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Brucellosis in livestock in and near the Maasai Mara National Reserve in Kenya

Seroprevalence and possible impact on animal and human welfare

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Brucellos hos tamboskap i och omkring nationalparken Maasai Mara i Kenya

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PREFACE

This study and report was completed as a master's thesis in Veterinary Medicine and Animal Science combined with a Minor Field Study, MFS, at the Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences, SLU. By getting the chance of performing the master thesis together with an MFS-scholarship gave me a great opportunity to combine my studies with research in another country and to give assistance in a project concerning developing sustainable livestock production. By staying in Kenya during the study it enabled that new experiences and insights could be obtained. I chose the subject of investigating the prevalence of antibodies against brucellosis in livestock in relation to the degree of contact with wildlife to get a deeper knowledge regarding how appropriate and sustainable animal husbandry should be sought and how it can be lived up to in low-income countries, in areas where human/wildlife conflicts are present. The subject also gave me the opportunity to learn more about the interesting culture of the country and the importance of animals being kept in pastoralists' societies.

In this study, the cattle selected for the study were sampled and blood from each one of them were collected to be analyzed for the presence of antibodies against Brucellosis. This master thesis was carried out in co-operation with a PhD-project about Foot and Mouth disease undertaken by Daniel Mutiso at the Department of Food Safety and Zoonoses, ILRI, in collaboration with ILRI (International Livestock Research Institute), aiming at investigating the prevalence of Foot and Mouth disease in domestic livestock and how it is transmitted. It also concerns the topic of how different livestock are affected by wildlife contact in pastoralists' societies in Kenya. This project focused on examining cattle from different villages which have applied different types of grazing strategies with aim of investigating whether there are any differences in prevalence of antibodies against brucellosis in serum based on the degree of contact with wildlife and other livestock. ILRI played an important role in this study through their great source of knowledge when it comes to livestock research in developing countries.

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SUMMARY

Brucellosis is caused by bacteria from the genus *Brucella.* The disease occurs worldwide and is of major importance in domestic animals, both for socio-economic reasons and because of the impact on animal and human welfare. Disease control programs in low-income countries are either inadequate or non-existent; a reason why brucellosis has got the chance of being as widely spread as it is as present. Not only does *Brucella* infect domestic animals, it also occurs in wildlife species, thus there is a risk of transmission between wild animals and domestic livestock in areas where these animal groups get in contact with each other. Many *Brucella* species in animals are proven to have the ability to also infect humans, causing human brucellosis, with different degrees of severity. The Maasai Mara ecosystem, in Narok County Kenya, is part of the arid and semi-arid lands which form about 80% of Kenya's landmass that support wildlife and livestock farming. Approximately, 68% of Narok County is rangelands with conservancies such as Lemek, Mara North, Koiyaki, Mara-Naboisho, Ol Kinyei, Olare Orok, Motorogi and Olarro which offer prime grazing for ranching and livestock production. While crop failure is common in many arid and semi-arid areas, livestock farming assumes a significant role. Maasai Mara is a well-known area, housing one of the most famous national reserves in Kenya and is surrounded by pastoral landscapes that are shared between wildlife, livestock and people. Such wildlife-livestock overlap may expose livestock to novel pathogens of wildlife origin and consequently reduce the food security of the Maasai pastoralists who derive their livelihood from livestock farming. A majority of livestock keepers in and around Maasai Mara depend entirely on livestock production for their livelihood and families' survival. By living in an area like the Maasai Mara, pastoralists have to settle for a limited agricultural practice due to wildlife occupying large areas, and also endure their livestock commingling with wildlife in search for pasture land and water sources, throughout the year. By mixing wildlife and livestock there is an increased danger of disease transmission, and the presence of zoonotic diseases, such as brucellosis, around the Maasai Mara may serve as a source of cross-transmission of disease not only between wildlife and livestock but also between livestock and humans.

The aim of this study was to investigate the seroprevalence of *Brucella* spp. in cattle in wildlife/livestock interfaces. A questionnaire was handed out to all participating farmers, including questions about animal keeping and management, experiences of wildlife contact and observed symptoms of disease in both livestock and people handling livestock, etc. Blood samples were collected from 225 cattle, from three villages with different distances to the Maasai Mara, and that engage in different strategies for grazing, allowing the animals to come in contact to wildlife to various extent. The purpose was to examine whether different farming strategies and the distance to wildlife-dense areas affect the incidence of infectious diseases. Antibodies against *Brucella abortus* was found, using ELISA, in 12.44% of the 225 sampled animals, with more females being infected than males (15% and 5%). Cattle from farms closer to Maasai Mara had a significantly higher prevalence of antibodies against *Brucella* spp. in serum (7.03 times higher odds of *Brucella* infection in Mara Rianta compared to Endoinyo Narasha, p=0.003), suggesting that a closer contact with wildlife may pose a risk of being afflicted by infectious diseases to a greater extent. Symptoms consistent with brucellosis were

reported to occur in both humans and animals. By studying the seroprevalence of a contagious and zoonotic disease as brucellosis, one may gain understanding of the disease extent as a whole, help identify mitigation strategies, and thereby improve both health and economic circumstances for affected farmers.

