in humans induces an acute febrile disease with undulant fever, joint and muscular pain and general malaise. These symptoms are not specific, therefore, many cases remain unrecognized and/or are misdiagnosed as other diseases such as malaria and typhoid fever [59].

Although many developed countries in Europe and Australia, Canada, Israel, Japan and New Zealand have eradicated brucellosis, it remains endemic in Africa, the Mediterranean, the Middle East, parts of Asia and Latin America [108]. In Tanzania, brucellosis has been documented since 1927 when an outbreak of abortion was reported in the Arusha region [113]. Since then, many livestock brucellosis studies have been conducted in various areas in the country. Relatively recent studies reported that prevalences of bovine brucellosis at the animal level were 14.3% in the Mikumi-Selous ecosystem [118], 6.8% in the Katavi-Rukwa ecosystem [11], 6.2% in Arusha and Manyara regions [114] and 12.0% in Tanga city [117].

In sub-Saharan Africa, 37.4% of the population lives in urban environments [42]. Control of zoonoses of livestock origin should take such rapid urbanization into account. A previous study in Kampala, the capital city of Uganda, found that brucellosis was one of the important zoonotic infections in humans [79], and brucellosis control in humans in a city should consider

risks carried through dairy value chain, as large proportion of risks comes from livestock production areas far outside the city [77]. Pasteurization of milk for all the dairy value chains should reduce overall risk for brucellosis to humans greatly, but generally zoonoses can be controlled most surely by controlling at animal reservoirs.

1.2. Historical perspective

Brucellosis was first suspected to occur in humans with flu-like symptoms like malaise, anorexia, fever and profound muscular weakness. Marston reported it in 1861 calling the condition "gastric remittent fever" [61]. Brucellosis was first diagnosed in human by British scientist Sir David Bruce in 1887 when he isolated a causative organism from the spleen of the patients and named it *Micrococcus melitensis* [113]. The disease is also named as “Mediterranean fever,” “Malta fever,” and “Undulant fever” in case of humans [61].

Brucella abortus was isolated from aborted cows in Denmark in 1897 by Bang, who linked the organism to infectious abortion in animals, calling the agent *Abortus Bacillus* of Bang. Alice Evans changed the genus and named it *Brucella* in honour of Sir David Bruce, owing to the close bacteriological and serological relationship between *M melitensis* and *Abortus Bacillus* of Bang [113]. The third member of the genus was isolated by Traum from swine abortions in 1914 and it was called *B. suis*. The fourth member of the genus was isolated from sheep by Buddle and Simmons in Australia and New Zealand in 1953 and was named *B. ovis* [120]. Stoenner and Lackman isolated another *Brucella* organism from desert wood rats in USA in 1957 and called it *B. neotomae*. *B. canis* was reported in the USA by Carmichael and Brunner in 1968 following isolation from dogs. In addition to these six species, currently other 4 species, *B. pinnipedialis* (had been isolated from pinned marine mammals), *B. ceti* (cetaceans), *B. microti* (vole) and *B. inopinata* were found [15].

1.3. Aetiology

Brucella organisms are small, non-motile, non-sporulating, non-toxigenic, non-fermenting facultative intracellular gram-negative bacteria. They are either coccobacilli or short bacilli with a size range of 0.5–0.7/μm wide by 0.6–1.5/μm long [99]. They can occur singly, in groups, or in chains, and grow well on media containing blood or serum [121]. Biochemically, *Brucella* organisms oxidise certain amino acids such as L-glutamic acid and L-asparagine and certain carbohydrates such as D-glucose and I-erythritol [121]. *B. abortus*, *B. melitensis*, *B. suis* and *B. neotomae* generally express smooth lipopolysaccharide (S-LPS), while *B. ovis* and *B.canis* do rough-lipopolysaccharide (R-LPS) [95].

The disease in cattle is usually caused by *Brucella abortus* and less frequently by *Brucella melitensis* where cattle are kept together with infected sheep or goat [99]. Occasionally, *B. suis* may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion [66]. According to Matope (2009), majority of cases of brucellosis in cattle worldwide are attributed to *B. abortus* biotype 1, while *B. abortus* biotype 2 has a worldwide distribution but considered less frequent than biotype 1 [82].

In humans, the disease is mainly caused by *B. melitensis* as the most pathogenic species, followed by *B. suis*, whereas *B*. *abortus* is considered as the mildest type of brucellosis [46].

1.4. Epidemiology of brucellosis in animals and humans

1.4.1 Distribution and prevalence

1.4.1.1 Livestock

Brucellosis is a widespread disease particularly among cattle. In small ruminants, the disease is more restricted to the Mediterranean region including southern Europe, West and Central Asia, South America and Africa [50, 97], with considerable variation between herds and between areas and countries. Although many developed countries in Europe and Australia,

Canada, Israel, Japan and New Zealand have eradicated brucellosis, it remains endemic in Africa, the Mediterranean, the Middle East, parts of Asia and Latin America [108].

In African countries, studies in cattle have reported the prevalence to be 4.2% in Ethiopia [17], 5.5–17.5% in Kenya [33], 15.8–18.1% in Uganda [19, 76], 9–61.8% in Egypt [23], 22% in Mali [84] and 6.6% in Ghana [67]. These data are difficult to compare due to variation in sampling techniques and serological tests used.

In Tanzania, brucellosis has been documented since 1927 when an outbreak of abortion was reported in the Arusha region [113]. Relatively recent studies reported that prevalences of bovine brucellosis at the animal level were 14.3% in the Mikumi-Selous ecosystem [118], 6.8% in the Katavi-Rukwa ecosystem [11], 6.2% in Arusha and Manyara regions [114] and 12.0% in Tanga city [117].

1.4.1.2 Humans

Despite a notifiable disease human brucellosis is in many countries, official figures do not reflect the actual number of human brucellosis cases each year. Thus, the true incidence has been estimated to be 10–25 times higher than that reflected in reports [125]. This discrepancy could be attributed to infections remaining unrecognized because of incorrect diagnosis or diagnoses of "pyrexia of unknown origin". Therefore, human brucellosis remains a public health burden in many developing countries, and its incidence in endemic areas varies from 1 to 200 per 100,000 people [73]. In Africa, Cameroon, Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Burkina Faso, Republic of Congo, Eritrea, Mali, Namibia, and Swaziland reported human cases of brucellosis [104]. In addition, Ghana, Togo and Chad are probably endemic according to sero survey [104]. The prevalence reported in some African countries ranges from 5 to 55% in countries such as Nigeria [2, 5, 14], Benin [44], Burundi [70] and Uganda [93]. In Tanzania, first report of the disease was in 1935 [7]. Further reports of human brucellosis in the

country were from the Medical Department of the Lake and Western Regions in 1959, 1960 and 1961 where three cases were confirmed [8]. The prevalences have been reported 0.6% in the Katavi-Rukwa ecosystem and 7.7% in northern part of Tanzania [11, 68]. However, isolation of the bacteria was not performed in the majority of these studies.

1.4.2 Source of infection and transmission

1.4.2.1 Livestock

Animals of all age groups are susceptible to brucellosis but persists more in sexually mature animals [113]. Transmission among animals is mainly through ingestion of contaminated pasture, water and feeds, licking infected placenta, aborted fetus or uterine discharges from infected animals [82]. Milk from infected animals is an important source of infection for calves. Transmission of *Brucella* organisms through inhalation, and via the conjunctiva, urogenital tract and teat canal has also been reported [20, 113]. Although males can be infected in early life and retain infection for life, they rarely contribute to the introduction or spread of infection to females by natural service, and transmission occurs when semen of infected bulls is used in artificial insemination [26, 82]. Therefore, in the pastoral and agro-pastoral areas where artificial insemination is not common in Tanzania, males are rarely responsible for the disease transmission. However, semen used for artificial insemination is usually collected from brucellosis free bulls. It is suggested that such bulls should be serologically and bacteriologically negative [106]. Vertical transmission was proved by Plommet, who states that between 60 and 70% of the fetuses born to infected mothers carry the infection in pregnancy [35].

1.4.2.2 Humans

Brucellosis in human is strongly related to that in animals and practices that expose humans

to infected animals or their products [55]. Major sources of human infection are consumption of unpasteurized milk, undercooked or fresh meat and blood from infected animals and handling of aborted materials without using protective items such as gloves [66, 125]. Transmission through direct contact of the bacteria is more likely to affect occupational group such as farmers, veterinarians, laboratory workers, butchers, milkers and inseminators [82, 113]. Furthermore, inhalation of the pathogens from dust or accidental self injection of S19 vaccine resulted in human infections [20, 101].

1.5. Pathogenesis

Brucella organisms enter to the body through ingestion, inhalation and invasion through skin, mucosa, conjunctiva and urogenital tract [31]. After the entry, the organisms are carried by neutrophils and macrophages and localize in the regional lymph nodes [31, 113]. In addition, the organisms invade and survive in both phagocytic and non-phagocytic cells, and localize in the rough endoplasmic reticulum. Although the bacteria are ingested by various local phagocytic cells, they can survive and multiply in the cells [27]. *Brucella* organisms are carried in the plasma and localize in various organs such as the pregnant uterus, udder, lymph nodes, spleen, testes, testicle and accessory male sex glands [82]. Erythritol in the placenta is considered as a strong growth stimulant of *B. abortus*, thereby accounting for its localization in the pregnant uterus [20]. Generally bacteremia is intermittent and often occur around parturition [113]. Although the reason of abortion is not understood well, it is considered to be due to the inflammation of placenta, or the direct effect of endotoxins, or the stress caused by the inflammation of fetal tissues [82].

1.6. Clinical manifestations

1.6.1 Livestock

The incubation period of brucellosis is variable and is defined as (i) period between exposure and abortion or (ii) the period between exposure and the first appearance of clinical disease or (□) the period between exposure and before the first serological evidence of infection [20, 113]. The length of incubation period in cows varies according to the time at which infection occurred, and it is also influenced by size of the exposure dose, age, sex, stage of gestation and immunity of the animal [20, 113]. Clinical findings depend on the immune status of the herd or flock. The major clinical signs are late term abortion, retained placenta, metritis and reduced milk production [66, 88]. Infected dams usually abort only once, and subsequent pregnancies are generally normal [74]. However, cows may shed the organism in milk and uterine discharges [74]. In males it causes orchitis and epididymitis with frequent sterility [57, 66].

1.6.2 Humans

Brucellosis in human has an acute, sub-acute or chronic forms of illness with clinical features including an intermittent or remittent fever, backache, headache, malaise, loss of appetite, muscular pain, joint pain and loss of weight [56, 89]. *Brucella* infection causes focal lesions in bones, urogenital tract and other organs. Complications such as arthritis, sacroiliitis, spondylitis and central nervous system disorders may occur [16, 126]. Diseases with similar clinical symptoms such as malaria, typhoid and joint diseases contribute to misdiagnosis of brucellosis, thereby resulting in mistreatments and underreporting [3].

1.7. Diagnosis

1.7.1 Isolation and characterization of disease causing organisms

1.7.1.1 Culture

Suitable specimens for culture in animals are fetal membranes, uterine discharges, milk, colostrum or blood from infected animals, liver and spleen from the aborted fetus. The most suitable specimen for isolation of *Brucella* are the supramammary lymph nodes, and retropharyngeal or prescapular lymph nodes are also used [20, 113]. Considering the risk of infection of laboratory worker, culturing procedure should be carried out in biohazard hood [30].

1.7.1.2 Microscopy

Smears of placental cotyledon, uterine discharges or fetal stomach contents are stained using Ziehl-Neelsen (stamp's staining) or Kosters' methods. The large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is regarded as an evidence of brucellosis. However, *Coxiella brunetii* and *Chlamidia* also express similar reaction to the methods [20, 127].

1.7.1.3 Molecular techniques

Brucella organism can be detected directly from specimen by molecular techniques hence shortening time required to identify the pathogen. The techniques are Polymerase Chain Reaction (PCR), Restriction Endonuclease Analysis (REA) and Restriction Endonuclease and Hybridization analysis [113]. These have high sensitivity and specificity. However, these techniques are too expensive for routine use [98]. They are more appropriate for differential diagnosis rather than for establishing prevalence.

1.7.2 Detection of specific antibody

In general, brucellosis in both animals and humans is diagnosed by serological methods [127]. Serological tests detect antibodies produced against lipopolysaccharides (LPS) of both smooth and rough *Brucella* spp [94]. The smooth species, *B. abortus, B. melitensis* and *B. suis* which contain the O-polysaccharide (OPS) as part of the lipopolysaccharides (LPS), are diagnosed serologically using either a whole cell antigen or smooth-lipopolysaccharide (S-LPS) prepared by chemical extraction, while the rough species, *B. canis* and *B. ovis* which contain no detectable OPS, are diagnosed using rough-lipopolysaccharides (R-LPS) or protein antigens.

According to Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009, following diagnosis tests are described for *Brucella abortus*: 1) identification of the agent by modified acid-fast staining of organisms, 2) PCR, 3) serological tests: Rose Bengal test (RBT), buffered plate agglutination test (BPAT), the complement fixation test (CFT), the enzyme-linked immunosorbent assay (ELISA), the fluorescence polarization assay (FPA), 4) milk ring test, 5) brucellin skin test [100].

Currently, the diagnosis of brucellosis is based on microbiological and serological laboratory tests [13, 109]. The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. All infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing.

PCR can provide both a complementary and biotyping method based on specific genomic sequences [127]. By PCR, *Brucella* biotyping and distinguishing vaccine strains can be accomplished satisfactorily but there has been limited validation for primary diagnosis. The first species-specific multiplex PCR for the differentiation of *Brcuella* was described by Bricker & Halling [21]. After that this methods has been improved, and new multiplex PCR (bruce-ladder) has been proposed for rapid and simple one-step identification of *Brucellra* species. The

detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. RBT is very sensitive and appears to be adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds. But like all other serological tests, it could sometimes give a positive result because of S19 vaccination or of false-positive serological reactions (FPSR), mostly due to prozoning and can sometimes be detected by diluting the serum sample or retesting after 4–6 weeks. Like the RBT, BPAT is also very sensitive, and positive samples should be retested using a confirmatory and/or complementary test(s). CFT is widely used and very specific, and accepted as a confirmatory test. But the procedure is complex and it needs good laboratory facilities and trained staff. Females that have been vaccinated with S19 between 3 and 6 months are usually considered to be positive if the sera give positive fixation at a titre of 30 or greater ICFTU (international complement fixation test units) /ml when the animals are tested at an age of 18 months or older. The indirect ELISA (I-ELISA) that uses smooth lipopolysaccharide (S-LPS) or the O-polysaccharide (OPS) are highly sensitive, but are not capable of fully resolving the problem of differentiating between antibodies resulting from S19 vaccination. The competitive ELISA (C-ELISA) eliminates some but not all reactions (FPSR) due to cross-reacting bacteria. The C-ELISA is capable of eliminating most reactions due to residual antibody produced in response to vaccination with S19, but it's not all. The diagnostic sensitivity could be equivalent to the BBAT and the I-ELISA in the testing of infected cattle. Like other serological tests, positive reactions should be investigated using suitable confirmatory and/or complementary tests because of positive results due to S19 or FPSR. The diagnostic sensitivity and specificity of the Fluorescence polarization assay (FPA) for bovine brucellosis are almost identical to those of the C-ELISA. The diagnostic specificity for cattle recently vaccinated with S19 is over 99% [96]. However the specificity of FPA in FPSR conditions is currently unknown. Like all other serological tests, positive reactions should be investigated using suitable confirmatory and/or

complementary strategies. Milk Ring Test is simple and effective agglutination test carried out in fresh cow's milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd [127]. Non-specific reactions are common with this test, especially in brucellosis free areas. The milk ELISA is far more specific than the MRT.

1.8. Treatment

1.8.1 Livestock

Treatment of brucellosis in animals is normally not undertaken and treatment trials have shown only partial success in eliminating the infection [83, 106]. In vitro treatment of *B. abortus* have been found to be sensitive to gentamycin, kanamycin, tetracyclines and rifampin. However, the effectiveness of these antimicrobials in vivo have not been comprehensively evaluated [82]. Some problems have been reported to be associated with the treatment of brucellosis. For example, the use of antibiotics such as penicillin and oxytetracycline causes L-transformation on the cell wall thereby possibly creating carrier animals and affecting future serological detection [20, 113]. Due to the fact that treatment has shown partial success, efforts are directed at control and prevention [12, 74].

1.8.2 Humans

The essentials in the treatment of human brucellosis is the administration of effective antibiotics for an adequate length of time. The goal of medical therapy in brucellosis is to control symptoms as quickly as possible, to prevent complications and relapses. Multidrug antimicrobial prescription is main therapy due to high relapse rates reported with mono therapeutic approaches [127]. The risk of relapse is not well understood, as resistance is not a significant issue in treating brucellosis. The World Health Organization recommends the following for adults and children older than 8 years:

(i) Doxycycline 100 mg PO bid and rifampin 600–900 mg/d PO: Both drugs are to be given for 6 weeks (more convenient but probably increases the risk of relapse).

(ii) Doxycycline 100 mg PO bid for 6 weeks and streptomycin 1 g/d IM daily for 2–3 weeks: This regimen is believed to be more effective, mainly in preventing relapse. Gentamicin can be used as a substitute for streptomycin and has shown equal efficacy [54].

(iii) Ciprofloxacin-based regimens have shown equal efficacy to doxycycline base regimens. For Children younger than 8 years: The use of rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) for 6 weeks is the therapy of choice. Relapse rate appears to be approximately 5% or less.