SAMMANFATTNING

Brucellos orsakas av bakteriearter från släktet *Brucella*. Sjukdomen förekommer över hela världen och är av stor betydelse framför allt hos tamdjur, både av socioekonomiska skäl och på grund av påverkan på djurs och människors välfärd. Bekämpningsprogram i utvecklingsländer är antingen otillräckliga eller obefintliga; en anledning till varför sjukdomen har fått chans att bli så utbredd som i nuläget. Brucellos drabbar inte enbart tamdjur, utan sjukdomen förekommer också hos vilda djurarter, vilket utgör en risk för överföring mellan vilda djur och tamdjur i områden där dessa djurgrupper kommer i kontakt med varandra. Många arter av *Brucella* som orsakar sjukdom hos djur har dessutom visat sig ha förmåga att infektera människor, och orsakar då human brucellos av varierande allvarlighetsgrad. Ekosystemet Maasai Mara som är beläget i Narok county i Kenya, är en del av de torra och halvtorra marker som utgör ca 80% av Kenyas landyta, och som nyttjas av vilt djurliv och stöder lantbruk och djurhållning. Nära 68% av Narok county utgörs av betesmarker med naturreservat såsom Lemek, Mara North, Koiyaki, Mara-Naboisho, Ol Kinyei, Olare Orok, Motorogi och Olarro, som erbjuder rika beten för boskapsuppfödning och animalieproduktion. Då missväxt av grödor är vanligt i många torra och halvtorra områden, har djurhållning en betydande roll. Maasai Mara är ett välkänt område, där ett av de mest kända reservaten i Kenya ligger, och är omgivet av pastorala landskap som delas mellan vilda djur, tamboskap och människor. Sådan överlappning mellan djurgrupper kan utsätta boskap för nya smittämnen som har sitt ursprung i de vilda djuren, vilket kan medföra en minskning i produktion och äventyra livsmedelsförsörjningen för de bönder som får sin inkomst från djuruppfödningen. En majoritet av djurhållare i och runt Maasai Mara är helt beroende av animalieproduktion för sin försörjning och sina familjers överlevnad. Genom att bo i ett område som Maasai Mara, måste djurhållare vara redo att kompromissa och nöja sig med en begränsad jordbrukspraxis på grund av de vilda djuren som ockuperar stora betesområden, och även tillåta sin boskap att beblandas med vilda djur i sökandet efter betesmarker och vattenkällor. Genom att blanda vilda djur och tamboskap finns en ökad risk för överföring av sjukdomar, och närvaron av zoonotiska sjukdomar, såsom brucellos runt Maasai Mara, leder till överföring av sjukdomar, inte bara mellan vilda djur och tamboskap, utan även till människor, då människor i dessa områden tenderar att leva mycket nära inpå sina djur.

Syftet med denna studie var att undersöka förekomsten av antikroppar mot *Brucella* spp. hos nötkreatur i gränsområden där tamboskap kan komma i kontakt med vilda djur. En enkät delades ut till alla deltagande bönder, som omfattade frågor om djurhållning, upplevelser om kontakten med vilda djur och upplevda sjukdomssymtom hos både djur och människor. Blodprover togs från 225 nötkreatur, från tre byar med olika avstånd till Maasai Mara, och med olika betesstrategier som tillåter djuren att komma i kontakt med vilda djur i olika omfattning. Målet var att se om det finns några skillnader i sjukdomsförekomst mellan dessa tre byar, och därmed kunna avgöra om olika jordbruksstrategier och avståndet till vilttäta områden påverkar förekomsten av smittsamma sjukdomar. I denna studie kunde antikroppar mot *Brucella* spp. påvisas med ELISA hos 12.44% av de 225 provtagna djuren, med fler honor än handjur infekterade (15% respektive 5%). Boskap från gårdar med mindre avstånd till Maasai Mara hade en signifikant högre prevalens av antikroppar mot *Brucella* spp. i serum (7.03 gånger

högre odds för *Brucella*-infektion i Mara Rianta jämfört med Endoinyo Narasha, p=0.003), vilket antyder att närheten till vilda djur kan innebära en risk att drabbas av infektionssjukdomar i större utsträckning. Djurägare rapporterade sjukdomssymtom hos både djur och människor som kan ha orsakats av brucellos. Genom att studera förekomsten av en så smittsam zoonotisk sjukdom som brucellos, kan man öka förståelsen för omfattningen av smittsamma sjukdomar som helhet, och påvisa fördelningen av sjukdom hos boskap och människor i byar som ligger i Maasai Maras’ gränsområden där kontakt mellan vilda arter och tamboskap ofta uppstår, vilket kan bidra till bättre bekämpningsstrategier, och därigenom förbättra både hälsa och ekonomiska förhållanden för drabbade bönder.

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INTRODUCTION

Brucellosis is a disease that according to the Centre for Disease Control and Prevention, USA (2012) occurs worldwide, but it's more in countries without effective health care and domestic animal health programs. It is one of the most severe diseases, especially for farmers since it does not only cause major suffering for their animals but also a great loss in production, at the same time as people handling the animals risk being affected and develop disease. There is no uncertainty that the farmers around Maasai Mara depend on livestock for their livelihood and families' survival, and in such livestock-dependent households, the health of humans, livestock and economic welfare are closely linked (Thumbi et al. 2015).

In the low-income countries of sub-Saharan Africa, it is estimated that about 300 million people has an income of less than 2 US $ dollar a day (Thumbi et al., 2015). A majority of those people are farmers depending on livestock for their own and families' survival. Livestock as a source of livelihood is held for mainly four reasons; source of food, monetary income, cultural values, and buying land. Presence of infectious diseases, such as brucellosis, in farm animals may jeopardize the economy of whole families, why it is of high importance to keep the animals healthy and reduce the risk of infection.

The disease is not only contagious and affects the livestock's health negatively, it is also zoonotic which means that even humans are likely to be infected if handling animals in herds where the disease is spread (McDermott & Arimi, 2002). The bacteria can be transmitted very fast between livestock within a herd, and in addition, also between wildlife and livestock that comes in contact with each other either physically or indirectly through contaminated environmental areas, and disease prevalence within a herd appears to have a tendency to increase with increasing herd sizes. Globally, there is a positive trend in the number of animals kept in the same herd, which may be one of the reasons for the increase seen in the number of new brucellosis cases documented in recent years (Sowjanya et al. 2013). Wildlife also provide a potential reservoir of brucellosis, increasing the risk of re-introduction into domestic livestock (Rhyan et al., 2013).

In livestock, the symptoms caused by bovine brucellosis are dominated by late-term abortions, stillbirths and weak calves, sometimes combined with retained placentas, metritis and other reproductive tract lesions. Bovine brucellosis primarily infects cattle, but it is a zoonotic disease and human cases are also reported regularly. Thus, the symptoms in humans are very diffuse and often mistaken for other diseases, such as malaria or influenza. Symptoms in humans vary and include recurrent fever, sweats, malaise, joint and back pain and other influenza-like signs. In both humans and animals, the symptoms may persist for long periods of days (Rhyan et al., 2013).

In Kenya, not so many studies on this topic has been published, despite the fact that it is important from both an economic and public health point of view. Awareness of the disease is relatively low among the local population and health care staff, and there is limited knowledge about the subject (Omemo et al., 2012). In addition, because of diffuse symptoms and the absence of accessible laboratory facilities, its presence often remained unrecognized even by veterinarians (Smits & Cutler, 2004). The aim and objectives with this study were to raise knowledge and awareness of brucellosis in three selected villages, to determine the current seroprevalence of bovine brucellosis and the associated exposure factors for transmission.

LITTERATURE REVIEW

Kenya

Country information

Kenya (officially the Republic of Kenya) is a country in East Africa, by the Indian Ocean, bordering Ethiopia, Somalia, South Sudan, Uganda and Tanzania. Its placement in the middle of the continental Africa, and its proximity to the sea makes the country an important hub of trading and tourism. The population consists of approximately 38.6 million people (2009), and a majority of Kenyans live in rural areas (KNBS, 2009). The population is divided into 42 officially acknowledged ethnical groups or *tribes*. Ethnicity is an important component in the personal identity in most parts of the Kenyan society (United Nations, 2014). The largest ethnical groups consist of Kikuyu (17.2%), Luhya (13.8%), Kalenjin (12.9%), Luo (10.5%), Kamba (10.1%), Somali (6.2%), Kisii (5.7%), Mijikenda (5.1%), Ameru (4.3%), Turkana (2.6%) and Maasai (2.2%) (PDNK, 2015). The area around Maasai Mara is inhabited predominantly by Maasai. In areas around national reserves, such as Maasai Mara, tourism is becoming an increasingly important source of income, for many indigenous people as well as the state (Thumbi et al., 2015). The tourism industry has exploited the Maasai for commercial purposes, making them a symbol of the country, although they only represent a small part of the Kenyan population (Fratkin, 2004).

Historically, Kenya was colonized by Britain in the 19th century (PDNK, 2015). During the British colonial rule, Kenya was part of British East Africa, and was originally planned as an Arab kingdom under British protection, but after an Arab uprising in 1895 Kenya was instead constructed as a colony. In 1896, the construction of a railway began, creating a connection between Uganda and the coast at Mombasa, promoting emigration from the UK to Kenya. This resulted in several thousand British farmers getting the opportunity to grow grains and plants on cheap and fertile land in the southwest. Kenya's independence was declared December 12, 1963 (United Nations, 2014). The railway is today an important route for export of goods to the coast and from the country, which facilitates trade considerably (PDNK, 2015).

There is no particular culture in Kenya that specifically identify the whole country, instead all 42 tribes have their own unique culture, but a majority of them have intertwined cultural expressions created through strong similarities in language, environment and tribal physical proximity (United Nations, 2014). The majority of indigenous people lives in the arid and semi-arid lands and forests, which about 80 per cent of Kenya's land mass consists of. These areas are also home to a substantial share of other Kenyans, operating within different socio-economic interests. The dry lands of Kenya hosts about 75 per cent of all the livestock in the country, in addition to providing sources of income and livelihoods to the natives. It is estimated that the arid and semi-arid lands and forests constitutes direct livelihoods to over 5 million pastoralists (PDNK, 2015). Cattle are the most important livestock, with major economic values in Kenya. Pastoralism, herding and raising livestock, is within Kenya, but also within the whole Africa and even globally, highly diverse. Still, they tend to share key cultural and institutional adaptations that promote perseverance in environments defined by high resource variability and limited productivity (Kaye-Zwiebel & King, 2014).