(iv) Pregnant women: Brucellosis treatment is a challenging problem with limited studies. The recommendation is a regimen of rifampin alone or in combination with TMP-SMX. However, TMP-SMX use by the end of pregnancy is associated with kernicterus. In patients with spondylitis, doxycycline and rifampin combined with an aminoglycoside (gentamicin) for the initial 2–3 weeks followed by 6 weeks of rifampin and doxycycline is usually recommended.

(v) Patients with meningoencephalitis may require doxycycline in combination with rifampin, TMP-SMX, or both. A brief course of adjunctive corticosteroid therapy has been used to control the inflammatory process, but studies are limited. Patients with endocarditis require aggressive therapy.

(vi) Aminoglycoside therapy in conjunction with doxycycline, rifampin, and TMP-SMX for at least 4 weeks followed by at least 2–3 active agents (without aminoglycosides) for another 8–12 weeks is preferred. Many other drugs have good in vitro activity against *Brucella*, including, but not limited to, chloramphenicol, imipenem-cilastatin, and tigecycline+Gentamicin-loaded microparticles and immune-response stimulates may hold future promise.

1.9. Control and eradication

1.9.1 Livestock

Several countries have been successful in eliminating brucellosis through control and eradication programmes, which were highly dependent on national strategies, priorities and policies [20, 125]. In sub Saharan Africa, due to various factors such as decreasing government budget for the disease control, brucellosis control programmes that require coordinated surveillance information exchange and application of control measures are not implemented in many sub Saharan countries [86].

Strategies based on the prevention of the spread of the disease between animals, monitoring of uninfected and suspected herds and zones, elimination of infected animals by test and slaughter, strict control of movement of infected and suspected animals, mass vaccination to reduce infection rate, and supporting specific education and training programmes have regarded effective in many countries [1, 20, 126]. The most effective control method for brucellosis in cattle is vaccination at early age [74, 122], between 3 to 10 months of age using S19 [82].
In Tanzania, vaccination for cattle using S19 was adopted in early 1980's. However, it was confined to government and parastatal dairy farms and no vaccination has been carried out in agro-pastoral and pastoral animals [113].

1.9.2 Humans

Control and prevention of brucellosis in human depends on its control in livestock. In addition, since main sources of human brucellosis are contaminated food and contact with infected animals, hygiene management and heating dairy products before consumption are crucial to avoid human infection where brucellosis in animals is endemic. Education of the population and especially those directly involved in the animal and food industries is important [127]. However, local customs, habits and beliefs may impede the wide application of potential

preventive measures of brucellosis in rural areas in many developing countries [27].

However, the vaccine strategy is currently applicable only in control of livestock disease [127]. In terms of *Brucella* vaccine for humans, various preparations have been used, including the live attenuated *B. abortus* strains 19-BA and 104M used in the USSR and China. In the cases of live vaccines, potential serious reactogenic were recognized [27, 34]. Therefore, since vaccination is a potential method of controlling brucellosis in humans, further research is necessary to develop vaccine that will be safe for human.

Chapter 2 Brucellosis risk in urban and agro-pastoral areas in Tanzania

2.1. Introduction

Brucellosis is one of the most-widespread bacterial zoonotic diseases in the world [127]. The genus *Brucella* consists of ten distinct species, *Brucella abortus*, *B. melitensis, B. suis, B. canis, B. neotomae, B. ovis, B. pinnipedialis, B. ceti, B. microti* and *B. inopinata* [15]. Among them, *B. abortus*, *B. melitensis* and *B. suis*, which mainly affect cattle, goats and swine, respectively, are considered to be serious pathogens of human brucellosis [45]. The disease causes reproductive losses in animals such as abortion, weak offspring, weight loss and reduced milk production, which inflict economical damage on farmers [86]. The major routes of human brucellosis infection are the consumption of contaminated raw dairy products, contact with infected animals, uterine secretions or aborted foetuses [27]. Brucellosis in humans induces an acute febrile disease with undulant fever, joint and muscular pain and general malaise. These symptoms are not specific, therefore, many cases remain unrecognized and/or are misdiagnosed as other diseases such as malaria and typhoid fever [59].

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In sub-Saharan Africa, 37.4% of the population lives in urban environments [42]. Control of zoonoses of livestock origin should take such rapid urbanization into account. A previous study in Kampala, the capital city of Uganda, found that brucellosis was one of the important zoonotic infections in humans [79], and brucellosis control in humans in a city should consider risks

carried through dairy value chain, as large proportion of risks comes from livestock production areas far outside the city [77]. Pasteurization of milk for all the dairy value chains should reduce overall risk for brucellosis to humans greatly, but generally zoonoses can be controlled most surely by controlling at animal reservoirs. Therefore, it is important to understand epidemiology and risk factors in different ecological settings, so that ecology specific disease control programmes can be planned.

The objectives of this study are to compare brucellosis prevalence and risk factors and behaviours associated with human brucellosis between urban and agro-pastoral areas in a brucellosis endemic region.

2.2. Materials and Methods

2.2.1 Study site

This study was carried out in Morogoro municipality and Mvomero district as representatives of urban and agro-pastoral areas, respectively, in Morogoro region, Tanzania. The region lies between latitude 5°58' and 10°S and longitude 35°25' and 38°30' E at an average altitude of 526 meters above sea level. Morogoro municipality is located around 200 km west of Dar es Salaam where the country's largest city and commercial centre. The distance between Morogoro municipality office and Mvomero district office is 37 km. The area has a sub-humid tropical climate with a mean annual rainfall of 870 mm (range, 610–1,180 mm). The area has a long rainy season that extends from March to June and a short rainy season from October to December [90]. The livestock types found in the region were comprised of cattle, goats, sheep, pigs, donkeys, birds and fish. The predominant livestock production system in Morogoro municipality is small holder dairy farms with zero-grazing, keeping cattle inside of the barn all the time with feeding cut grass, and pastoral and agro-pastoralism are also observed. In Mvomero district, crop production is the dominant farming system, and mixed crop-livestock

system with grazing, here defined as going outside for grass and water, is also common.

2.2.2 Study design and sample size estimation

A cross-sectional study involving blood sampling and a structured interview using a questionnaire was designed.

Individual animal sample size was calculated by the following formula (Equation 1) for a comparison of two proportions, to detect differences between the prevalences of urban and agro-pastoral areas [119]:

$$n = \frac{\left\{M_{\alpha/2}\sqrt{2p(1-p)} + M_{\beta}\sqrt{p_1(1-p_1)+p_2(1-p_2)}\right\}^2}{(p_2-p_1)^2} \quad \text{Equation 1}$$

where n is sample size for each population, p_1 and p_2 are true individual cattle prevalences in agro-pastoral areas and urban areas, respectively, p is $(p_1 + p_2)/2$, $M_{\alpha/2}$ is the multiplier associated with α, the required significance level, and M_{β} is the multiplier associated with β, the probability of a Type II error. We set p_1 as 14.9% based on the results from a previous study [118], p_2 as 9.9% to detect a 5% difference from p_1, $M_{\alpha/2}$ as 1.96 and M_{β} as 0.84. Thus, the calculated individual cattle sample size was 680 from each area.

In agro-pastoral areas, Mvomero and Dakawa wards in Mvomero district were selected as study areas considering the reachable distance from Morogoro municipality, where Sokoine University of Agriculture is located. Mvomero ward has five villages with cattle farmers. However, one village in the ward is located too far from Morogoro municipality compared to the other villages, and list of farms of another village was not available. Therefore, only the other three villages in Mvomero ward and all four villages in Dakawa ward were included. Two-stage cluster sampling was designed for these areas, selecting farms as primary sampling

units, and cattle as secondary. The number of farms to be sampled in agro-pastoral areas, *g*, was calculated by the following formula described by Thrusfield [119]:

$$g = \frac{1.96^2 \times Ts \times Vc}{d^2 \times Ts - 1.96^2 \times P_{\exp} \times \left(1 - P_{\exp}\right)} \quad \text{Equation 2}$$

where *Vc* is between cluster variance, *Ts* is total number of animals to be sampled calculated from Equation 1, $P_{\exp}$ is expected prevalence, and *d* is desired absolute precision, 0.05. *Vc* was calculated from the associated data of a published study conducted in and around Kampala, Uganda in 2007 [78]. $P_{\exp}$ was set as 14.9% based on the previous report in Mvomero district [118]. The number of farms to be sampled from the agro-pastoral area was calculated as 18.2, thus 19 farms. However, due to time constraints, only 17 farms were sampled.

In this study, cattle older than 3 months of age were targeted to avoid the effect of maternal antibody to new-born calves. All eligible cattle were sampled at farms with less than or equal to 50 cattle. For larger farms, maximum 50 cattle were purposely selected to reflect the age structure of the herd. This was because cattle blood sampling was conducted in the early morning before cattle go grazing in agro-pastoral areas, and it was difficult to sample more than 50 cattle from a herd in a day. Farms to be sampled were proportionally allocated to the seven villages according to the numbers of farms within them, and study farms were randomly selected from the lists of farms.

For urban areas, all 17 cattle-raising wards where cattle farmer lists were available in Morogoro municipality were selected. Unlike agro-pastoral areas, the common raising style was small-scale zero-grazing. Therefore, instead of fixing the number of farms to be sampled, the number of cattle to be sampled was proportionally allocated to these wards, and farms were randomly selected. As a result, 667 and 673 cattle were selected from 106 and 17 farms in

Morogoro municipality and Mvomero district, respectively.

2.2.3 Field survey

A field survey was conducted between August and December 2015. Blood was collected from either jugular or sacral medial vein using vacutainer tubes. Blood samples were brought to the laboratory in Sokoine University of Agriculture every day and were left for 24 hours at ambient temperature to separate serum from the blood. Serum was collected and stored in a freezer at −20°C until serological testing.

Information on farm owner, farm characteristics and the animals kept was collected using a structured questionnaire written in English pre-tested with both urban and agro-pastoral farmers. The questionnaire was administered by face-to-face interviews and translated into the national language Swahili at the interviews mainly by the authors, who have the ability to communicate with cattle owners through the language. In cases when the owners were absent, their family members or employees involved in cattle raising answered the questionnaire instead.

2.2.4 Serological tests

All sera were screened using the Rose Bengal plate test (RBPT) (IDvet, Grabels, France), and a competitive enzyme-linked immunosorbent assay (C-ELISA) (Boehringer Ingelheim Svanova, Uppsala, Sweden) was conducted for RBPT-positive samples in duplicate. RBPT and C-ELISA were performed following the manufacturers' instructions at Sokoine University of Agriculture. For C-ELISA, the optical density was measured at 450 nm using an ELISA reader, Multiskan RC version 6.0 (Thermo Labsystems, Helsinki, Finland). A sample with positive results for both RBPT and C-ELISA was regarded as sero-positive, and a farm with at least one seropositive cow was regarded as an infected farm.

2.2.5 Prevalence estimation

RBPT generally causes false positives and false negatives, and the animal level test prevalence obtained from serological tests needs to be adjusted to calculate the true prevalence. As this study used RBPT and C-ELISA, animal level true prevalence was calculated as Equation 3 using serial testing calculation [36, 119]:

$$P = \frac{P^T + Sp - 1}{Se + Sp - 1} \quad \text{Equation 3}$$

where

$$Se = Se_1 \times Se_2$$

$$Sp = 1 - \{(1 - Sp_1) \times (1 - Sp_2)\}$$

where P is true prevalence, P^T is test prevalence, Se_1 and Se_2 are sensitivities of RBPT and C-ELISA, 0.812 and 1.000, and Sp_1 and Sp_2 are their specificities, 0.863 and 0.999, respectively [47, 105].

For urban areas, the animal level true prevalence was calculated by Equation 3 using serological test results. The 95% confidence interval was calculated using the one-sample chi-squared test using the prop () function in statistic software R version 3.3.2, and adjusted using Equation 3. For agro-pastoral areas, as the maximum number of animals sampled was 50, the number of sero-positive cattle if all cattle would have been tested within each sampled herd was stochastically estimated for the herds larger than 50, by binomial distribution using the rbinom (n, p) function, using the total number of cattle in a herd as n, and the test prevalence in the herd, p. Then, a beta distribution was modelled, and its median, 2.5th and 97.5th percentiles were calculated using qbeta (quantile, $s + 1$, $N - s + 1$) function in R, where s is the total number

of test positive animals modelled, and *N* is the total number of animals in the studied herds. Finally, the overall test prevalence and 2.5th and 97.5th percentiles estimated were adjusted to gain the true prevalence and 95% confidence interval using Equation 3. This procedure was iterated for 5,000 times in R. The farm-level prevalences and 95% confidence intervals in urban and agro-pastoral areas were calculated using the one-sample chi-square test. Within-herd prevalence of positive farms and the 95% confidence interval were estimated using a Generalized Linear Model (GLM) with binomial errors using R.

2.2.6 Statistical analysis

For comparisons of farm characteristics between urban and agro-pastoral areas and univariable risk factor analyses, the Wilcoxon rank-sum test was performed for count and score data. Pearson's chi-squared test with Yates' continuity correction was performed for binary and categorical data, and Fisher's exact test was used when at least one cell included expected frequencies less than 5.

For the risk factor analysis at the farm level, data from both urban and agro-pastoral areas were used, as the farm level risk factors may be associated with the ecological difference between these areas. However, socio-economic factors were not included, as this may simply show the difference in socio-economic characteristics between these areas. On the other hand, as explained later in the Results section, only one cow in a farm was infected with *Brucella* in the urban areas, and only data from the agro-pastoral areas were analysed at the animal level. In this analysis, male and female cattle in agro-pastoral areas were separately analysed in order to correctly investigate the sex specific risk factors.

In multivariable analysis, GLM with binomial errors was used for the farm level risk factor analysis. At the animal level, generalized estimating equations (GEE) was performed to take into account farm-level covariates, selecting serological test results as response variables, and

variables with P values < 0.2 in the univariable analyses as explanatory variables, using geepack [53] in R. Collinearity was evaluated for all combinations of these explanatory variables with a cut-off correlation = 0.9; no collinearity was found among these variables. Backward stepwise simplification was conducted using the likelihood ratio test. For all the statistics, the computer software R was used.

2.3. Results

2.3.1 Characteristics of urban and agro-pastoral livestock production systems

Tables 1, 2 and 3 show the comparisons of farming style, socio-economical characteristics and knowledge associated with brucellosis between urban and agro-pastoral areas. The proportion of farmers using zero-grazing was significantly greater in urban areas (71.7%) than agro-pastoral areas (0%, $P < 0.01$, Table 1). In contrast, the proportions of farmers keeping goats and sheep were significantly greater in agro-pastoral areas ($P < 0.01$, Table 1). The numbers of animals were also significantly larger in agro-pastoral areas than urban areas ($P < 0.01$, Table 2), suggesting that traditional farming style is common in agro-pastoral areas, whereas intensive farming is predominant in urban areas. However, the proportion of income from livestock farming out of total income was not significantly different between the two areas ($P = 0.32$). In urban areas, significantly more farms were owned by women (39.6%, $P < 0.01$, Table 1), and the mean age of owners was higher (55.9 years, $P < 0.01$, Table 2) than in agro-pastoral areas (5.9% and 45.5 years, respectively).

The proportion of farms that experienced abortion in cattle was significantly higher in agro-pastoral areas (82.4%) than in urban areas (9.4%, $P < 0.01$, Table 1). All farmers in agro-pastoral areas used their own bulls for breeding, while 39.6% of farmers in urban areas used their own bulls. In urban areas, 51.9% of farmers used bulls from other farms, but none did this in agro-pastoral areas. Artificial insemination (AI) was common (34 farmers, 32.1%) in urban

areas (Table 1), but 25 of them used bull as well (not shown in Table 1). AI was not used in agro-pastoral areas. The proportion of farmers who purchased cattle within 1 year (58.8%) was significantly higher in agro-pastoral areas than that in urban areas (30.2%, $P = 0.04$, Table 1). Urban farmers (92.5%) received significantly more veterinary services than agro-pastoral farms (64.7%, $P < 0.01$). The *Brucella* vaccine was not used in any farms studied in both areas (Table 1).