Pastoralism

Pastoralists are people who rely on livestock for most of their subsistence. Within Kenya, cattle keepers are the most common pastoralists, and they mostly consist of people from Maasai and Samburu tribes (Fratkin, 2004). There are mainly two different types of pastoralism, determined by living area; adaptive migrators who adapted a voluntary form of mobility, and those who are forced to move by changed climate and environmental factors. Migration is well-known to be the primary coping strategy, especially in times of drought and other processes that are encroaching the pastoralist's living space (PNDK, 2015). Mobility is a characteristic adaptation that permits pastoralists to protect themselves against fortuitously variable environmental conditions, and to get access to resources that are scattered across wide areas. Approximately 74.4% of the population in Kenya live in rural areas (2015), and about 78.6% were during 2013 involved in agriculture of some kind (FAOSTAT, 2016). Most farming is small scale and food products are mainly prepared for own consumption. Livestock-keeping pastoralists occupy approximately 70% of Kenya's land, some of them are constantly moving as a strategy of always having access to adequate resources and not wearing pastures down, some are forced to move due to climate changes, and others are able to remain in the same location due to a more stable climate, which may result in longer-standing communities (Fratkin, 2004).

Challenges and obstacles

Despite resilient social-ecological adaptations by Kenyan pastoralists, many systems are failing to meet the needs of household livelihood while maintaining ecological resources. Due to drought and other environmental condition, in combination with a rapidly increasing population, more resources are consumed than what is produced (Kaye-Zwiebel & King, 2014). Kenyas' population growth rate is one the highest in the world, with an annual increase of 3.0% (KNBS, 2009), affecting both urban and rural populations, sedentary as well as pastoral, who are forced to move and raise their families onto less productive land. Due to movement of farmers into the plains, pastoralists such as Maasai lose herding lands and water sources. In areas where agriculture only is possible in isolated highlands, and since many pastoralists in these areas are forced to constant movement, conflicts with pastoral neighbors about land ownership is an uprising problem and sometimes it may even result in armed fighting for disputed lands (Fratkin, 2004).

Drought and famine are major problems in Africa, which during the second half of the 20th century increased dramatically. Due to larger climatic events, such as global warming, and volcanic eruptions, drought became more apparent, and in combination with the war situation and politic dislocation at that time, hundreds of thousands of people lost their lives by starvation (Fratkin, 2004). During this period, pastoralists lost half of their cattle population, mostly because of starvation, but also due to infectious diseases that were given opportunity to infect weakened herds, and transmit between large numbers of animals when captured in smaller areas. Disease incidence increased violently, particularly after rain resumed, becoming a growing problem in pastoralist societies (PDNK, 2014). Since then, predisposing factors for disease transmission, such as restrictions on mobility in relation to increasing human and livestock densities, has increased. In addition, these factors create year-round pressures on remaining pasture land, leading to a decrease of grazing resources and land degradation. Climate change is now even manifested in many of these areas, resulting in more erratic rainfall, harsher temperature and more frequent droughts. With the changing patterns in rainfall comes direct effects on ecosystems by a decrease in primary productivity, which in turn complicates the livelihood and resource management further (Kaye-Zwiebel & King, 2014). The risk of the spread and transmission of infectious diseases is now inherent in pastoralists way of life. During migration in transhumance, herds have frequent contact with other livestock, and will also be nearer wildlife, at common feeding grounds and in the search of water sources. Prevalence of infectious diseases in these areas are high, and many types of bacteria and viruses get the opportunity of cross-transmission between animal species. One pathogen of particular importance is *Brucella* spp. since it has the ability to spread between all types of mammals, including humans, and may have increased incidence during periods of this kind (Smits, 2013).

Brucella

Overview of disease

Brucellosis is a contagious disease that occurs worldwide. It is caused by bacteria, mainly *Brucella abortus* and *Brucella melitensis*, which are gram-negative coccobacilli bacteria of the genus *Brucella*. The disease is one of the world's most important zoonotic diseases, especially in low-income countries, and for farmers it results in serious economic losses when the lesions in livestock caused by *B. abortus* consist of necrotic placentitis and mastitis in pregnant cows, which leads to late-term abortion, stillbirth, decrease in milk production and weak-born calves (Neta et al. 2010). In small ruminants, such as sheep and goats, brucellosis is mainly caused by *B. melitensis* with symptoms clinically similar to infection with *B. abortus* in cattle, dominated by orchitis and epididymitis in males and abortion in late-term pregnancy in females, fever and depression (Epiwebb, 2008). Nine recognized species, or biovars, are included within the genus *Brucella* (Seleem et al., 2010). Five of them can infect humans, all types are infective to different species of mammals, but usually keep within certain preferential hosts (Godfroid et al. 2011). The bacteria have their predilection site in certain cells, mainly macrophages and trophoblasts in the pregnant uterus, and cells within organs in the reproductive tract, but can survive and multiply successfully in both phagocytic and non-phagocytic cells as well, which explains their pathogenicity and high virulence.

Pathogenesis

Bovine brucellosis, caused by *B. abortus*, is a chronic granulomatous infectious disease (Sowjanya et al., 2013) which, in cattle, demonstrates tropism for the placental tissues. The clinical manifestations of the bacteria vary and depends on route of infection, age, immunological and reproductive status, virulence and dose of the *Brucella* strain (Xavier et al., 2009), but attacking cells in the pregnant uterus is the most common, resulting in gross lesions especially in placentomes, which become very friable and covered with fibrinous exudate, ultimately leading to the abortion of the fetus. These clinical manifestations and lesions are results of bacterial replication and overgrowth, and even after abortion, *B. abortus* can continue to replicate, multiplying into large numbers inside the fetus and placenta. Aborted fetuses demonstrate variable degrees of autolysis and organs in the abdominal cavity is often covered with fibrin, as a result of the bacterial activity (Xavier et al., 2009). One explanation for this phenomenon may be access to a certain sugar alcohol, erythritol, which has been found in large amounts in bovine fetuses, concentrated in fetal fluids, cotyledons and the chorion. Erythritol acts as an important and totally unique carbon and energy source for bacterial metabolism, hence enable the bacteria to keep replicating even long after the death of the calf-fetus. Smith et al. (1965) showed how it is possible, both in vitro and in newborn calves, to stimulate the growth of *B. abortus* by manipulating the access to erythritol, and suggested that the presence of the molecule is a major factor which enhances the susceptibility of the reproductive tract in cattle to be infected with *B. abortus* (Smith et al., 1965). Further studies conducted on the subject provide evidence of the contribution to the localization of infection, when the concentration of erythritol seems to be high even in reproductive tissues in bulls, which are susceptible for infection in the genitalia in the same way as cows, especially through orchitis.

Meanwhile, studies have not been able to prove the presence of erythritol in seminal vesicles or the placenta in species such as humans, which corroborates the theory since humans are not susceptible to acute orchitis and placentitis caused by brucellosis (Goodwin & Pascual, 2016).

Strategy of escaping immune system and surviving in host

Godfroid et al. (2011) describes that *Brucella* spp. has an intracellular life within mammalian hosts. It can infect different kinds of phagocytes, adjust and conform their intracellular trafficking so that the bacteria eventually can reach their replicative niche. By living inside of phagocytes *Brucella* spp. resists different types of environmental stresses and can compromise host immune responses (Roop et al., 2009). Furthermore, *Brucella* spp. avoid apoptosis by parasitizing macrophages when the bacteria have their long-term survival in the reticuloendothelial system of bone marrow, spleen and liver. As long as *Brucella* spp. sustain within the macrophages, the host mammal is considered chronically infected (Gorvel & Moreno, 2002). *Brucella* spp. is then able to replicate to large numbers in placental trophoblasts during gestation of host mammal. By living inside cells in the pregnant uterus, the bacterium is allowed to rapidly and uninterruptedly increase in numbers owing to the local immune response which is modulated during the time of gestation to prevent the fetus from being rejected. (Neta et al., 2010). Furthermore, the exact mechanisms behind presiding to the attack and colonization of the uterus in different pregnant female animal species are not yet completely known (Godfroid et al., 2011), but there are some theories discussing that once *Brucella* spp. has invaded the host cell, certain survival mechanisms are being activated that enables for effectively avoiding extracellular killing and the natural defense mechanisms of the host (Goodwin & Pascual, 2016). In comparison to other gram-negative bacteria, *Brucella* spp. expresses a molecule on the outer layer of the cell membrane, a “non-classical” lipopolysaccharide molecule (LPS) that has a different formation from other LPS structures. Acting as a major virulence factor for the bacteria’s pathogenicity, the LPS-molecule enhances intracellular survival by resisting the acidified environment inside phagosomes and counteracting the bacteria-containing phagosome from fusing with lysosomes (Neta et al., 2010). This makes the bacteria highly resistant to host immune responses. Despite the ability to survive intracellularly within many different cell types, such as epithelial cells, macrophages, male and female reproductive tissues and respiratory tissue, the intracellular trafficking of the bacteria and its capability to remodel certain cell signal pathways is not identical in each cell type. All cells aren’t as susceptible as the macrophage, which plays the role of *B. abortus* most preferable host before proliferation phase. For instance, neutrophils, which are also phagocytic cells, are not tolerating intracellular growth of bacteria in any extent, complicating intracellular survival of *Brucella* spp, even though the intracellular trafficking is similar to other retentive cells (Neta et al., 2010).

For bovine brucellosis, the placenta in pregnant females is the most common site for infection and proliferation of bacteria, but studies have demonstrated that *B. abortus* even can survive long time in aborted fetuses, replicate into large amounts in fetal fluids and cotyledons, constituting as a major source of infection if ingested by other animals (Xavier et al., 2009). Aborted fetuses, along with fetal membranes and uterine discharges eliminated after abortion thus are the main sources of infection, and the most important to withhold from the risk of transmitting disease (Neta et al., 2010).

Epidemiology and transmission routes

In afterbirth, amniotic fluid, placentas and stillborn fetuses from infected animals there is a large amount of bacterial antigen that can infect both humans and other animals through small skin lesions or mucus membranes, via aerosol by inhalation of airborne agents or from oral intake and ingestion (SVA, 2015). Even sperm, ejaculate, vaginal discharge, urine, faeces and milk from infected cattle contains lots of bacteria. Unpasteurized milk is therefore a risk product in areas where brucellosis occurs in ruminants, and where it's likely for milk to be consumed without cooking.

Brucella abortus is mainly transmitted through ingestion of contaminated feed, water or milk, or by penetration of the skin, conjunctiva and mucous membranes. Infected bulls excrete bacteria in semen and thus can infect cows during breeding, since the disease is venereal. Trans-placental transmission with congenital infection often occurs as a result (Epiwebb, 2008).