Table 1. Comparisons of characteristics of farms between urban and agro-pastoral areas (proportional data)

Categories	Percentages (number of farms)		Test statistics	*P* value
	Urban areas (Total = 106)	Agro-pastoral areas (Total = 17)		
Grazing system: zero grazing	71.7 (76)	0 (0)	$x^2 = 28.9$, df = 1	< 0.01
Cattle herded with goats or sheep	35.8 (38)	100 (17)	$x^2 = 21.9$, df = 1	< 0.01
Cattle herded with goats	35.8 (38)	94.1 (16)	$x^2 = 17.9$, df = 1	< 0.01
Cattle herded with sheep	10.4 (11)	64.7 (11)	Fisher's exact test	< 0.01
Breeding system				
Own bull	39.6 (42)	100 (17)	Fisher's exact test	< 0.01
Bull from other farms	51.9 (55)	0 (0)	Fisher's exact test	< 0.01
Artificial insemination	32.1 (34)	0 (0)	Fisher's exact test	< 0.01
Purchase of cattle	30.2 (32)	58.8 (10)	$x^2 = 4.1$, df = 1	0.04
History of abortion of cattle	9.4 (10)	82.4 (14)	Fisher's exact test	< 0.01
Contact with wild animals	0 (0)	23.5 (4)	Fisher's exact test	< 0.01
Use of *Brucella* vaccine	0 (0)	0 (0)	-	-
Receiving veterinary services	92.5 (98)	64.7 (11)	Fisher's exact test	< 0.01
Male-owned farms	60.4 (64)	94.1 (16)	$x^2 = 5.9$, df = 1	0.01

Table 2. Comparisons of characteristics of farms between urban and agro-pastoral areas (count data)

Categories	Urban areas Median (2.5th and 97.5th percentiles)	Agro-pastoral areas Median (2.5th and 97.5th percentiles)	*P* value
Owner's age, year	57 (32–74)	45 (24–76)	< 0.01
Family size	6 (3–10)	10 (5–25)	< 0.01
Number of cattle	6 (2–18)	64 (11–396)	< 0.01
Number of goats	0 (0–24)	28 (6–85)	< 0.01
Number of sheep	0 (0–24)	10 (0–120)	< 0.01
Number of cattle bought per year	0 (0–2)	2 (0–8)	< 0.01
Number of cattle sold per year	1 (0–5)	5 (0–38)	< 0.01
Proportion of income from livestock farming	0.50 (0.10–0.75)	0.50 (0.11–0.76)	0.32

Table 3. Comparisons of education level and knowledge associated with brucellosis between urban and agro-pastoral areas

Categories	Percentages (number of farms)		Test statistics	*P* value
	Urban areas (Total=106)	Agro-pastoral areas (Total=17)		
Level of education			Fisher's Exact Test	< 0.01
No education	0.9 (1)	35.3 (6)		
Primary	21.7 (23)	52.9 (9)		
Secondary	36.8 (39)	5.9 (1)		
Diploma	17.0 (18)	0 (0)		
University	23.6 (25)	5.9 (1)		
Knowledge on brucellosis				
Name	34.0 (36)	5.9 (1)	$x^2 = 4.2$, df = 1	0.04
Symptoms	8.5 (9)	0 (0)	Fisher's Exact Test	0.36
Transmission from cattle to human	8.5 (9)	0 (0)	Fisher's Exact Test	0.36
Brucellosis vaccine	6.6 (7)	0 (0)	Fisher's Exact Test	0.59

2.3.2 Knowledge

The education level of farmers was significantly higher in urban areas than in agro-pastoral areas ($P < 0.01$, Table 3). Regarding knowledge about brucellosis, urban farmers (34.0%) knew significantly more about the disease name than agro-pastoral farmers (5.9%, $P = 0.04$, Table 3). However, the proportions of farmers with knowledge of symptoms, transmission from cattle to humans and availability of vaccine were low, and not significantly different between the two areas.

2.3.3 Prevalence

Figure 1 shows the distributions of sero-positive and sero-negative farms in urban (b) and agro-pastoral areas (a). There was only one infected farm in the urban area. The northern area than the northernmost road in Figure 1a was a mountainous area, and the other areas were plain. In the mountainous area, sero-negative farms were concentrated. Farm-level sero-prevalences were 0.9% (1/106, 95% CI: 0.0–5.9) and 52.9% (9/17, 95% CI: 28.5–76.1) in urban and agro-pastoral areas, respectively, and the prevalence in agro-pastoral areas was significantly higher than in urban areas (Fisher's exact test, $P < 0.01$). At the animal level, test prevalences were 0.1% (1/667) and 4.2% (28/673) in urban and agro-pastoral areas, respectively, and true prevalences were estimated to be 0.2% (95% CI: 0.0–1.1) in urban areas. Table 4 shows the estimated total number of sero-positive cattle and adjusted animal level prevalence in agro-pastoral areas, and the median of the overall true prevalence in agro-pastoral areas was 7.0 (95% CI: 5.7–8.4). The confidence intervals of urban and agro-pastoral areas estimated did not overlap. In agro-pastoral areas, adjusted within-herd prevalence of positive farms ranged between 3.0% and 27.3%, and adjusted mean within-herd prevalence was 8.5% (95% CI: 5.8–11.8).

Table 4. The estimated total number of positive cattle and adjusted animal level prevalence in agro-pastoral areas

Percentile	Estimated total number of positive cattle	Adjusted values with 50th, 2.5th and 97.5th percentiles
50th	91	7.0 (5.7–8.3)
2.5th	74	5.7 (4.5–6.9)
97.5th	109	8.4 (7.0–9.9)

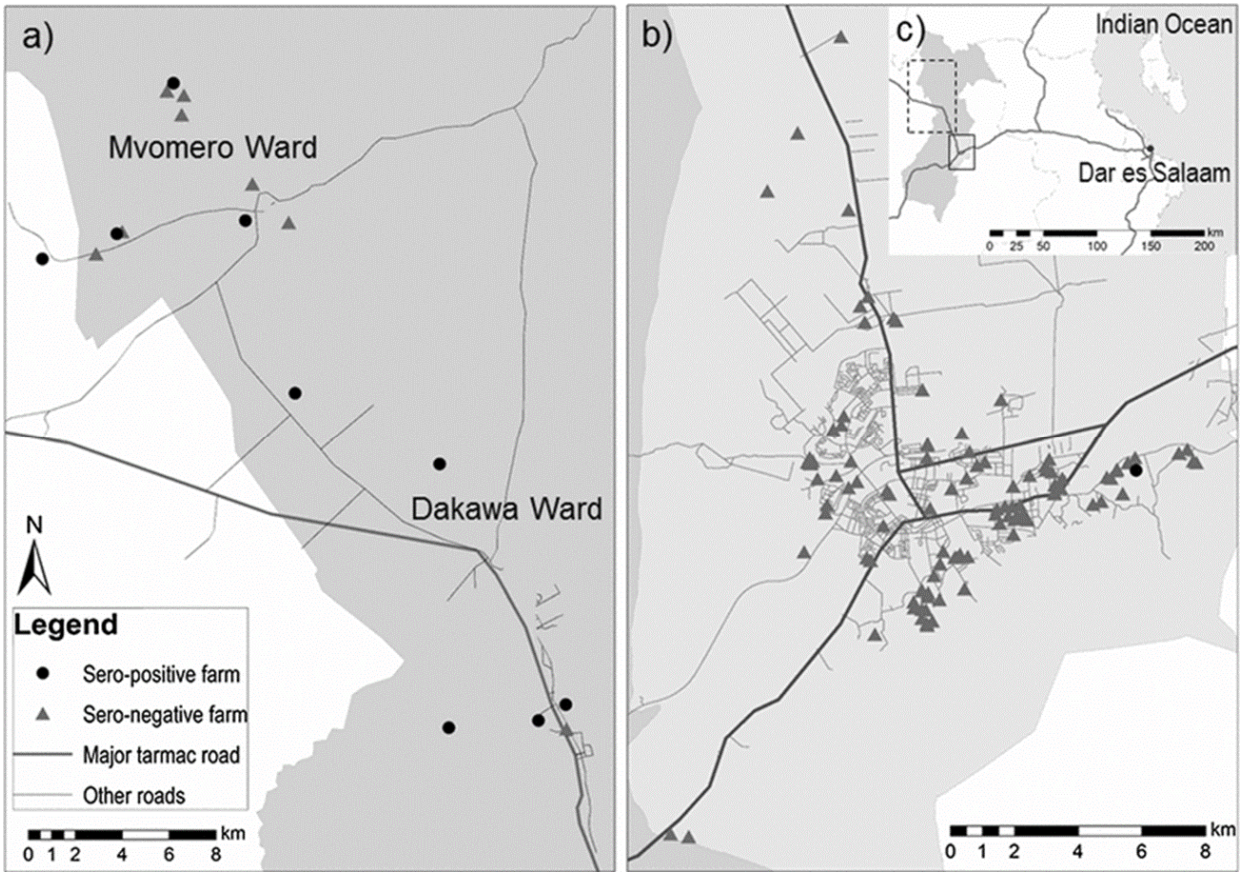

Figure 1. The distributions of *Brucella* sero-positive and negative herds studied. Panels (a): agropastoral areas in Mvomero district (shaded grey); (b) urban areas in Morogoro municipality (shaded pale grey); and (c) a square with dotted line shows the locatino of panel (a), while with solid line that of (b).

2.3.4 Risk factors for brucellosis at the farm level

Table 5 shows the results of the univariable analyses for categorical data at the farm level. The herd size in infected herds (median number of cattle 85 (2.5th and 97.5th percentiles: 21–424) was significantly larger than healthy herds (median 6 (2.5th and 97.5th percentiles: 2–48, $P < 0.01$). The other factors significantly associated with bovine brucellosis were cattle grazing ($P < 0.01$), keeping goats or sheep ($P < 0.01$), and history of abortion in the herd ($P < 0.01$, Fisher's exact tests, Table 5).

At first, multivariable analysis was conducted including herd size and it was the only factor remained in the final model. In fact, the herd size of infected farms (median 146) was significantly larger than that of healthy farms (median 40) in agro-pastoral areas ($P = 0.04$). However, as 71.7% of urban farmers were zero-grazers and most of agro-pastoral farmers grazed large numbers of cattle, herd size had a strong relationship with such farming styles (Table2). For this reason, multivariable analysis was performed excluding the factor, herd size. In this analysis, the final model included two risk factors: history of abortion in the herd (odds ratio (OR) = 13.0 (95% CI: 2.4–71.9), $P < 0.01$) and cattle grazing (OR = 7.7 (95% CI: 0.8–70.7), $P = 0.07$). Although cattle grazing demonstrated a P value > 0.05, this was retained based on biological plausibility.

Table 5. Farm-level univariable analysis (binary and categorical data, Fisher's exact tests)

Factors	Response	Infected herds	Healthy herds	Prevalence (%)	*P* value
Cattle grazing	Yes	9	38	19.1	< 0.01
	No	1	75	1.3	
Keeping goats or sheep	Yes	10	45	18.2	< 0.01
	No	0	68	0	
Abortion	Yes	8	16	33.3	< 0.01
	No	2	97	2	
Bought-in cattle	Yes	5	37	11.9	0.31
	No	5	76	6.2	
Breeding system	Bull	10	104	8.8	1
	AI	0	9	0	

2.3.5 Risk factors for brucellosis at the animal level

In the univariable analysis at the animal level using female cattle data in agro-pastoral areas, history of abortion ($P < 0.01$, Table 6) and age were significant factors. The median age of infected female cattle (7.0 years, 2.5th and 97.5th percentiles: 3.7–13.4) was significantly older than healthy female cattle (5.0 years, 2.5th and 97.5th percentiles: 0.3–14.0, $P < 0.01$). In terms of male cattle, only one out of 163 bulls was positive. The infected bull was indigenous breed raised in an agro-pastoral area. Out of the other healthy bulls, 160 were indigenous and 2 were other breeds. The infected bull was bought-in, and bought-in bulls accounted for 7.4% (12/163) of total bulls sampled. The age of the infected bull and the median age of healthy male cattle were 3.0 and 1.5 (2.5th and 97.5th percentiles: 0.3–5.0) years. All female and male cattle were born by natural mating.

In the multivariable analysis for female cattle in agro-pastoral areas, two risk factors were identified: age (OR = 1.1 (95% CI: 1.01–1.21), $P = 0.04$) and history of abortion (OR = 3.7 (95% CI: 1.2–11.8), $P = 0.03$).

Table 6. Animal-level univariable analysis for female cattle in agro-pastoral areas (binary and categorical data, Fisher's exact tests)

Factors	Response	Infected animals	Healthy animals	Prevalence (%)	*P* value
Abortion	Yes	6	26	18.8	< 0.01
	No	21	457	4.4	
Breed	Indigenous	27	472	5.4	1
	Other	0	11	0	
Bought-in	Yes	6	48	11.1	0.054
	No	21	435	4.6	

2.3.6 Human risks against brucellosis

The questionnaire survey found that agro-pastoral farmers practiced significantly more risky behaviours for human brucellosis such as drinking raw milk (17.6%, $P < 0.01$), drinking blood (35.3%, $P < 0.01$) and helping cattle birth (100%, $P = 0.04$) than urban farmers (0%, 0% and 79.2%, respectively, not shown in tables). Amount of milk consumption was not significantly different between urban (median 500 ml/person/day, 2.5th and 97.5th percentiles: 200–1,688) and agro-pastoral farmers (1000, 250–1000, $P = 0.42$). Human fever cases were significantly higher among agro-pastoral farmers (1: low to 5: high frequency, median 4.0, 2.5th and 97.5th percentiles: 1.0–5.0) than urban farmers (2.0, 1.0–4.4, $P < 0.01$).

2.4. Discussion

In the present study, brucellosis prevalence and risk factors and behaviours associated with human brucellosis between urban and agro-pastoral areas were evaluated. Previous studies reported that the animal prevalence was 1.9% (2/104) in small-scale dairy farms in Morogoro district [87] and 14.9% (25/168) in Mvomero district [118], supporting our result of the lower prevalence in urban areas than in agro-pastoral areas in Morogoro region. It was speculated that the low prevalence in smallholder dairy cattle in Morogoro region was due to confinement of animals and low stocking rates, which led to reduced contamination of pastures [87]. In our study, the difference in the prevalences between the two areas was explained by the practice of grazing. Through grazing, cattle have contact with other livestock, wild animals, aborted foetus and placenta that potentially contain *Brucella* bacteria. Moreover, even though grazing was practiced, mountainous areas had only a few infected farms, and this suggested extensive movements allowing overlap of herd territories facilitated between-herd brucellosis transmission. Cattle grazing is officially prohibited in the urban areas of Morogoro municipality by the local government (personal communication with officers). The real-life situation was

that although some farmers conducted grazing, the majority of urban farmers were zero-grazers. Conversely, all agro-pastoral farmers grazed their cattle. In the agro-pastoral area, pastures are limited especially during the dry season. Hence, grazing is inevitable and it is not prohibited by the local government. Previous studies also reported that grazing was a risk factor associated with brucellosis in other countries [4, 62, 78]. However, the higher prevalences were observed in intensive production systems than extensive systems in other reports in the Lake Victoria zone in Tanzania and other African countries [39, 40, 57, 58], and findings of this study may not be generalized to other areas.

In our study, the presence of goats or sheep was a risk factor of bovine brucellosis in univariable analysis but not in multivariable analysis. Several studies have shown that keeping small ruminants with cattle was a risk factor for brucellosis transmission between different animal species [6, 57, 63, 111]. The previous research conducted in Mvomero district showed the brucellosis prevalence in small ruminants was 1.4% [118]. Although this prevalence was low, the magnitude of *B. melitensis* infection in animal brucellosis is not known well in this area. Cross-species transmission of *Brucella* infection may occur, and further investigations such as identification of the *Brucella* species and its biotypes are recommended to understand the epidemiology better [57].

Abortion was identified as a risk factor at both farm and animal levels. *Brucella* infection was considered to contribute to some of the abortion cases of cows in the study area. In case of bovine brucellosis, abortion is not only the consequence of *Brucella* infection, but also a cause of infection, as aborted foetus and placenta from infected cows are highly contaminated with the bacteria.

Another risk factor identified in animal-level analysis was age. Given that the level of exposure to *Brucella* bacteria may be constant over time, older cattle are expected to have a higher risk of sero-positivity to *Brucella*. A previous study also suggested that older age was a

risk factor for brucellosis in cattle in Kenya [62].

In addition, although bought-in cattle was not a significant factor in animal-level multivariable analysis, this factor demonstrated a *P* value close to 0.05 in the univariable analysis. A hypothesis arises from this is that farmers tend to sell cows that have a history of abortion to livestock markets, which may contribute to the spread of brucellosis in the areas. Conversely, farmers may mitigate the risk of brucellosis infection among their herds by selling cattle with a history of abortion. Further research is needed to understand human behaviours associated with cattle sales.

This study provided useful information on the control of bovine brucellosis. First, very low prevalence in the urban areas suggested that intensification of dairy farming itself may be the effective control options for bovine brucellosis, providing opportunities for better control measures with adequate infrastructure and training. Although other reports indicated intensification is associated with high prevalence due to increased stocking densities, animal contacts and a higher birth index [39, 40, 62, 85], strictly confined dairy system may overcome these risks.

On the other hand, agro-pastoralists still dominate in Tanzania, and play an important role in food production. Strict biosafety and management measures, vaccination and a test-and-slaughter strategy are recognized as the most effective methods to control brucellosis in livestock [81]. However, such control options require intensive veterinary service, which is a great challenge in the setting. In the present study, none of farmers had ever used the *Brucella* vaccine, and most of them were even not aware of its existence. In addition, veterinary officers, who have their own veterinary medicine shops for more than 15 years in the study areas, had never administered *Brucella* vaccine in the areas (personal communications). Therefore, second, vaccine related socio-economic researches, such as willingness-to-pay, cost benefit analysis, potential policy support, and attitude change by education, are necessary. Third, as mentioned

above, abortion and cattle trade are associated with between- and within-herd brucellosis transmission. Quantification of transmission dynamics using mathematical modelling would provide clearer ideas in the disease control. Such approach also provides a-priori evaluation of strategic vaccination to avoid accidental human infection with vaccine isolates from cows vaccinated since the current common vaccines, S19 and RB51 are live vaccines [18]. Furthermore, S19 has a difficulty in using with test-and-slaughter programme as S19 produces the antibody that cannot be distinguished from that induced by infection with field strains, thereby making serological diagnostic tests invalid, although RB51 overcomes the problem [103].

From the results of bovine brucellosis prevalence and human practices of drinking cattle blood and raw milk, agro-pastoral farmers were found to be at risk of human brucellosis, lacking knowledge about brucellosis. It was reported that the human brucellosis prevalence in Mvomero district was 36.1% [56]. In the present study, frequency of human fever cases was perceived higher among agro-pastoral farmers than among urban farmers, and brucellosis may be associated with this. Change of traditional dietary habits is generally a challenge, and socio-economic studies are needed to plan successful and sustainable control programmes.