For all of the nine *Brucella* species it is known that the risk of disease spreading in laboratories handling the positive samples is very high (Epiwebb, 2008). Therefore, it is of high importance that every suspected case of brucellosis is reported to the laboratory so that educated personnel can handle the samples as highly contagious material and don't risk the disease being transmitted to other people in the surroundings. Veterinarians are considered particularly exposed to infection because they often come in contact with infected material, associated with obstetrics as well as removing retained afterbirths. Scratches on hands and arms is in this context usually the gate to infection but the bacteria can also penetrate intact skin. Another risk factor for veterinarians is performing vaccinations with live vaccines.
Brucellosis can travel long distances within hosts since it is capable to be carried and spread between species, even the ones migrating. Caron et al. (2016) investigated the prevalence in African buffalos in relation to their movement pattern and found that they are likely to carry the disease within national parks and conservation areas, through community land and across country borders. Their behavior thus constitutes a risk for livestock and human health in areas surrounded by wildlife. Other studies have been made on other species of both wildlife and livestock with similar results. Since *Brucella* also can infect humans, and the signs of illness are diffuse, there is a high risk of disease transmission in another direction; humans infecting domestic animals in their care, which in turn spread the infection further to wild animals not yet exposed to disease.

Symptoms of disease

In cattle, brucellosis is mainly caused by *B. abortus* and usually causes abortion during late-term pregnancy, followed by retained afterbirth and metritis (Epiwebb, 2008). The latter symptom can appear acute and then lead to septicemia and even death, or a stage where the animals remain chronically infected and temporarily infertile may occur. Following pregnancies generally progress normally, but it may happen that affected cows abort a second, or even a third time (Neta et al., 2010). The bacteria may even manifest in udder tissue, affecting the udder health negatively, causing mastitis and decreased milk production (Xavier et al., 2009). Males that are infected usually develop orchitis and epididymitis. One or both testicles is then severely swollen and painful (Epiwebb, 2008). The swelling often remains for a long period of time and the testicle will eventually go into necrosis. Affected animals are sterile during acute phase and for some time after, but if the infestation of disease is one-sided the fertility can be regained. The time of incubation varies between 30 and 60 days and a certain level of reversed age-resistance is present, which means that younger animals are less likely to be infected than older (Epiwebb, 2008). Even a certain difference in resistance in various breeds has been reported. Animals that have undergone infection can become permanent carriers of the infectious agent when the infection is carried intracellularly.

Brucellosis in humans is in an early stage characterized by undulating fever, chills, sweats, malaise, general weakness and pain from back and joints. These symptoms are often mistaken for influenza or other infectious diseases with similar signs of illness, why the diagnosis is easy to miss in an early stage. After a couple of days, the symptoms often disappear but may reoccur after a while and then restore or be repeated several times. The recovery takes long time and the disease can pass to a chronic stage. The symptoms are then more diffuse and may include small undulations in temperature, joint pain, malaise, nausea, headache, back pain and problem sleeping. The incubation period may vary from 1-3 weeks up to a couple of months (Epiwebb, 2008).

Diagnostic methods

If brucellosis is suspected, the disease must be confirmed with laboratory analyses and diagnostics (OIE, 2014). The bacteria can be isolated by cultivation, and smears from sampled materials can be examined microscopically with the objective of finding bacteria or bacterial antigen. Also the blood or serum and even milk can be investigated with certain techniques with the aim of finding antibodies against *Brucella* spp. Direct methods that prove live bacteria or bacterial antigens are very specific and reliable, since detectable bacteria implies active infection, but also time-consuming and methods such as culturing require laboratories with appropriate biosafety degree (Xavier et al., 2009). The method of searching for antibodies is the most common for the detection of *Brucella* infection in both humans and animals. They are less expensive and often quicker. However, serology does not have as high specificity as direct methods, when serology only tells that the sampled individual once has been infected, and positive results does not mean that the individual is carrying infection by the time of examination. In addition, there is a risk of results being false negatives as well (Lindahl-Rajala et al., 2016). Although, for investigating the prevalence of disease within an area or a herd, serology is a very useful analysis method. There are different designs of tests that search for different complexes or structures of the antibodies (Hop et al., 2015). Several of these serological tests have been evaluated worldwide (Muktaderul et al., 2011), such as enzyme-linked immunosorbent assay (ELISA), Rose-Bengal test (RBT), *Brucella* fluorescent antibody test (FAT), serum agglutination test (TAT) and complement fixation test (CFT). Since there are no solid evidence of antibodies responding similarly to the same antigen or stage of infection twice, different methods may need to be used to detect infection. Responses from antibodies are generally variable, why these different methods have been designed for the same cause, to adjust to the activity of the antibodies in certain stages of disease, but may vary in accuracy depending on the type of antigen, stage of infection and antibody response. It is suggested that two or more of these tests should be combined to maximize the efficacy, in order to add up the potential of an individual antigen with high specificity with another antigen with high sensitivity, and thus increase the chance of detecting positive samples from truly infected animals (Muktaderul et al., 2011).

Zoonotic aspect

According to The World Health Organization (WHO, 2010) an estimated 500,000 new people worldwide gets diagnosed with brucellosis annually. In 2014 brucellosis was the most frequently reported disease in humans in Kenya (96,571 cases was reported during that year), and it tops the lists over reported zoonoses in humans in a majority of the African countries today (OIE, 2014). However, it is likely that the number of new disease cases is underestimated because symptoms are easily mistaken for other diseases, such as malaria which is a very common disease in these particular areas.