This study clearly showed the necessity of One Health [131] and ecohealth approaches [130], which involves engagement with different level of stakeholders including communities, in controlling this long lasting zoonosis in endemic countries.

2.5. Summary of Chapter 2

Epidemiology of human and animal brucellosis may depend on ecological conditions. A cross-sectional study was conducted to compare prevalence and risk factors of bovine brucellosis, and risky behaviours for the human infection between urban and agro-pastoral areas in Morogoro region, Tanzania. Cattle blood sampling and interviews using a structured

questionnaire were conducted with farmers. Rose-Bengal test was conducted for the cattle sera, and positive samples were confirmed with competitive ELISA.

Farm-level sero-prevalences were 0.9% (1/106, 95% CI: 0.0–5.9%) and 52.9% (9/17, 95% CI: 28.5–76.1%) in urban and agro-pastoral areas, respectively. The animal-level adjusted prevalences were 0.2% (1/667, 95% CI: 0.0–1.1%) and 7.0% (28/673, 95% CI: 5.7–8.4%) in those areas. The final farm-level model including both areas found two risk factors: history of abortion in the herd ($P < 0.01$) and cattle grazing ($P = 0.07$). The animal-level risk factors in agro-pastoral areas were age ($P = 0.04$) and history of abortion ($P = 0.03$).

No agro-pastoral farmer knew about *Brucella* vaccine. Agro-pastoralists generally had poorer knowledge on brucellosis, and practiced significantly more risky behaviours for human brucellosis such as drinking raw milk (17.6%, $P < 0.01$) and blood (35.3%, $P < 0.01$), and helping cattle birth (100%, $P = 0.04$) than urban farmers (0%, 0% and 79.2%, respectively). Intervention programmes through education including both human and animal health particularly targeting agro-pastoralists would be needed.

Chapter 3 Perception and behaviours associated with bovine brucellosis control among agro-pastoralists in Morogoro region, Tanzania

3.1. Introduction

Brucellosis is a worldwide zoonotic disease caused by several species of the genus *Brucella*. Although many developed countries have eradicated the disease, it is endemic in some regions such as Latin America, Mediterranean, Middle East, Africa, and Asia, causing large economic losses due to the problems of livestock production and human health [28]. In domesticated animals, the disease is characterised by abortion, infertility in adult animals, and reduced milk yields. Although most infected cows will only abort once, they remain a source of infection during subsequent normal calvings [52]. In humans, clinical symptoms include fever, headache, weakness, malaise, arthralgia, and other less common clinical manifestations [112]. *Brucella* species have also been detected from a variety of wildlife, such as bison (Bison bison), red deer (Cervus elaphus), feral swine, wild boar (Sus scrofa), African buffalo (Syncerus caffer), and lion (Panthera leo), which may act as reservoirs for livestock and human infection [11, 49, 75, 127]. The sources of infection for animals include aborted foetus, placenta, milk, and semen from infected animals [127]. The most common sources of human infection are unheated livestock products [22]. Contact among livestock, wildlife, and human is common for pastoral and agro-pastoral farmers in Tanzania [11]. In addition, risky behaviours for human infection such as eating raw meat and drinking raw milk have been observed among some farmers [60].

Zoonoses can be controlled most efficiently and surely by tackling animal reservoirs. As control strategies, biosecurity at the farm level, test-and-slaughter programmes, and immunisation have been demonstrated as notable tools for brucellosis control in livestock [81]. However, these control measures may conflict with the customs of affected communities, such as pastoralists and extensive agro-pastoralists, and may be challenging due to the high cost for surveillance, slaughter of infected animals, and general compensation in developing countries [80].

In terms of immunisation, *Brucella abortus* vaccines have been successfully used

worldwide for bovine brucellosis control for decades. In sub-Saharan Africa, national brucellosis control programmes involving vaccination were performed in southern African countries. However, outside of southern Africa, vaccination was rarely conducted and if done, it was a makeshift effort rather than a coordinated national programme [86]. One of the reasons of underuse of vaccines in developing countries is lack of public resources. Considering the circumstances, this study seeks an opportunity for a community-based disease control strategy wherein cattle farmers pay for *Brucella* vaccination by themselves, which would be sustainable if accepted.

Another concern about the maintenance and transmission of bovine brucellosis is cattle trade. Selling out cattle that experienced abortion may occur due to moral hazards; thus, investigation into the perception and behaviour of cattle keepers would provide useful information in planning brucellosis control strategies. Abortion is not only caused by *B. abortus* in cattle but also by several other pathogens such as *Neospora caninum*, bovine viral diarrhoea virus, bovine herpesvirus type 1, and *Leptospira interrogans* [10, 25]. However, previous reports including the findings in Chapter 2 have shown a significant association between abortion and infection with *B. abortus* in eastern and southern Africa [9, 78, 88, 92], supporting the hypothesis that selling out cattle that experienced abortion may be a risk factor for disease spread.

In this study, we used the item count technique (ICT), introduced by Droitcour et al. [38], to understand the behaviour of selling out cows that experienced abortion. The ICT is an indirect questioning technique used to estimate the proportion of people who have engaged in a sensitive behaviour. Estimation using the ICT is expected to be higher than that from conventional direct questioning. For example, Tsuchiya et al. [121] reported that the ICT indicated significantly more shoplifting activities compared with direct questioning. In animal health, Randrianantoandro et al. [107] studied illegal sales of African swine fever-infected pork in

Madagascar using the ICT.

The objective of this study was to investigate the farm-level prevalence and risk factors of bovine brucellosis and perception and behaviours related to its control, including willingness to pay for vaccination, among agro-pastoralists in Morogoro Region, Tanzania, where brucellosis is endemic [56].

3.2. Materials and Methods

3.2.1 Study site

This study was conducted in Mvomero District in Morogoro Region, Tanzania (Figure. 2). Two villages in Dakawa ward and four villages in Mvomero ward were selected from the district, based on the availability of the lists of cattle farms and the reachable distance from Morogoro municipality, where Sokoine University of Agriculture is located. The district is located between latitudes 8°0′ and 10°0′S and between longitudes 37°0′ and 28°22′E. The altitude of the district is between 380 and 1,520 meters above sea level, providing a suitable climate for a variety of tropical and subtropical crops. The district receives a bimodal type of rainfall with peaks in April and December for long and short rains, respectively, while May to October remains relatively dry [90]. The average rainfall amounts to 1,200 mm per annum with variations from 800 to 2,000 mm [91]. The district's economy depends on agriculture, mainly from crop production. The livestock types found in the district comprise cattle, goats, sheep, pigs, donkeys, and birds. Most cattle are indigenous breeds raised with a semi-extensive or extensive system, and a few improved dairy cattle are reared mainly with an intensive system.

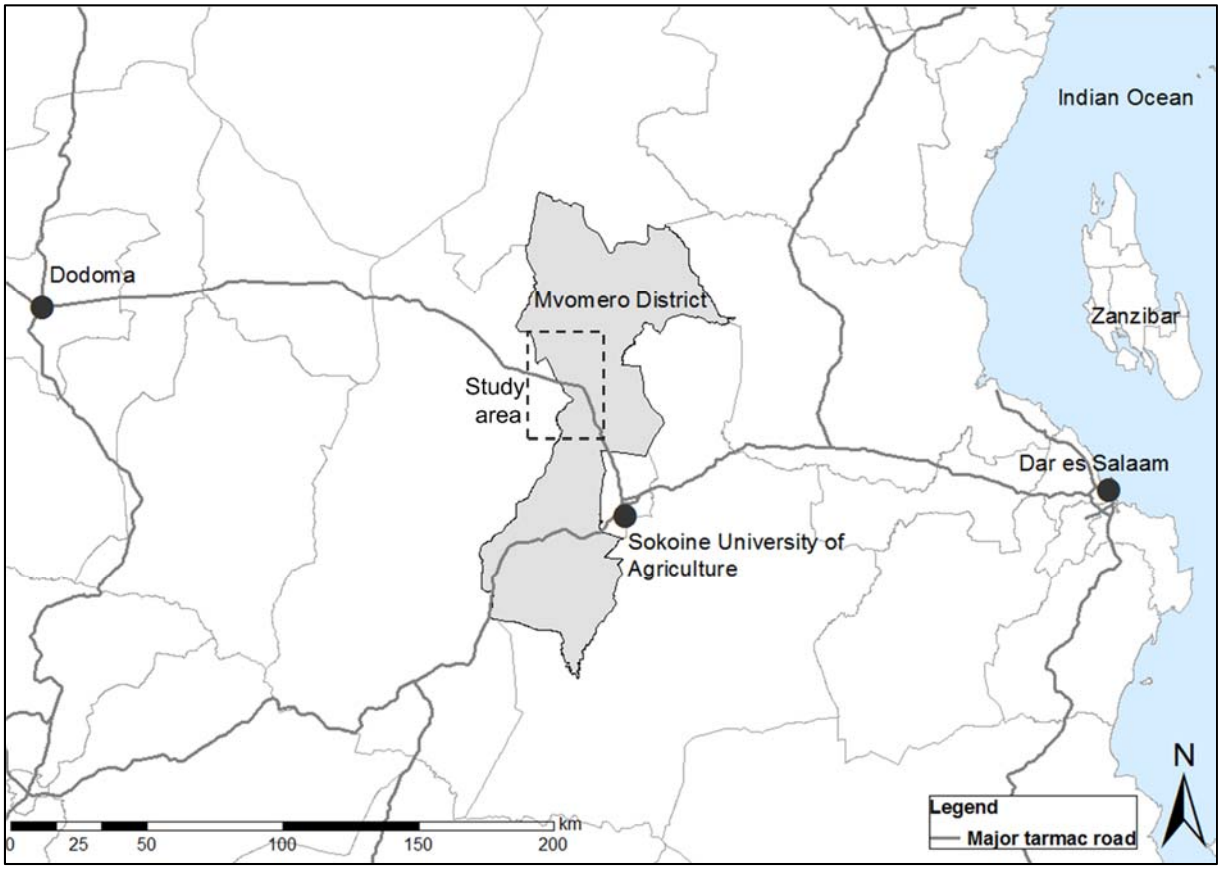

Figure 2. Map showing Mvomero District in Morogoro Region, Tanzania. A square with dotted lines shows the study area (See Figure 3).

3.2.2 Study design and sample size estimation

A cross-sectional study involving herd milk sampling and a structured interview using a questionnaire was designed. Although this study had multiple purposes, the farm sample size was calculated by the following formula for estimating farm-level prevalence [119]:

$$n = \frac{1.96^2 * P_{\exp}(1 - P_{\exp})}{d^2} \quad \text{Equation 4}$$

where n is the required sample size based on an infinite population, $P_{\exp}$ is the expected prevalence, and d is desired absolute precision. We set $P_{\exp}$ as 0.529 based on the findings in Chapter 2 [9] and d as 0.05. The calculated sample size was 383. However, in the case of small populations, the required sample size, n_{adj}, given by the following formula can be adopted:

$$n_{adj} = \frac{N \times n}{N + n} \quad \text{Equation 5}$$

where n, obtained from Equation 4, is the sample size and N is the number of cattle farms based on the lists of farms, which was 170. As a result, n_{adj} was calculated as 118. Farms to be sampled were proportionally allocated to the villages according to the numbers of cattle farms within them, and study farms were selected from the lists by random sampling using runif() function in statistical software R. After a recruitment process by the veterinary officers based on the random sampling, 124 farms were included in our study.

3.2.3 Field survey

A field survey was conducted from September to October 2016. Herd milk was collected in Falcon tubes and brought to the laboratory in Sokoine University of Agriculture. Milk samples were stored in a freezer at −20°C until diagnostic testing.

Information on farm owner, farm characteristics, the animals kept, and willingness-to-pay for *Brucella* vaccine was collected using a structured questionnaire written in English that had

been pre-tested with cattle farmers. Translation of the questions into the national language, Swahili, was validated in advance by cross-checking with Swahili-speaking individuals, and the questionnaire was administered by face-to-face interviews in Swahili. In cases when the owners were absent, their family members or employees involved in cattle raising answered the questionnaire instead, except for the question on willingness to pay for vaccination, which was asked of the owners by telephone. Farmers were asked questions related to knowledge about brucellosis prior to the explanation of brucellosis. Willingness to pay for vaccination was surveyed after the explanation of brucellosis and the vaccine. The vaccine price was set as 3,000 Tanzania shilling (approximately 1.3 USD at the time of writing) per shot in this study, taking into account pricing information obtained from a veterinary officer who owned his own veterinary medicine shop.

3.2.4 Diagnostic test

All herd milk samples were tested in duplicate using an indirect enzyme-linked immunosorbent assay (I-ELISA) (Boehringer Ingelheim Svanova, Uppsala, Sweden). I-ELISA was performed following the manufacturers' instructions at Sokoine University of Agriculture. For I-ELISA, the optical density was measured at 450 nm using an ELISA plate reader, Multiskan RC version 6.0 (Thermo Labsystems, Helsinki, Finland). A farm with a positive milk I-ELISA result was regarded as an infected farm.

3.2.5 Statistical analysis

The sensitivity and specificity of milk I-ELISA for individual milk samples was assumed to be 99.6% and 99.1%, respectively [123]. However, in this study, herd milk samples were tested. As no information was available for herd-level sensitivity and specificity of milk I-ELISA, this study used the apparent prevalence, and the 95% confidence interval (CI) was

calculated.

For univariable risk factor analyses, the Wilcoxon rank-sum test was performed for count and score data. Pearson's chi-squared test with Yates' continuity correction was performed for binary and categorical data, and Fisher's exact test was used when at least one cell included an expected frequency of less than 5. In addition, education and knowledge scores were established based on the level of education of the farmers and the number of knowledge items on brucellosis the farmers knew (farmers' education level and knowledge items are shown in Table 8).

In multivariable analysis, a generalised linear model (GLM) with binomial errors was used, selecting I-ELISA results as response variables, and variables with P values < 0.2 in univariable analyses were considered as explanatory variables. Collinearity was evaluated for all combinations of these explanatory variables with a cut-off correlation = 0.9; no collinearity was found among these variables. Backward stepwise simplification was conducted using the likelihood ratio test.

To identify factors associated with willingness to pay for vaccination, univariable and multivariable analyses were performed using the same method and procedure for risk factor analysis of brucellosis. However, brucellosis status was excluded from the analysis because farmers did not know the test results at the time of interview. Associations between conduct of risky behaviours for human brucellosis infection and bovine brucellosis status and between conduct of risky behaviours and tribes were analysed using Pearson's chi-squared test. Fisher's exact test was used in cases in which at least one cell included an expected frequency of less than 5. Statistical analyses were performed using the computer software R version 3.3.2. Statistical significance was considered to exist at P values < 0.05.

3.2.6 The item count technique

There are two types of ICT, the single list and double list. The double list technique was

performed in this study since it cuts the variance of the estimate in half, consequently providing a more accurate estimate [38]. Two baseline lists, X and Y, that each contained different items were prepared so that the key item was presented to all respondents. The key item was ‘sell out cattle that experienced abortion to cattle markets’.

Baseline list X was as follows:

(1) Use bulls for breeding

(2) Drink raw milk

(3) Ask a veterinary officer when cattle have fever

(4) Send cattle for grazing

Baseline list Y was as follows:

(1) Use chemical insecticides on cattle to kill ticks

(2) Feed commercial concentrates to cattle

(3) Sell manure to others

(4) Have a biogas plant

Table 7 shows subsamples used in this study. The respondents were told that the questionnaire was anonymous in order to yield honest answers, and they were asked to report the number of items in each list that were true for them without mentioning which ones. According to a published guideline [29], at least 40–50 respondents are needed for each subsample to assure stability and accuracy of the estimate. Thus, the total of 124 farmers was equally divided into three subsamples, and farmers were randomly assigned to a subsample.

The proportion of farmers engaged in the key item as assessed using the ICT was calculated by the following formula:

$$p_1 = \frac{1}{2}\left[\left(\overline{X}_{5A} - \overline{X}_{4B}\right) + \left(\overline{Y}_{5B} - \overline{Y_{4A}}\right)\right] \quad \text{Equation 6}$$

where p_1 is the proportion of farmers who sell out cattle that experienced abortion, X_{5A} is the mean number of items on "baseline list X plus key item" engaged in by Subsample A, X_{4B} is the mean number of items on "baseline list X" engaged in Subsample B, Y_{5B} is the mean number of items on "baseline list Y plus key item" engaged in by Subsample B, and Y_{4A} is the mean number of items on 'baseline list Y' engaged in by Subsample A. The variance of the estimate was calculated using the formula explained by Droitcour et al. [38]. The estimation from a direct question, p_2 was obtained from the following equation:

$$p_2 = \frac{n_y}{N_{dq}} \quad \text{Equation 7}$$

where n_y is the number of 'yes' responses and N_{dq} is the number of respondents of the direct question, which is the sample size of Subsample C. If p_1 is significantly higher than p_2, then human behaviour in the key item is considered to be sensitive.

The binomial test named Twobinom [71, 129] was used for the comparison of p_1 and p_2. The factor score was calculated by dividing the estimation from the ICT by that from direct questioning, therefore showing the efficiency of the ICT compared with direct questioning.

Table 7. Questionnaire and sample size for each subsample of the ICT

	Subsample A	Subsample B	Subsample C
Questionnaire	Baseline X + key item	Baseline X	Direct question
	Baseline Y	Baseline Y + key item	
Sample size	41	41	42

3.3. Results

3.3.1 Characteristics of the study farms

Table 8 shows the socio-economical characteristics, knowledge about bovine brucellosis, and farming style of cattle owners. Only one farm operated a zero-grazing system; the others used semi/free grazing. One farm used both its own bull and artificial insemination for cattle breeding.