The infection is mainly transmitted to humans from animals through ingestion of contaminated animal products such as raw milk and cheese. There is also a risk for humans to be infected by handling infected materials like afterbirth or stillborn calves, when the placenta contains large amounts of bacterial antigen. *Brucella* spp. is thus able to penetrate through skin, easier if not intact. Even inhalation of airborne bacteria is a potential risk for infection (SVA, 2015). In rural areas, in which a majority of the African population lives, humans are highly correlated with livestock populations, and live very closely to their animals. The proportion of people relying on livestock for their livelihood is in some places in Africa up to 90%, depending on country, climate, and the type of livestock production system. By living close to livestock the exposure to brucellosis is high, and thus they are at high risk of infection (McDermott & Arimi, 2002). In 2014, brucellosis was the most reported zoonotic disease among humans worldwide, later reports are not to be found in statistics. In the whole of sub-Saharan Africa, the numbers are similar. It follows a trend where brucellosis seems to top the lists of most important zoonotic diseases reported (OIE, 2014). Even in high-income countries brucellosis is a disease of great importance, but due to successful control programs it has been managed to be kept under control (SVA, 2015).

Brucellosis in humans is a disease with very diffuse symptoms, and that often gets misdiagnosed. It is multisystemic, may vary between acute to chronic, and is characterized by undulating fever, sweats, malaise, headache, joint and back pain and body wasting. Hence the signs of illness are non-specific, the disease has caused enormous problems with the clinical diagnosis of infection, especially in sub-Saharan Africa, where it constantly gets misdiagnosed as malaria, which is a disease that is very prevalent and gives the same diffuse symptoms, thus they are very difficult to distinguish clinically (McDermott & Arimi, 2002). To diagnose brucellosis, it requires methods that often aren’t present in many areas of low-income countries, and since the disease can't be diagnosed correctly through just clinical signs, it results in significant underreporting of cases (Sowjanya et al., 2013). The incubation period, the time after infection and before symptoms are experienced varies between a week and up to 60 days (Epiwebb, 2008), why people may walk around for long time, unaware of infection. However, inefficiency in affected people may appear and avert from working, resulting in loss of personal finances, but also charging healthcare, causing increased public health costs (Seleem et al., 2010).

Treatment

In animals, the infection may be chronic and responses poor to treatment (Rabinowitz & Conti, 2010). Since the bacteria are intracellular they're difficult to reach and kill with antibiotic treatment. However, the only medication available against brucellosis is antibiotics and even after prolonged administration of antibiotics the treatment can be unsuccessful. Despite treatment, animals may still be able to pass the infection to other animals and humans, which should be considered before commencing a medication regime. Because of this, it is unusual to begin antibiotic treatment in animals. Instead the use of culling or euthanasia is encouraged to prevent the disease from spreading, when *Brucella* is found in a few individuals within a herd (McDermott & Arimi, 2002). If *Brucella* is widespread within an area, the implementation of mass vaccination is used as a tool for lowering the prevalence (FAO, 2010). Although, this requires all animals being tested for *Brucella* in affected herds where the bacteria have been detected.

In cattle, the use of antibiotic treatment is not considered justifiable because of the risk of drug resistance, low efficiency and high cost. Therefore, the procedure of culling to prevent proliferation is often implemented as a last step after that prevalence has been lowered to for example <5% (often lower, depending on the epidemiological situation) using vaccination, as long as it is economically affordable (Ducrotoy et al., 2015; SANCO/6095/2009). In humans, the treatment strategy usually requires three different types of antibiotics to get rid of the infection. There are different drug regimens described but all includes high dosages of doxycycline, gentamicin and streptomycin for a long period of time. Some regimens are even combined with trimethoprim-sulfamethoxazole. If exposed during pregnancy, their obstetric care provider should be consulted. People that are exposed to brucellosis and are under treatment should regularly be monitored and kept supervised to ensure that the symptoms are under control (Rabinowitz & Conti, 2010).

Control

To keep the disease under control, slightly different strategies have been performed in different parts of the world. Some of them are simply not applicable in low-income countries, such as culling, even though it is used as a very effective method in wealthy parts of the world (Godfroid et al., 2011). In high-income countries, the main focus is put on eradication of disease in combination with risk analysis to avoid the bacterium from getting re-introduced. In the developing world on the other hand, the information related to brucellosis and its prevalence is still scarce, and the implementation of advanced control programs is difficult (Seleem et al., 2010). Therefore, the use of vaccinations programs is a valuable strategy in those countries where more drastic methods aren't economically justifiable. Vaccinations ought to be the cornerstone of control programs where livestock are kept in close contact to humans. There are vaccines available for cattle, sheep and goats that have been successfully used worldwide, but unfortunately no vaccines for pigs and wildlife have yet been produced. For humans there are neither any vaccines available, why prevention relies on controlling the animal reservoir.

At the animal/ecosystem/human interface it is critical to reduce opportunities for *Brucella* to jump host species as already seen in livestock, wildlife and humans. This task is a challenge for the future in terms of veterinary public health, as for wildlife and ecosystem managers and will need a "One Health" approach to be successful (Godfroid et al., 2011).