Table 8. Characteristics of the study farms

Categories	Response (n = 124 farms)	Percentage
Characteristics of owner		
Tribe: Maasai	60	48.3
Male-owned farm	107	86.3
Level of education		
No education	59	47.6
Primary	46	37.1
Secondary	14	11.3
Diploma	1	0.8
University	4	3.2
Knowledge on brucellosis		
Name of the disease	17	13.7
Symptoms	4	3.2
Transmission from cattle to human	3	2.4
Brucella vaccine	3	2.4
Characteristics of farming		
Grazing system: semi/free grazing	123	99.2
Conducting agriculture	105	84.7
Cattle herded with goats or sheep	94	75.8

Table 8. Characteristics of the study farms (continued)

Categories	Response (n = 124 farms)	Percentage
Breeding system		
Own bull	114	91.9
Bull from other farms	9	7.3
Artificial insemination	2	1.6
Purchase of cattle	116	93.5
History of abortion of cattle	68	54.8
Contact with other livestock herd	113	91.1
Contact with wild animals	17	13.7
Use of any kind of vaccine	41	33.1
Use of *Brucella* vaccine	0	0
Using a veterinary service	84	67.7

3.3.2 The farm-level prevalence

Figure 3 shows the geographical distributions of brucellosis positive and negative herds in the study area. The apparent farm-level prevalence was 44.4% (55/124, 95%CI: 35.5–53.5).

3.3.3 Risk factors for brucellosis

Table 9 and 10 show the results of the univariable analyses for brucellosis for binary data, and count and score data, respectively. The significant variables were using a veterinary service ($P = 0.03$) as a preventive factor and larger herd size ($P = 0.049$) a risk factor.

In the multivariable analysis, the final model included one preventive factor, using a veterinary service (OR = 0.39, 95%CI: 0.18–0.84, $P = 0.02$).

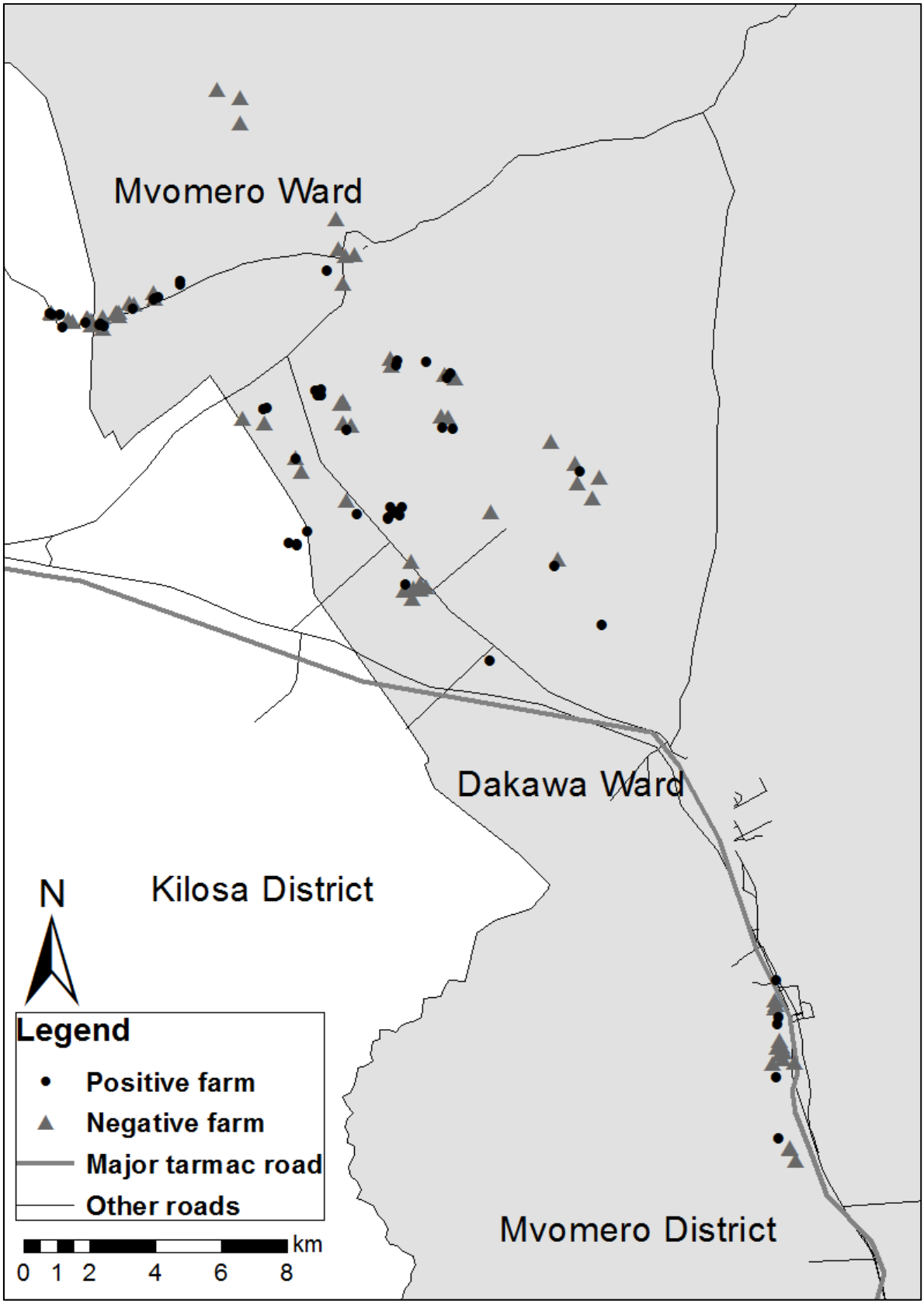

Figure 3. The distributions of *Brucella* positive and negative herds studied.

Table 9. Univariable risk factor analysis on binary response variable

Factors	Response	Infected herds	Healthy herds	Prevalence (%)	Test statistics	*P* value
Presence of goats or sheep	Yes	39	55	41.5	x^2 =0.86 , df = 1	0.35
	No	16	14	53.3		
Contact with other livestock	Yes	53	60	46.9	Fisher's exact test	0.11
	No	2	9	18.2		
Contact with wildlife	Yes	7	10	41.2	Fisher's exact test	1
	No	48	59	44.9		
Bought-in cattle	Yes	52	64	44.8	Fisher's exact test	1
	No	3	5	37.5		
Abortion of cattle	Yes	32	36	47.1	x^2 = 0.24 , df = 1	0.63
	No	23	33	41.1		
Using a veterinary service	Yes	31	53	36.9	x^2 = 4.96, df = 1	0.03
	No	24	16	60.0		
Public vet treatment	Yes	13	25	34.2	x^2 = 1.73, df = 1	0.19
	No	42	44	48.8		
Owners' sex	Male	49	58	45.8	x^2 = 0.30 , df = 1	0.58
	Female	6	11	35.3		
Tribe	Maasai	30	30	50.0	x^2 = 1.09 , df = 1	0.30
	Others	25	39	39.1		

Table 10. Univariable risk factor analysis for brucellosis on count and score data

Factor	Infected herds (2.5 and 97.5th percentiles)	Healthy herds (2.5 and 97.5th percentiles)	*P* value
Number of cattle	50 (8–219)	30 (4–230)	0.049
Age of owner	42 (20–75)	45 (21–71)	0.41
Education score (0: no education; 1: primary; 2: secondary; 3: diploma; 4: university)	1 (0–3)	1 (0–4)	0.84
Knowledge score: 0–4	0 (0–1)	0 (0–3)	0.07

3.3.4 Willingness-to-pay for vaccination

Approximately 59.7% and 89.5% of farms were willing to pay for *Brucella* vaccine for all cattle and newborn calves, respectively. For vaccinating all cattle option, the Maasai significantly refused to pay for vaccination compared with other tribes ($P = 0.02$, Table 11), and the number of cattle of accepted farmers was significantly lower than that of refused farmers ($P < 0.01$, Table 12). In addition, education and knowledge scores were significantly higher in the group that accepted vaccination than the group that refused ($P = 0.02$ and 0.01, respectively, Table 6). Out of these factors, tribe was the single significant factor in the multivariable analysis for all cattle vaccination (Maasai: OR = 0.39, 95%CI: 0.19–0.83, $P = 0.01$). For vaccinating calves option, a significant factor in the univariable analysis was using a veterinary service ($P = 0.03$, Table 11), which was also the single significant factor in the multivariable analysis (OR = 4.0, 95%CI: 1.2–13.0, $P = 0.02$). Proportions of the acceptance to pay for *Brucella* vaccine among study farms were 59.7% for all cattle vaccination option, and 89.5% for new-born calves option, respectively. For vaccinating all cattle option, Maasai significantly refused to pay for vaccination when compared with the other tribes ($P = 0.02$, Table 11), and the number of cattle of accepted farmers was significantly lower than that of refused farmers ($P < 0.01$, Table 12), and education and knowledge scores were significantly higher in the group that accepted vaccination than that refused ($P = 0.02$ and 0.01, respectively, Table 12). Out of these factors, tribe was the single significant factor in the multivariable analysis for all cattle vaccination (Maasai: OR = 0.39, 95%CI: 0.19–0.83, $P = 0.01$). For vaccinating calves option, a significant factor in the univariable analysis was using a veterinary service ($P = 0.03$, Table 11), which was also a single significant factor in the multivariable analysis (OR = 4.0, 95%CI: 1.2–13.0, $P = 0.02$).

Table 11. Factors associated with willingness-to-pay for *Brucella* vaccination on binary response

		Vaccinate all cattle				Vaccinate calves			
Factors	Response	Accept	Not accept	Accept (%)	*P* value	Accept	Not accept	Accept (%)	*P* value
Abortion in	Yes	39	29	57.4	0.69	60	8	88.2	0.83
Cattle	No	35	21	62.5		51	5	91.1	
Using a veterinary	Yes	53	31	63.1	0.35	79	5	94.0	0.03
service	No	21	19	52.5		32	8	80.0	
Tribe of the	Maasai	29	31	48.3	0.02	52	8	86.7	0.48
owner	Others	45	19	70.3		59	5	92.2	

Table 12. Factors associated with willingness-to-pay for *Brucella* vaccination on count and score data

	Vaccinate all cattle			Vaccinate calves		
Factors	Accept (2.5 – 97.5th)	Not accept (2.5 – 97.5th)	*P* value	Accept (2.5 – 97.5th)	Not accept (2.5 – 97.5th)	*P* value
Number of cattle	30 (3–203)	50 (12–234)	<0.01	45 (4–218)	30 (12–229)	0.54
Age of owner	45 (21–70)	42 (20–76)	0.39	43 (21–75)	50 (28–69)	0.43
Education score: 0–4	1 (0–3.2)	0 (0–3.6)	0.02	1 (0–3)	0 (0–3)	0.13
Knowledge score: 0–4	0 (0–2.4)	0 (0–0.8)	0.01	0 (0–2.5)	0 (0–0.7)	0.49

3.3.5 Behaviour of selling cows with an experience of abortion

Based on the ICT, 45.1% (SE = 7.4%) of farmers sold cattle with a history of abortion to the cattle market. This estimate was not statistically different from that obtained by direct questioning (34.1%, SE = 7.5%, binomial *P* value = 0.27, factor score = 1.32). Thus, farmers sold out cattle with a history of abortion without hesitation.

3.3.6 Human risks against brucellosis

Table 13 shows the risky behaviours for brucellosis infection in humans for binary data. No significant association was observed between bovine brucellosis status and risky behaviours. However, the Maasai significantly conducted risky behaviours compared with other tribes (drinking raw milk: $P = 0.06$, drinking blood: $P < 0.01$, using gloves to help delivery: $P = 0.03$, Table 14).

Table 13. Proportions of infected and non-infected herd households conducting risky behaviour for human infections with *Brucella*

Factors	Response	Infected herds	Healthy herds	Percentage	*P* value
Drinking raw milk	Yes	33	38	46.5	0.71
	No	22	31	41.5	
Drinking blood	Yes	18	20	47.4	0.80
	No	37	49	43.0	
Using gloves to help delivery	Yes	5	8	38.5	0.88
	No	50	61	45.0	

Table 14. Proportions of Maasai and the other tribe households conducting risky behaviour for human infections with *Brucella*

Factors	Maasai (*n* = 60)	Percentage	Other tribes (*n* = 64)	Percentage	*P* value
Drinking raw milk	40	66.7	31	48.4	0.06
Drinking blood	38	63.3	0	0	<0.01
Using gloves to help delivery	2	3.3	11	17.2	0.03

3.4. Discussion

This study was performed from diversified perspectives towards brucellosis control focusing on agro-pastoral areas in Tanzania. In this study, the result of bovine brucellosis prevalence revealed that the disease is endemic in the study area, as suggested in Chapter 2 and other study conducted in the same district [9, 118].

Risk factor analysis indicated using a veterinary service as a preventive factor of bovine brucellosis. Other studies mentioned that the lower prevalence of bovine brucellosis in herds under the supervision of a veterinarian is likely due to improved monitoring and preventive health measures for the disease such as proper disposal of aborted materials and hygienic procedures [4, 32, 65]. It is well known that delivering adequate animal health services contributes to a low incidence of diseases. Veterinary extension is considered to play a key role in zoonosis mitigation through education of sanitary procedures and rearing measures for livestock farmers, raising awareness of the diseases.

Vaccination plays a major role in brucellosis control in endemic areas. *B. abortus* vaccines have been successfully used worldwide for bovine brucellosis control for decades. S19 and RB51 have been most commonly used for cattle. Safety duration of immunity induced by S19 in calves has proven to be quite long, reaching almost the entire productive lifespan of the animal [37]. In addition, the efficacies of calfhood vaccination of S19 and RB51 are regarded to be similar, although S19 is considered to be slightly more effective than RB51 in experimental conditions [102, 129]. S19 induces antibody responses that cannot be distinguished from the antibody response by natural infection with field strains, thereby making serological diagnostic tests invalid [103], while RB51 lacks the expression of the O-side chains in lipopolysaccharide, overcoming the serologic problems observed after S19 vaccination. Although S19 had been widely used previously, currently RB51 is used in many countries including Sub-Saharan African countries instead of S19 [13].

National brucellosis control programmes involving vaccination have been performed in many countries [43, 85, 86]. However, to our knowledge, there were no reports about a community-based *Brucella* vaccination strategy in which farmers pay the cost themselves. Serological testing with slaughter is also the main method for disease control. However, it is not always cost-effective in cattle [43] and compensation for slaughtered cattle is difficult for developing countries where financial resources are scarce. Considering the limited resources and endemic situation of brucellosis in Tanzania [11, 58, 116, 124], we studied willingness to pay for *Brucella* vaccination by farmers themselves as a community-based intervention.

In general, brucellosis mass vaccination is targeted at the entire cattle population of the target area. However, vaccination of adult cows causes infertility and shedding of *Brucella* bacteria in milk [24, 68]. Thus, this study investigated the cattle farmers' willingness to pay for vaccination in two scenarios: all cattle vaccination and calf vaccination. The results clarified that around 90% of farmers would be willing to pay for vaccination for calves, while around 60% of them agreed to pay for all cattle, indicating the feasibility of a calf vaccination programme. In addition, although the question of willingness to pay for calf vaccination assumed to include both sexes, vaccination is performed only for female calves when implemented, as vaccination in male calves results in testicular infection and infertility [37]. Thus, the acceptance rate of a vaccination programme targeting only female calves is expected to be even higher than that observed in our study. Similar to the analysis of preventive factors of bovine brucellosis, the analysis of factors associated with willingness to pay for vaccination showed that veterinarians were motivators for farmers to accept calf vaccination. A previous report also showed that advice from veterinarians was a promoting factor for farmers to have an intention towards zoonotic disease control [41]. For the all cattle vaccination option, being a Maasai tribe member was a hesitating factor. This may be due to their traditional culture, which is very different from modern culture. Although most farmers

including the Maasai agreed to calf vaccination, calf vaccination may have been temporarily accepted by the Maasai because a very limited number of cattle are supposed to be vaccinated initially. Since brucellosis and *Brucella* vaccine were new concepts for most of the farmers at the interview, the Maasai may be sceptical about using the vaccine.

This study investigated human behaviour of selling aborted cows using the ICT. The ICT found that around half of the study farms sold out cows that experienced abortion without hesitation. The farmers in this study sell their cattle at the Mkongeni cattle market, where most adult cattle traded are brought to Dar es Salaam for slaughter and meat consumption (personal communication with district livestock officers). Selling out infected cattle will decrease the brucellosis within-herd prevalence. However, farmer-to-farmer trades of cattle were also observed in the market, and selling out cows that experienced abortion at the market may contribute to the spread of brucellosis to other farms. Although abortion in cattle can occur for several different reasons, abortion is strongly associated with bovine brucellosis in endemic areas as reported in Chapter 2 and other studies [9, 78, 88, 92]. Therefore, suggesting farmers to sell out cows that experienced abortion for slaughter, not for raising in other farms, and admitting meat consumption of slaughtered cattle might be practical control methods of brucellosis in developing countries, as long as farmers agree to participate.

Our study found poor knowledge about brucellosis among farmers in the study area, whereas other studies conducted in similar settings reported that agro-pastoralism was associated with high knowledge of zoonoses such as brucellosis [64] and pulmonary tuberculosis [48, 72]. The farmers in the study area had few opportunities to learn about the disease (personal communication), thus promotion of health education is required.

In terms of the risk of human infection, the Maasai tended to conduct risky behaviours. This is due to their cultural background and traditional habits; thus, it may be difficult to change these customs. The Maasai had previously regarded education as less important and

the education level of Maasai was significantly lower than that of other tribes ($P < 0.01$). However, the Tanzania government is currently providing a premium on education and the number of Maasai children who go to school has increased in the study area (personal communication with Maasai participants). For this reason, the probability of success in changing risky behaviours against human infection by health education programmes may increase.

Among animal brucellosis, we studied only bovine brucellosis. Although research focusing on human and small ruminants has been conducted in Tanzania [11, 60, 68, 115], the information is still limited. Moreover, isolation and identification of *Brucella* species have not been performed in more than five decades in Tanzania [11, 51]. Recently, the Tanzania government has selected brucellosis as one of the prioritised zoonotic diseases for the country, confirming the plan to conduct One Health surveillance for both humans and animals for rapid and effective response to improve current public health situations (National Workshop on Prioritization of Zoonotic diseases, 2017). By using the knowledge generated in this study, supplemented by such current research opportunities, a feasible plan for community-based control of brucellosis may become available in Tanzania in the near future. Such protocol may be of great need in other developing countries where resources are limited.

3.5. Summary of Chapter 3

This study showed that bovine brucellosis is endemic in agro-pastoral areas in Morogoro Region, Tanzania. Veterinary service was a preventive factor of bovine brucellosis, suggesting that regular preventive health measures may reduce the prevalence. Surveyed cattle farmers were willing to pay for brucellosis vaccination, particularly by limiting calves to be vaccinated, indicating the feasibility of community-based calf vaccination programmes. Receiving education from veterinarians was again critical towards accepting vaccination for calves.

Farmers were selling cows that experienced abortion without hesitation, which may have contributed to the maintenance of the disease; however, this practice likely also suppressed the within-herd prevalence. Lastly, knowledge about brucellosis was poor among surveyed farmers, and the Maasai conducted risky behaviours for human infection. Taken together, this study showed that a One Health approach for joint planning and actions of community-based brucellosis intervention, including health education, is feasible in Tanzania.

Chapter 4 General discussion

This study was performed from diversified perspectives towards brucellosis control in Morogoro region, Tanzania. Chapter 2 evaluated prevalence and risk factors of bovine brucellosis, and behaviours associated with human brucellosis between urban and agro-pastoral areas. In terms of knowledge on brucellosis, both urban and agro-pastoral farmers were poor on it and there had been few or no opportunity to learn about brucellosis correctly for the study farmers. It was clarified that bovine brucellosis prevalence was quite low in urban areas but the disease was endemic in agro-pastoral areas in the region, suggesting strict confinement is the effective control options for bovine brucellosis since grazing was a risk factor of brucellosis. In addition, bought-in cattle and abortion were suspected to be associated factors with brucellosis. A hypothesis arisen from this is that farmers tend to sell cows that have a history of abortion to livestock markets, which may contribute to the spread of brucellosis in the areas. Further research had been needed to understand human behaviours associated with cattle sales. In addition, the number of farmers studied in agro-pastoral areas where the disease was endemic was only 17 which was small. For these reasons, the study conducted in chapter 3 focused agro-pastoralists who still dominate in Tanzania. Furthermore, willingness-to-pay for *Brucella* vaccination by farmers themselves was investigated to explore the possibility of community-based intervention.

The study in Chapter 3 confirmed the endemic situation of bovine brucellosis in the agro-pastoral areas. Veterinary service was a preventive factor of bovine brucellosis, being likely due to the better monitoring and preventive health measures for the disease [33−35]. The results of willingness-to-pay for vaccination showed that around 90% of the farmers accepted to pay for vaccination for calves, while around 60% of them agreed to pay for all cattle, indicating the feasibility of calf vaccination programme. Similar to the preventive factor for bovine brucellosis, the analysis of the factors associated with willingness-to-pay for the vaccination showed that veterinarians were motivators for farmers to accept vaccination for calves. Thus veterinary

extension is considered to play a key role for zoonosis mitigation through education of the sanitary procedures and rearing measures for livestock farmers and the recommendation of the vaccination to the farmers where it is needed.

Maasai were found to conduct risky behaviours against human infection and their education level were significantly lower than other tribes' one. Those behaviours may associate with their traditional culture, but health education may change the practices.

This study investigated human behaviour of selling aborted cows using the ICT. The ICT found that around half of the study farms sold out cows that caused abortion, and farmers were selling cows experienced abortion without hesitation, which may have contributed to the maintenance of the disease but at the same time suppressed within-herd prevalence.

The following recommendations were made for the brucellosis control both in animals and humans:

i) Brucellosis research in small ruminants

The magnitude of brucellosis in small ruminants is not known well not only in the study areas but also in other areas in Tanzania. Cross-species transmission of *Brucella* infection may occur and since *B. melitensis* causes heavy symptoms to humans, further investigations in small ruminants including identification of the *Brucella* species and its biotypes are recommended to understand the epidemiology more.

ii) Brucellosis research in humans

From the results of bovine brucellosis prevalence and human practices of drinking cattle blood and raw milk, agro-pastoral farmers especially Maasai were at the risk of human brucellosis. It was reported that the human brucellosis prevalence in Mvomero district was 36.1% [56]. However, human brucellosis information is still limited in Tanzania. To estimate the real burden

of brucellosis, further research on human brucellosis is necessary.

iii) Public health education

Effective education is necessary for the disease control programme. For this, collaboration among veterinary departments, medical departments and local authorities is vital, especially in agro-pastoral areas where bovine brucellosis is endemic and many farmers conduct risky behaviours of human infection and lack the knowledge on brucellosis. To organize short seminar at village with the explanation of animals associated with brucellosis, clinical signs both in animals and humans, transmission routes, preventive ways of infection may be useful.

iv) Expansion of veterinary service

In this study veterinary service was found as a preventive factor of bovine brucellosis and promoting factor to accept calf vaccination. Veterinary extension is considered to play a key role for zoonosis mitigation through education of the sanitary procedures and rearing measures for livestock farmers with raising awareness of the diseases.

v) Infectious disease modelling

Quantification of transmission dynamics using mathematical modelling would provide clearer ideas in the disease control. Such approach also useful to select the prioritized group or areas of vaccination.

vi) Economic evaluation

The disability caused by human brucellosis with expenses incurred during seeking medical services are huge [69]. The economic benefit and cost-effectiveness of mass vaccination programme including human brucellosis was estimated in Mongolia [110]. This kind of

research may be effective to make decision for disease control at any levels such as farm, district or nation.

vii) Community-based control

Even if the intervention from the government towards the disease is not expected, some action should be taken to reduce the disease. The feasible plan for the disease control should be made considering the limited resources and endemic situation in Tanzania. This research showed the acceptance of farmers to pay for *Brucella* vaccine for calves and thus feasibility of community-based intervention. This protocol may be also helpful in other areas where resources are limited and national intervention is not expected.

viii) Intensification of cattle farming as a disease control

Considering the information obtained in this research, different strategies are considered for the disease control between urban and agro-pastoral areas in the study areas. In urban areas, strict confinement, which is normal in zero-grazing system, is important to keep low prevalence or to eradicate the disease. Moreover, it is ideal to avoid buying cattle and mixing them to other herd if possible. The use of *Brucella* vaccine is not necessary considering the quite low prevalence. On the other hand, in agro-pastoral areas, vaccination will play an important role for the disease control because of the endemic situation.

ix) One Health approach

One Health approach, which involves engagement with different level of stakeholders including communities, is necessary in controlling this long lasting zoonosis in endemic countries [131]. Brucellosis was selected as one of the prioritized important zoonotic diseases in Tanzania by the government with the plan to conduct One Health surveillance for both humans and animals

(National workshop on Prioritization of Zoonotic diseases, 2017). The surveillance and other further actions are expected to contribute to the disease control.

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REFERENCES

1. Abdussalam, M. and Fein, D.A. 1976. Brucellosis as a world problem, ed., Karger, Basel.
2. Adesokan, H. K., Alabi, P. I. and Ogundipe, M. A. 2016. Prevalence and predictors of risk factors for Brucellosis transmission by meat handlers and traditional healers' risk practices in Ibadan, Nigeria. *J. Prev. Med. Hyg.* **57**: E164−e171.
3. Adesokan, H. K., Alabi, P. I., Stack, J. A. and Cadmus, S. I. 2013. Knowledge and practices related to bovine brucellosis transmission amongst livestock workers in Yewa, south-western Nigeria. *J. S. Afr. Vet. Assoc.* **84**: E1−5.
4. Al-Majali, A. M., Talafha, A. Q., Ababneh, M. M. and Ababneh, M. M. 2009. Seroprevalence and risk factors for bovine brucellosis in Jordan. *J. Vet. Sci.* **10**: 61−65.
5. Alausa, O. and Awoseyi, A. 1976. Brucellosis: the situation in Western Nigeria. *Trop. Geogr. Med.* **28**: 54−59.
6. Alvarez, J., Saez, J. L., Garcia, N., Serrat, C., Perez-Sancho, M., Gonzalez, S., Ortega, M. J., Gou, J., Carbajo, L., Garrido, F., Goyache, J. and Dominguez, L. 2011. Management of an outbreak of brucellosis due to *B. melitensis* in dairy cattle in Spain. *Res. Vet. Sci.* **90**: 208−211.
7. Anon. 1935. Tanganyika Annual Report. Department of Veterinary Services.
8. Anon. 1962. Monthly Reports. Department of Medical Services. Lake and West Provinces.
9. Asakura, S., Makingi, G., Kazwala, R. and Makita, K. 2017. Brucellosis risk in urban and agro-pastoral areas in Tanzania. *Ecohealth*
10. Asmare, K., Regassa, F., Robertson, L. J., Martin, A. D. and Skjerve, E. 2013. Reproductive disorders in relation to *Neospora caninum*, *Brucella* spp. and bovine

viral diarrhoea virus serostatus in breeding and dairy farms of central and southern Ethiopia. *Epidemiol. Infect.* **141**: 1772−1780.

11. Assenga, J. A., Matemba, L. E., Muller, S. K., Malakalinga, J. J. and Kazwala, R. R. 2015. Epidemiology of *Brucella* infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. *BMC. Vet. Res.* **11**: 189.
12. Australia Animal Health. 2005. Disease Strategy: Bovine Brucellosis (version 3.0) Australian Veterinary Emergency Plan (AUSVETPLAN), 3rd Edition, Primary Industries Ministerial Council, Canberra.
13. Avila-Calderon, E. D., Lopez-Merino, A., Sriranganathan, N., Boyle, S. M. and Contreras-Rodriguez, A. 2013. A history of the development of *Brucella* vaccines. *Biomed. Res. Int.* **2013**: 743509.
14. Baba, M. M., Sarkindared, S. E. and Brisibe, F. 2001. Serological evidence of brucellosis among predisposed patients with pyrexia of unknown origin in the north eastern Nigeria. *Cent. Eur. J. Public Health* **9**: 158−161.
15. Banai, M. and Corbel, M. 2010. Taxonomy of *Brucella*. *Open. Vet. Sci. J.* **4**: 85−101.
16. Benjamin, B. and Annobil, S. H. 1992. Childhood brucellosis in southwestern Saudi Arabia: a 5-year experience. *J. Trop. Pediatr.* **38**: 167−172.
17. Berhe, G., Belihu, K. and Asfaw, Y. 2007. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *Int. J. Appl. Res. Vet. M.* **5**: 65−71.
18. Berkelman, R. L. 2003. Human illness associated with use of veterinary vaccines. *Clin. Infect. Dis.* **37**: 407−414.
19. Bernard, F., Vincent, C., Matthieu, L., David, R. and James, D. 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Prev. Vet. Med.* **67**: 267−281.

20. Bishop, G. C., Bosman, P. P. and Herr, S. 1994. Bovine Brucellosis: *In*: Infectious Diseases of Livestock with Special Reference to Southern Africa, Oxford University Press, UK.

21. Bricker, B. J. and Halling, S. M. 1995. Enhancement of the *Brucella* AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. *J Clin. Microbiol.* **33**: 1640−1642.

22. Busch, L. A. and Parker, R. L. 1972. Brucellosis in the United States. *J. Infect. Dis.* **125**: 289−294.

23. Campbell, B. M. and Luckert, M. K. 2002. Uncovering the Hidden Harvest: Valuation Methods for Woodland and Forest Resources, Earthscan, UK.

24. Chand, P., Chhabra, R. and Nagra, J. 2015. Vaccination of adult animals with a reduced dose of *Brucella abortus* S19 vaccine to control brucellosis on dairy farms in endemic areas of India. *Trop. Anim. Health Prod.* **47**: 29−35.

25. Fraser, C. M. 1991. The Merck Veterinary Manual, Rahway, NJ, Merck.

26. Corbel, M. J. 1988. Brucellosis. In: Fertility and Infertility in Veterinary Practice, Bailliere Tindall, London.

27. Corbel, M. J. 1997. Brucellosis: an overview. *Emerg. Infect. Dis.* **3**: 213−221.

28. Corbel, M. J., Elberg S. S. and Cosivi O. 2006. Brucellosis in humans and animals. World Health Organization, Geneva.

29. Dalton, D. R. and Wimbush, J. C. 1994. Using the unmatched count technique (UCT) to estimate base rates for sensitive behaviour. *Pers. Psychol.* **47**: 817–829.

30. David, L. H. and Arthur, M. F. 1998. Brucellosis. *In*: Medical Aspects of Chemical and Biological Warfare, Office of the Surgeon General, Dept of the Army, USA.

31. Davis, D. S., Templeton, J. W., Ficht, T. A., Williams, J. D., Kopec, J. D. and Adams, L. G. 1990. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis,

and transmission to cattle. *J. Wildl. Dis.* **26**: 360−371.

32. de Alencar Mota, A. L. A., Ferreira, F., Ferreira Neto, J. S., Dias, R. A., Amaku, M., Hildebrand Grisi-Filho, J. H., Telles, E. O. and Picao Goncalves, V. S. 2016. Large-scale study of herd-level risk factors for bovine brucellosis in Brazil. *Acta Trop.* **164**: 226−232.

33. Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. and Courbious, C. 2001. Livestock to 2020: the next food evolution. *Outlook Agr.* **30**: 27−29.

34. Deqiu, S., Donglou, X. and Jiming, Y. 2002. Epidemiology and control of brucellosis in China. *Vet. Microbiol.* **90**: 165−182.

35. Diaz Aparicio, E. 2013. Epidemiology of brucellosis in domestic animals caused by *Brucella* melitensis, *Brucella* suis and *Brucella abortus*. *Rev. Sci. Tech.* **32**: 43−51, 53−60.

36. Dohoo, I. R., Martin, W. and Stryhn, H. E. 2003. Veterinary Epidemiologic Research. Charlottetown, P.E.I. University of Prince Edward Island.

37. Dorneles, E. M., Sriranganathan, N. and Lage, A. P. 2015. Recent advances in *Brucella abortus* vaccines. *Vet. Res.* **46**: 76.

38. Droitcour, J., Caspar, R. A., Hubbard, M. L., Parsley, T.L., Visscher, W. and Ezzati, T. M. 2004. The item count technique as a method of indirect questioning: A review of its development and a case study application. *In*: P. Biemer, R. Groves, L. Lyberg, N. Mathiowetz, S. Sudman editors. Measurement errors in surveys. New York, Wiley.

39. Ducrotoy, M. J., Bertu, W. J., Ocholi, R. A., Gusi, A. M., Bryssinckx, W., Welburn, S. and Moriyon, I. 2014. Brucellosis as an emerging threat in developing economies: lessons from Nigeria. *PLoS Negl. Trop. Dis.* **8**: e3008.

40. Ducrotoy, M. J., Ammary, K., Ait Lbacha, H., Zouagui, Z., Mick, V., Prevost, L., Bryssinckx, W., Welburn, S. C. and Benkirane, A. 2015. Narrative overview of animal

and human brucellosis in Morocco: intensification of livestock production as a driver for emergence? *Infect. Dis. Poverty* **4**: 57.

41. Ellis-Iversen, J., Cook, A. J., Watson, E., Nielen, M., Larkin, L., Wooldridge, M. and Hogeveen, H. 2010. Perceptions, circumstances and motivators that influence implementation of zoonotic control programs on cattle farms. *Prev. Vet. Med.* **93**: 276−285.
42. FAO. FAOSTAT: the statistical database of FAO, Annual population, ed.
43. FAO. 2015. Regional workshop on brucellosis control in Central Asia and Eastern Europe. *FAO Animal Production and Health Report* **8**.
44. Fayomi, B., Laudat, P., Audurier, A. and Zohoum, I. 1987. Human brucellosis in Benin: results of a serological survey among exposed workers. *Med. Trop. (Mars).* **47**: 145−148.
45. Ficht, T. 2010. *Brucella* taxonomy and evolution. *Future Microbiol.* **5**: 859−866.
46. Galinska, E. M. and Zagorski, J. 2013. Brucellosis in humans-etiology, diagnostics, clinical forms. *Ann. Agric. Environ. Med.* **20**: 233−238.
47. Gall, D. and Nielsen, K. 2004. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Rev. Sci. Tech.* **23**: 989−1002.
48. Gele, A. A., Bjune, G. and Abebe, F. 2009. Pastoralism and delay in diagnosis of TB in Ethiopia. *BMC Public Health* **9**: 5.
49. Godfroid, J., Garin-Bastuji, B., Saegerman, C. and Blasco, J. M. 2013. Brucellosis in terrestrial wildlife. *Rev. Sci. Tech.* **32**: 27−42.
50. Godfroid, J., Cloeckaert, A., Liautard, J. P., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B. and Letesson, J. J. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet. Res.* **36**: 313−326.

51. Godfroid, J., Al Dahouk, S., Pappas, G., Roth, F., Matope, G., Muma, J., Marcotty, T., Pfeiffer, D. and Skjerve, E. 2013. A "One Health" surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comp. Immunol. Microbiol. Infect. Dis.* **36**: 241−248.

52. Goodwin, Z. I. and Pascual, D. W. 2016. Brucellosis vaccines for livestock. *Vet. Immunol. Immunopathol.* **181**: 51−58.

53. Halekoh, U., Højsgaard, S. and Yan, J. 2006. The R package geepack for generalized estimating equations. *J. Stat. Softw.* **15**: 1−11.

54. Hasanjani Roushan, M. R., Mohraz, M., Hajiahmadi, M., Ramzani, A. and Valayati, A. A. 2006. Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. *Clin. Infect. Dis.* **42**: 1075−1080.

55. Jaber, L., Dahan, S. and Harari, I. 1999. Control of brucellosis in Taibe: multi-central collaboration. *Harefuah* **137**: 454−456, 511, 510.

56. James, L. W. 2013. Studies of human brucellosis in Mikumi–Selous ecosystem, Morogoro, Tanzania. M.Sc. dissertation. Sokoine University of Agriculture, Morogoro, Tanzania.

57. Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H. and Kindahl, H. 2009. Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Rev. Sci. Tech.* **28**: 933−943.

58. Jiwa, S. F. H., Kazwala, R.R., Tungaraza, R., Kimera, S. I. and Kalaye, W.J. 1996. Bovine brucellosis serum agglutination test prevalence and breed disposition according to prevalent management systems in the Lake Victoria zone of Tanzania. *Prev. Vet. Med.* **26**: 341–346.

59. John, K., Kazwala, R. and Mfinanga, G. S. 2008. Knowledge of causes, clinical

features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC Infect. Dis.* **8**: 162.

60. John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G. S., MacMillan, A. and Cleaveland, S. 2010. Quantifying risk factors for human brucellosis in rural northern Tanzania. *PloS One* **5**: e9968.

61. Joklik, W. K., Willet, H.P. and Amos, D.B. 1980. Zinsser Microbiology 17th edition, Appleton-Century-Crofts, New York.

62. Kadohira, M., McDermott, J. J., Shoukri, M. M. and Kyule, M. N. 1997. Variations in the prevalence of antibody to *brucella* infection in cattle by farm, area and district in Kenya. *Epidemiol. Infect.* **118**: 35−41.

63. Kahler, S. C. 2000. *Brucella* melitensis infection discovered in cattle for first time, goats also infected. *J. Am. Vet. Med. Assoc.* **216**: 648.

64. Kansiime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I. B., Sempa, J., Kiwanuka, S. N., Asiimwe, B. B. and Rutebemberwa, E. 2014. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. *BMC Public Health* **14**: 242.

65. Kaoud HA, Z. M., Shimaa ARD, Nasr A. 2010. Epidemiology of brucellosis among farm animals. *J. Nat. Sci.* **8**.

66. Karimuribo, E. D., Ngowi, H. A., Swai, E. S. and Kambarage, D. M. 2007. Prevalence of brucellosis in crossbred and indigenous cattle in Tanzania. *Livestock Res. Rural Dev.* **19**.

67. Kubuafor, D. K., Awumbila, B. and Akanmori, B. D. 2000. Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: public health implications. *Acta Trop.* **76**: 45−48.

68. Kunda, J., Fitzpatrick, J., Kazwala, R., French, N. P., Shirima, G., Macmillan, A.,

Kambarage, D., Bronsvoort, M. and Cleaveland, S. 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health* **7**: 315.

69. Kunda, J., Cleaveland, S., Fitzpatrick, J., French, N., Shirima, G., Kazwala, R. and Kambarage, D. 2004. Diagnostic and therapeutic implications of human brucellosis in Arusha and Arusha and Manyara regions, Tanzania. *Tanzan. Health Res. Bull.* **6**: 1−4.

70. Laroche, R., Petat, E. and Hajayandi, P. C. 1987. Human brucellosis in Burundi. Serologic survey in Rusizi Plain. *Med. Trop. (Mars)* **47**: 35−38.

71. LaBrie, J. W. and Earleywine, M. 2000. Sexual risk behaviors and alcohol: higher base rates revealed using the unmatched-count technique. *J. Sex. Res.* **37**: 321−326.

72. Legesse, M., Ameni, G., Mamo, G., Medhin, G., Shawel, D., Bjune, G. and Abebe, F. 2010. Knowledge and perception of pulmonary tuberculosis in pastoral communities in the middle and Lower Awash Valley of Afar region, Ethiopia. *BMC Public Health* **10**: 187.

73. Lopez-Merino, A. 1989. Brucellosis in Latin America, CRC Press, Boca Raton Florida.

74. Lyimo, B. E. 2013. Prevalence of bovine brucellosis in smallholder dairy farms in Morogoro Municipality, Tanzania. M.Sc. dissertation. Sokoine University of Agriculture, Morogoro, Tanzania.

75. Madsen M, A. E. 1995. Serologic survey of Zimbabwean wildlife for brucellosis. *J. Zoo. Wildl. Med.* **26**: 240−245.

76. Magona, J. W., Walubengo, J., Galiwango, T. and Etoori, A. 2009. Seroprevalence and potential risk of bovine brucellosis in zerograzing and pastoral dairy systems in Uganda. *Trop. Anim. Health. Prod.* **41**: 1765−1771.

77. Makita, K., Fevre, E. M., Waiswa, C., Eisler, M. C. and Welburn, S. C. 2010. How human brucellosis incidence in urban Kampala can be reduced most efficiently? A

stochastic risk assessment of informally-marketed milk. *PloS One* **5**: e14188.

78. Makita, K., Fevre, E. M., Waiswa, C., Eisler, M. C., Thrusfield, M. and Welburn, S. C. 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC veterinary research* **7**: 60.

79. Makita, K., Fevre, E. M., Waiswa, C., Kaboyo, W., Eisler, M. C. and Welburn, S. C. 2011. Evidence-based identification of the most important livestock related zoonotic diseases in Kampala, Uganda. *J. Vet. Med. Sci.* **73**: 991−1000.

80. Marcotty, T., Matthys, F., Godfroid, J., Rigouts, L., Ameni, G., Gey van Pittius, N., Kazwala, R., Muma, J., van Helden, P., Walravens, K., de Klerk, L. M., Geoghegan, C., Mbotha, D., Otte, M., Amenu, K., Abu Samra, N., Botha, C., Ekron, M., Jenkins, A., Jori, F., Kriek, N., McCrindle, C., Michel, A., Morar, D., Roger, F., Thys, E. and van den Bossche, P. 2009. Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Ann. Trop. Med. Parasitol.* **103**: 401−411.

81. Marta Pérez-Sancho, T. G.-S., Lucas Domínguez, Julio Álvarez. 2015. Control of animal brucellosis—the most effective tool to prevent human brucellosis, updates on brucellosis. Manal Mohammad Baddour (Ed.), In Tech.

82. Matope, G. 2009. Epizootological studies and diagnostic approaches towards cattle brucellosis in the smallholder dairy sector of Zimbabwe, ed., University of Zimbabwe.

83. Matope, G., Bhebhe, E., Muma, J. B., Oloya, J., Madekurozwa, R. L., Lund, A. and Skjerve, E. 2011. Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. *Trop. Anim. Health Prod.* **43**: 975−982.

84. Mayoral, J. W. 1992. Production performance under rotational (strip) grazing system, ed., Alabama University, USA.

85. McDermott, J., Grace, D. and Zinsstag, J. 2013. Economics of brucellosis impact and control in low-income countries. *Rev. Sci. Tech.* **32**: 249−261.

86. McDermott, J. J. and Arimi, S. M. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet. Microbiol.* **90**: 111−134.

87. Mdegela, R. H., Kusiluka, L. J., Kapaga, A. M., Karimuribo, E. D., Turuka, F. M., Bundala, A., Kivaria, F., Kabula, B., Manjurano, A., Loken, T. and Kambarage, D. M. 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in Eastern Tanzania. *J. Vet. Med. B. Infect. Dis. Vet. Public Health.* **51**: 123−128.

88. Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J. and Skjerve, E. 2011. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Trop. Anim. Health Prod.* **43**: 651−656.

89. Minas, M., Minas, A., Gourgulianis, K. and Stournara, A. 2007. Epidemiological and clinical aspects of human brucellosis in Central Greece. *Jpn. J. Infect. Dis.* **60**: 362−366.

90. Mkoma, S. L. and Mjemah, I C. 2011. Influence of meteorology on ambient air quality in Morogoro, Tanzania. *Int. J. Environ. Sci.* **1**: 1107−1115.

91. Movek, D. S. 2008. Small farmer productivity through increased access to draught power opportunities. Consultancy report stakeholder mapping in Morogoro region.

92. Muma, J. B., Samui, K. L., Siamudaala, V. M., Oloya, J., Matop, G., Omer, M. K., Munyeme, M., Mubita, C. and Skjerve, E. 2006. Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. *Trop. Anim. Health Prod.* **38**: 195−206.

93. Ndyabahinduka, D. G. and Chu, I. H. 1984. Brucellosis in Uganda. *Int. J. Zoonoses*

11: 59−64.

94. Nielsen, K. 2002. Diagnosis of brucellosis by serology. *Veterinary microbiology* **90**: 447−459.

95. Nielsen, K., Smith, P., Widdison, J., Gall, D., Kelly, L., Kelly, W. and Nicoletti, P. 2004. Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7. *Vet. Microbiol.* **100**: 25−30.

96. Nielsen, K., Gall, D., Jolley, M., Leishman, G., Balsevicius, S., Smith, P., Nicoletti, P. and Thomas, F. 1996. A homogeneous fluorescence polarization assay for detection of antibody to *Brucella abortus*. *J. Immunol. Methods.* **195**: 161−168.

97. Nielsen, K. and Duncan, J. R. 1990. Animal Brucellosis, CRC Press, Boca Raton.

98. Nimri, L. F. 2003. Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. *BMC Infect. Dis.* **3**: 5.

99. OIE. 2009. Bovine Brucellosis. *In*: World Assembly of Delegates of the OIE Chapter 2.4.3. *Rev. Sci. Tech.*

100. OIE. 2017. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017 part2 Section 2.1. Chapter 2.1.4.

101. Olle-Goig, J. E. and Canela-Soler, J. 1987. An outbreak of *Brucella melitensis* infection by airborne transmission among laboratory workers. *Am. J. Public. Health.* **77**: 335−338.

102. Olsen, S. C. 2000. Immune responses and efficacy after administration of a commercial *Brucella abortus* strain RB51 vaccine to cattle. *Vet. Ther.* **1**: 183−191.

103. Olsen, S. C. and Stoffregen, W. S. 2005. Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert Rev. Vaccines* **4**: 915−928.

104. Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E. V. 2006. The new global map of human brucellosis. *Lancet Infect. Dis.* **6**: 91−99.

105. Portanti, O., Tittarelli, M., Di Febo, T., Luciani, M., Mercante, M. T., Conte, A. and Lelli, R. 2006. Development and validation of a competitive ELISA kit for the serological diagnosis of ovine, caprine and bovine brucellosis. *J. Vet. Med. B. Infect. Dis. Vet. Public Health* **53**: 494−498.

106. Radostits, O. M., Gay, C. C., Blood, C. D. and Hinchcliff, K. W. 2000. Veterinary Medicine, Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. (9th Edition), WB Saunders Company Ltd, New York.

107. Randrianantoandro, T. N., Kono, H. and Kubota, S. 2015. Knowledge and behavior in an animal disease outbreak - Evidence from the item count technique in a case of African swine fever in Madagascar. *Prev. Vet. Med.* **118**: 483−487.

108. Refai, M. 2002. Incidence and control of brucellosis in the Near East region. *Vet. Microbiol.* **90**: 81−110.

109. Romero, C., Gamazo, C., Pardo, M. and Lopez-Goni, I. 1995. Specific detection of *Brucella* DNA by PCR. *J. Clin. Microbiol.* **33**: 615−617.

110. Roth, F., Zinsstag, J., Orkhon, D., Chimed-Ochir, G., Hutton, G., Cosivi, O., Carrin, G. and Otte, J. 2003. Human health benefits from livestock vaccination for brucellosis: case study. *Bull. World Health Organ.* **81**: 867−876.

111. Samaha, H., Al-Rowaily, M., Khoudair, R. M. and Ashour, H. M. 2008. Multicenter study of brucellosis in Egypt. *Emerg. Infect. Dis.* **14**: 1916−1918.

112. Sauret, J. M. and Vilissova, N. 2002. Human brucellosis. *J. Am. Board Fam. Pract.* **15**: 401−406.

113. Shirima, G. M. 2005. The epidemiology of brucellosis in animals and humans in Arusha and Manyara regions in Tanzania. Ph.D. thesis. University of Glasgow, UK.

114. Shirima, G. M., Cleaveland, S., Kazwala, R. R., Kambarage, D. M., Nigel, F., McMillan, A., Kunda, J., Mfinanga, G. S. and Fitz, Patrick, J. S. 2007. Sero-

prevalence of brucellosis in smallholder dairy, agropastoral, pastoral, beef ranch and wildlife animals in Tanzania. *Bull. Anim. Health Prod. Afr.* **55**: 13−21.

115. Swai, E. S. and Schoonman, L. 2009. Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. *Zoonoses Public Health* **56**: 183−187.

116. Swai, E. S. and Schoonman, L. 2010. The use of rose bengal plate test to asses cattle exposure to *Brucella* infection in traditional and smallholder dairy production systems of tanga region of Tanzania. *Vet. Med. Int.* **2010**.

117. Swai, E. S. and Schoonman, L. 2012. A survey of zoonotic diseases in trade cattle slaughtered at Tanga city abattoir: a cause of public health concern. *Asian Pac. J. Trop. Biomed.* **2**: 55−60.

118. Temba, P. 2012. Seroprevalence of Brucella species infections and associated risk factors in wildlife-livestock interface: A case study of Mikumi-Selous ecosystem. M.Sc. dissertation. Sokoine University of Agriculture, Morogoro, Tanzania.

119. Thrusfield, M. 2005. Veterinary Epidemiology Third edition. Blackwell Science ltd, Oxford, UK.

120. Topley, W. W. C. and Wilson, G.S. 1990. Topley and Wilson's Principles of Bacteriology, Virology, and Immunity 7th Edition, Edward Arnold, London.

121. Tsuchiya, T., Hirai, Y. and Ono, S. 2007. A Study of the Properties of the Item Count Technique. *Public Opin. Q.* **71**: 253−272.

122. Tun, T. N. 2007. Prevalence Survey of Bovine Brucellosis (*Brucella abortus*) in Dairy Cattle in Yangon, Myanmar. M.Sc. dissertation. Chiang Mai University and Freie University at Berlin.

123. Vanzini, V. R., Aguirre, N., Lugaresi, C. I., de Echaide, S. T., de Canavesio, V. G., Guglielmone, A. A., Marchesino, M. D. and Nielsen, K. 1998. Evaluation of an

indirect ELISA for the diagnosis of bovine brucellosis in milk and serum samples in dairy cattle in Argentina. *Prev. Vet. Med.* **36**: 211−217.

124. Weinhäupl, I., Schöpf, K. C., Khaschabi, D., Kapaga, A. M. and Msami, H. M. 2000. Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es salaam Region and in zebu cattle in Lugoba area, Tanzania. *Trop. Anim. Health Prod.* **32**: 147−154.

125. WHO. 1997. Brucellosis. Fact Sheet N173. World Health Organisation, Geneva.

126. WHO. 1998. The development of new/Improved brucellosis vaccines, Geneva, switzerland 11−12 Dec 1997.

127. WHO. 2006. The control of neglected zoonotic diseases: a route to poverty alleviation. WHO press.

128. Wilcox, R. R. 1996. Statistics for the social sciences. San Diego, CA: Academic Press.

129. Yang, X., Skyberg, J. A., Cao, L., Clapp, B., Thornburg, T. and Pascual, D. W. 2013. Progress in *Brucella* vaccine development. *Front. Biol. (Beijing)* **8**: 60−77.

130. Zinsstag, J. 2012. Convergence of EcoHealth and One Health. *EcoHealth* **9**: 371−373.

131. Zinsstag, J., Schelling. E., Waltner-Toews, D., Whittaker, M. and Tanner, M. 2015. One Health: the theory and practice of integrated health approaches. Wallingford, UK: CABI Publishing.

ABSTRACT

Brucellosis is a zoonotic disease spreading across the world. Although the cases and burden of the disease are tremendous, the disease control by the government is not expected due to the lack of recourses in many developing countries. This study was carried out to explore the sustainable and affordable control options of bovine brucellosis in Morogoro region, Tanzania.

First study was conducted as a cross-sectional study to compare prevalence and risk factors of bovine brucellosis, and risky behaviours for the human infection between urban and agro-pastoral areas. Cattle blood sampling and interviews using a structured questionnaire were conducted with farmers. Rose-Bengal test was conducted for the cattle sera, and positive samples were confirmed with competitive ELISA.

Farm-level sero-prevalences were 0.9% (1/106, 95% CI: 0.0–5.9%) and 52.9% (9/17, 95% CI: 28.5–76.1%) in urban and agro-pastoral areas, respectively. The animal-level adjusted prevalences were 0.2% (1/667, 95% CI: 0.0–1.1%) and 7.0% (28/673, 95% CI: 5.7–8.4%) in those areas. The final farm-level model including both areas found two risk factors: history of abortion in the herd ($P < 0.01$) and cattle grazing ($P = 0.07$). The animal-level risk factors in agro-pastoral areas were age ($P = 0.04$) and history of abortion ($P = 0.03$). No agro-pastoral farmer knew about *Brucella* vaccine. Agro-pastoralists generally had poorer knowledge on brucellosis, and practiced significantly more risky behaviours for human brucellosis such as drinking raw milk (17.6%, $P < 0.01$) and blood (35.3%, $P < 0.01$), and helping cattle birth (100%, $P = 0.04$) than urban farmers (0%, 0% and 79.2%, respectively). Intervention programmes through education including both human and animal health particularly targeting agro-pastoralists would be needed.

Thus second study was performed focusing on agro-pastoral areas, investigating the farm level prevalence and risk factors for bovine brucellosis, and perception and behaviours related

with brucellosis control among agro-pastoralists.

A cross-sectional study involving herd milk diagnosis by Indirect ELISA and questionnaire survey was conducted in 124 farms. The questions included potential risk factors, knowledge of brucellosis, willingness-to-pay for cattle vaccination, and Item Count Technique (ICT) for selling behaviour of cows experienced abortion.

The knowledge on brucellosis among study farmers was poor (name of the disease: 13.7%, symptoms: 3.2%, transmission from cattle to human: 2.4%, and *Brucella* vaccine: 2.4%). The farm-level bovine brucellosis prevalence was 44.4% (55/124, 95% CI: 35.5–53.5). There was no risk factor for bovine brucellosis but a preventive factor, using veterinary service (OR = 0.39, 95%CI: 0.18−0.84, $P = 0.02$). For the scenarios of vaccinating all cattle and only calves, 59.7% and 89.5% of farmers were willing to pay for vaccination. Being Maasai tribe was a hesitating factor for vaccinating all cattle (OR = 0.39, 95%CI: 0.19−0.83, $P = 0.01$) and using a veterinary service an encouraging factor for vaccinating calves (OR = 4.0, 95%CI: 1.2−13.0, $P = 0.02$). The ICT found that 45.1% of the farmers sold out cows that caused abortion. The estimate was not statistically different from that obtained by direct questioning (34.1%, SE = 7.5%, binomial P value = 0.27, factor score = 1.32), suggesting that farmers did not hesitate to do the activity. Maasai conducted the risky behaviours against human infection such as drinking raw milk ($P = 0.06$) or blood ($P < 0.01$), and helping delivery with bear hands ($P = 0.03$) than the other tribes.

The results showed that bovine brucellosis is endemic in agro-pastoral areas in Morogoro region, Tanzania. Veterinary service was a preventive factor of bovine brucellosis, suggested that regular preventive health measures may reduce the prevalence. The cattle farmers were willing to pay for brucellosis vaccination, particularly by limiting calves to be vaccinated, indicating the feasibility of community-based calf vaccination programme. Receiving education from veterinarians was again a key to accept vaccination for calves. Farmers were

selling cows experienced abortion without hesitation, and this may have contributed to the maintenance of the disease but at the same time suppressed within-herd prevalence. This study showed that One Health approach for joint planning and actions of community-based brucellosis intervention, including health education, is necessary and feasible in Tanzania.

ABSTRACT IN JAPANESE（和文要旨）

ブルセラ病は世界中に広く蔓延している人獣共通感染症である。ブルセラ病は実際の発生数及び被害は甚大である一方、多くの途上国では政府による疾病制御は予算及び資源の不足により期待できない。本研究はタンザニアモロゴロ州における牛ブルセラ病の持続的かつ支払い可能な制御法の検討を目的に実施された。初回は横断研究により都市部と農業・放牧混合地域における牛ブルセラ病の有病率の比較及びリスク因子、ヒト感染に関わるリスク行動の調査研究を行った。牛の採血及び農家への質問票調査を実施した。牛血清の診断にはローズベンガル試験を用い、陽性サンプルにはC-ELISA を実施した。

農場レベル有病率は都市部、農業・放牧混合地域でそれぞれ 0.9% (1/106, 95% CI: 0.0–5.9%)、52.9% (9/17, 95% CI: 28.5–76.1%)であった。牛個体レベル調整有病率はそれぞれ 0.2% (1/667, 95% CI: 0.0–1.1%)、7.0% (28/673, 95% CI: 5.7–8.4%)であった。農場レベルリスク因子は農場における牛の流産 ($P<0.01$) 及び放牧 ($P=0.07$) であった。ブルセラワクチンに関する知識のある農業・放牧混合地域の農家は皆無であり、同農家らはブルセラ病に関する知識が乏しく、生乳飲用(17.6%, $P<0.01$ や牛血飲用(35.3%, $P<0.01$)、牛の助産(100%, $P=0.04$)等のヒト感染リスク行動を都市部農家と比較して有意に実施していた (各 0%、0%、79.2%)。特に農業・放牧混合農家を対象としたヒト及び動物の健康に関する教育を通した介入プログラムの必要と考えられた。

そこで、二回目の調査は農業放牧混合農家に焦点を当て、牛ブルセラ病の農場レベル有病率及びリスク因子、農家のブルセラ病制御に関する知識と行動を明らかにすることを目的として実施した。横断研究を用い I-ELISA によるバルク乳診断と質問票調査を 124 農家に対して実施した。質問票にはブルセラ病に関わるリスク因子及び知識、ワクチン支払意欲及び Item Count Technique (ICT)法による流産牛売却行動の項目を設定した。

調査農家のブルセラ病に関する知識は乏しかった (疾病名: 13.7%、症状: 3.2%、 牛からヒトへの伝染: 2.4%、ワクチン: 2.4%)。農場レベル有病率は 44.4% (55/124, 95% CI: 35.5–53.5)であった。牛ブルセラ病リスク因子は無かったが、獣医サービスの利用が防除因子であった (OR = 0.39, 95%CI: 0.18–0.84, $P = 0.02$)。ワクチン支払について、すべての牛対象の場合 59.7%、子牛のみ対象の場合 89.5%の農家が受諾した。マサイ族はすべての牛対象のワクチン接種を有意に断る傾向がみられ (OR = 0.39, 95%CI: 0.19–0.83, $P = 0.01$)、獣医サービス利用農家は子牛のみ対象のワクチン接種を有意に受け入れる傾向がみられた (OR = 4.0, 95%CI: 1.2–13.0, $P = 0.02$)。ICT 法による解析では 45.1%の農家が流産牛を市場に売却しており、直接質問による回答(34.1%, SE = 7.5%, binomial P value = 0.27, factor score = 1.32)と有意な差はみられず、売却行動に隠蔽性は無いと考えられた。マサイ族は生乳飲用 ($P = 0.06$)、牛血飲用 ($P < 0.01$)、素手による牛の助産 ($P = 0.03$)等のヒトブルセラ病感染リスク行動を他部族に比べ有意に取っていた。

これらの結果から、タンザニア国モロゴロ州の農業・放牧混合地域では牛ブルセラ病は蔓延していることが分かった。獣医サービスは牛ブルセラ病の防除因子であり、継続的な制御・衛生対策が有病率を減少させる可能性が示された。農家の、特に子牛のみ対象の場合のワクチン支払意欲は高く、コミュニティ主体の子牛ワクチン接種プログラムの実現性が示された。獣医からの教育を受ける機会は、子牛ワクチン接種受け入れにも重要であることが分かった。また、農家は流産牛の市場への売却にためらいはなく、売却行動により他農場への感染を広げている可能性がある一方、農場内有病率を減少させている可能性も考えられた。本研究から、衛生教育を含めたコミュニティ主体によるブルセラ病介入プログラムの作成と実施のためのワンヘルスアプローチの必要性及び実行可能性が示された。

APPRENDICES

Appendix 1: Questionnaire template used in the study of Chapter 2

Questionnaire on brucellosis for farmers in Morogoro region

2015, Rakuno Gakuen University, Japan, Sokoine University of Agriculture, Tanzania

<Purpose of the study>

The purpose of the study is to investigate the best-bet control methods of brucellosis in zero-grazing and Agro-pastoral farms in Morogoro region, Tanzania. We would like to ask a few questions and sample blood from cattle.

The study is conducted by Rakuno Gakuen University and Sokoine University of Agriculture.

<Agreed to participate?> □

1. BACKGROUND DATA

Farm No._____ Date of interview (dd/mm/yy)_____

1-1 Head of household_____Sex □ Male □ Female Age_____

1-2 Respondent_____ Sex □ Male □ Female Age_____

1-3 Street_____Village_____Ward_____

District_____

GPS coordinates_____S_____E

1-4 Distance to nearest farm (meters/km) _____

1-5 Distance from village center (meters/km) _____

1-6 Highest level of education completed of the household head:

□ No school attendance

□ Schooling attendance ending primary school

□ Schooling attendance ending secondary school

□ Schooling attendance ending college or specialized training college

□ Schooling attendance with high education (university)

□ Other

1-7 Number of family member _____

1-8 Other source of income □ Yes _____ □No

1-9 If yes, proportion of livestock farming in total income _____%

2. HERD INFORMATION AND MANAGEMENT

2-1 Number of livestock

Cattle_____ (Bull _____ Milking cows_____ Heifers_____

Calves_____)

Goats_____ Sheep_____

2-2 Which grazing system? □Zero-grazing □Semi-intensive □Extensive □Other_____

2-3 Do you cultivate crops or vegetables? □ Yes □ No

2-4 Do you conduct migration? □ Yes → which months_____ □ No

2-5 Do you use communal grazing? □ Yes □ No

2-6 How many cattle do you buy-in and sell out per year? Buy_____ Sell_____

2-7 How often do you visit cattle market? _____/month

2-8 Do you purchase cattle from other farmers directly? □ Yes □ No

2-9 Where do you sell your cattle or where do the buyers come from?

□ Within the neighboring villages □ Within the ward_____

□ Outside the region_____ □ All of the above

□ Others_____

2-10 Please provide proportions of stages of cattle which you buy-in (out of 100%).

Adult_____ Heifer_____ Calves_____

2-11 Do you use AI service or natural mating? □ AI □ Natural mating □ Both

2-12 If you use natural mating, whose bull do you use? □ Yours □ Other farmers' □ Both

2-13 How many abortions are there in your herd?

Cattle_____ /year Goats_____/year Sheep_____/year

2-14 Contact with other animals (1=often, 2=occasionally, 3=never)

HERDS	DRY SEASON		WET SEASON	
	Grazing areas	Watering points	Grazing areas	Watering points
Cattle from other herds				
Sheep/Goats from other herds				
Wild animals				

3. ANIMAL HEALTH MANAGEMENT

3-1 Have you ever used any vaccine? □ Yes □ No

3-2 If yes, when did you conduct the last vaccination and which type?

Year _____ Type_____

3-3 Have you used brucellosis vaccine? □ Yes □ No

3-4 Which type of veterinary treatment do you use?

□Public service □Private service □From other farmers □Self treatment

□Other _____ □None

4. MILK VALUE CHAIN

4-1 Do you sell milk? □ Yes □ No

4-2 If yes, how often? _____/week

Where?

□ Within the neighboring villages □ Within the ward_____

□ Outside the region_____ □ All of the above

□ Others_____

5. HUMAN HEALTH

5-1 Which milk do you consume? □Raw milk □Boiled milk □Other_____

5-2 How much milk do you consume/day _____ml

5-3 Do you drink blood of cattle? □ Yes □ No

5-4 Do you help in the birthing of animals and touch placenta or fetus? □ Yes □ No

5-5 If yes, how do you deal with? _____

5-6 How often do you have febrile disease? (1=very often, 2=often, 3=occasionally, 4=very occasionally, 5= almost no) _____

5-7 Which type of medical facility do you use?

□Hospital □Dispensary □Traditional healers □Others_____

Place_____ Distance _____km

6. KNOWLEDGE

6-1 Do you know the name of brucellosis? □ Yes □ No

6-2 Do you know the symptoms of brucellosis? □ Yes □ No

6-3 Do you know that brucellosis can be transmitted from cattle to human? □ Yes □ No

6-4 Do you know brucellosis vaccine? □ Yes □ No *Explain about brucellosis

7. WILLINGNESS OF CONTROLLING BRUCELLOSIS

7-1 If you have brucellosis positive cattle, which method do you want to conduct?

□Cull □Sell to other farmers □Continue keeping

□Use vaccine (ex. vaccination cost: 2000Tsh/shot)

7-2 Do you agree to shot vaccine to bull and calves which were born in this year? □ Yes □ No

Thank you so much for cooperation.

Appendix 2: Questionnaire template used in the study of Chapter 3

Questionnaire on brucellosis for farmers in Morogoro region

2016, Rakuno Gakuen University, Japan, Sokoine University of Agriculture, Tanzania

<Purpose of the study>

The purpose of the study is to investigate the best-bet control methods of brucellosis in Agro-pastoral farms in Morogoro region, Tanzania. We would like to ask some questions and sample bulk milk from cattle.

The study is conducted by Rakuno Gakuen University and Sokoine University of Agriculture.

<Agreed to participate?> □

1. BACKGROUND DATA

Farm No._____ Date of interview (dd/mm/yy)_____

1-1 Head of household_____Sex □ Male □ Female Age_____

Tribe_____

1-2 Respondent_____ Sex □ Male □ Female Age_____

Tribe_____

1-3 Street_____Village_____Ward_____

District_____

GPS coordinates_____S_____E

1-4 Distance to nearest farm (meters/km) _____

1-5 Distance from village center (meters/km) _____

1-6 Highest level of education completed of the household head:

□ No school attendance

□ Schooling attendance ending primary school

□ Schooling attendance ending secondary school

□ Schooling attendance ending college or specialized training college

□ Schooling attendance with high education (university)

□ Other

1-7 Number of family member _____

1-8 Other source of income □ Yes _____ □No

1-9 If yes, proportion of livestock farming in total income _____%

2. HERD INFORMATION AND MANAGEMENT

2-1 Number of livestock

Cattle_____ (Bull _____ Milking cows_____ Heifers_____

Calves_____)

Goats_____ Sheep_____

2-2 Which grazing system? □Zero-grazing □Semi-intensive □Extensive □Other_____

2-3 Do you cultivate crops or vegetables? □ Yes □ No

2-4 Do you conduct migration? □ Yes → which months_____ □ No

2-5 How many newborn calves do you have per year? _____

2-6 How many cattle do you buy-in and sell out per year? Buy_____ Sell_____

2-7 How often do you visit cattle market? _____/month

2-8 Do you purchase cattle from other farmers directly? □ Yes □ No

2-9 Where do you sell your cattle or where do the buyers come from?

□ Within the neighboring villages □ Within the ward_____

□ Outside the region_____ □ All of the above

□ Others_____

2-10 Please provide proportions of stages of cattle which you buy-in (out of 100%).

Adult_____ Heifer_____ Calves_____

2-11 Do you use AI service or natural mating? □ AI □ Natural mating □ Both

2-12 If you use natural mating, whose bull do you use? □ Yours □ Other farmers' □ Both

2-13 How many abortions are there in your herd?

Cattle_____ /year Goats_____/year Sheep_____/year

2-14 Contact with other animals (1=often, 2=occasionally, 3=never)

HERDS	DRY SEASON		WET SEASON	
	Grazing areas	Watering points	Grazing areas	Watering points
Cattle from other herds				
Sheep/Goats from other herds				
Wild animals				

3. ANIMAL HEALTH MANAGEMENT

3-1 Have you ever used any vaccine? □ Yes □ No

3-2 If yes, when did you conduct the last vaccination and which type?

Year _____ Type_____

3-3 Have you used brucellosis vaccine? □ Yes □ No

3-4 Which type of veterinary treatment do you use?

□Public service □Private service □From other farmers □Self treatment

□Other _____ □None

3-5 How do you handle female cattle which have history of abortion?

□Continue keeping □Sell to the cattle market □Slaughter

□Others_____

4. MILK VALUE CHAIN

4-1 Do you sell milk? □ Yes □ No

4-2 If yes, how often? _____/week

Where?

□ Within the village □ Within the neighboring villages

□ Within the ward_____ □ Outside the region_____

□ Others_____

4-3 Do you boil milk before selling it? □ Yes □ No

5. HUMAN HEALTH

5-1 Which milk do you consume? □Raw milk □Boiled milk □Other_____

5-2 How much milk do you consume/day _____ml

5-3 Do you drink blood of cattle? □ Yes □ No

5-4 Do you help in the birthing of animals and touch placenta or fetus? □ Yes □ No

5-5 If yes, how do you deal with? _____

5-6 How often do you have febrile disease? (1=very often, 2=often, 3=occasionally, 4=very occasionally, 5=almost no) _____

5-7 Which type of medical facility do you use?

□Hospital □Dispensary □Traditional healers □Others_____

Place_____ Distance _____km

6. KNOWLEDGE

6-1 Do you know the name of brucellosis? □ Yes □ No

6-2 Do you know the symptoms of brucellosis? □ Yes □ No

6-3 Do you know that brucellosis can be transmitted from cattle to human? □ Yes □ No

6-4 Do you know brucellosis vaccine? □ Yes □ No *Explain about brucellosis

7. WILLINGNESS OF CONTROLLING BRUCELLOSIS

7-1 If you have brucellosis positive cattle, which method do you want to conduct?

□Sell for culling □Sell to other farmers □Continue keeping

7-2 Do you agree to shot vaccine to bull and calves which were born in this year? □ Yes □ No

7-3 Do you agree to shot vaccine to all catlle? □ Yes □ No

7-4 Do you agree to put on ear tags to the catlle which will be vaccinated? □ Yes □ No

(vaccination cost: 3000Tsh/shot)

Thank you so much for cooperation.