OIE (2009) describes that there are certain factors responsible for the incidence of infection with brucellosis in both humans and animals. The difficulties with the disease are its capacity of quickly spreading between individuals and also the lack of knowledge in contamination routes and adequate hygiene, especially in low-income countries. In addition, necessary vaccinations to achieve immunity in livestock are not widespread in most low-income countries, and many of the existing vaccines against brucellosis are not adapted to be used in tropical conditions since all available vaccines contains attenuated live bacterial antigen, and may be inactivated in high temperatures. Although, there are several vaccines available that are adapted to different animal species. In cattle, there are vaccines developed from a strain (strain 19) of *B. abortus* (called S19 or RB51) that evidently increases resistance to infection (Rabinowitz & Conti, 2009). When using these vaccines, it both stimulates the innate and adaptive immune response, which is similar to the response activated during real infection (Goodwin & Pascual, 2016). The same vaccine has been used in attempts to control outbreaks in wildlife, but without successful evidence of its effectiveness. The way the animal responses after vaccination is variable and associated with how the vaccine is formulated and administrated. For successful vaccination, both the route of administration and the frequency are considered important factors.

To prevent brucellosis, a "One Health" approach is required, where human and animal health disciplines work together. The risk of human infection can only be reduced if the disease in animals is controlled (Rabinowitz & Conti, 2009). Since *Brucella* is able to pass between livestock and wildlife it will remain difficult to control, and preventive measures must be based on keeping the level of disease in livestock as low as possible to avoid people becoming infected. Today there are no human vaccines against brucellosis, and the treatment is rather extensive, why it is essential to avoid infection (Godfroid et al., 2011).

In high-income countries the method of culling is successfully applied to control the disease, after the implementation of vaccination has lowered the prevalence of *Brucella* in herds. Unfortunately, in low-income countries that is not an option when it would jeopardize whole families only source of income and livelihood (Godfroid et al., 2011). These parts of the world would rather serve in extensive vaccination programs, but it is dependent on all affected farmers getting involved and are motivated to achieve a mutual goal, otherwise there is a risk that the attempts fail and all the effort would then be in vain. Insisting on good collaboration between farmers, and between cattle owners and veterinarians, are key factors for successful control of brucellosis (OIE, 2009).

The global situation

In general

Brucellosis is distributed worldwide, but due to eradication and control programs, it is now a rare disease in wealthy countries. Its prevalence is largely dependent on the level of control in domestic animals. For instance, in Europe, several countries have managed to totally eradicate the bacteria. Through extensive and widespread surveillance methods these countries even avoid bacteria crossing the land border and thus remain free from infection despite the disease is circulating in nearby or neighboring countries (Seleem et al., 2010). However, the eradication and control programs of brucellosis in different species have been more or less successfully implemented in different parts of the world. This has resulted in an extremely diverse epidemiological situation in animal brucellosis among different regions (Godfroid & Käsbohrer, 2002). Many north European and Scandinavian countries, including Sweden, Norway, France and Germany, are declared officially bovine brucellosis free, while the situation is less advantageous in parts of the southern Europe. In the USA, the prevalence of bovine brucellosis in cattle have reduced drastically, and the disease is soon to be eradicated, thus expensive control programs in both cattle and humans have been manifested with great success. However, there are areas in which the disease remains a problem due to free-ranging wildlife that serves as reservoirs, mostly around the Greater Yellowstone Area, maintaining the disease from passing between wildlife and livestock (Rabinowitz & Conti, 2009). Since 2002, 17 events where bovine brucellosis has been transmitted from wildlife in the Greater Yellowstone Area have been investigated. Before that, brucellosis was nearly eliminated from the area since no cases at all were reported between 1990 and 2002 (Rhyan et al., 2013). The free-ranging elk and bison now seems to serve as reservoirs for mostly *B. abortus,* passing the disease between each other and domestic cattle, maintaining the infectious cycle, and makes the disease a remaining problem in that particular area (Seleem et al., 2010). On the other hand, in several European Union countries, *B. abortus* has been successfully eradicated due to adequate surveillance and eradication programs that have managed to totally exterminate the disease. Even countries like Australia, Canada, Israel, Japan and New Zeeland have managed to succeed by the same procedure. In these countries, the disease has not been encountered for several years, unlike portions of Asia, some countries in Europe and the Middle East and the entire African continent where *Brucella* currently is considered endemic (Jajere et al., 2016). In some parts of the world it seems to be an up-going trend, and may be correlated to the increasing herd sizes. In large parts of Asia, the disease is still considered endemic, even though many countries have put large effort and resources in surveillance and control programs. The actual prevalence of Brucellosis in Africa is not yet totally established (Seleem et al., 2010).

In Africa

Brucellosis is considered endemic in many African countries. It's a serious obstacle to farmers, livestock and rural economies in the whole of the African continent, where it does not only affect public health, but also food security and animal welfare negatively. There are prevention and control mechanisms present that could be of great help to contain brucellosis both in livestock and humans but they are not yet properly applied in the majority of the African countries (OIE, 2009). In fact, many of these strategies could actually even prevent any other infectious animal disease if used properly. The actual, true prevalence of brucellosis in most African countries is not yet fully established (Seleem et al., 2010). However, geomedical literature surveys by Thimm & Wundt (1975) showed early that brucellosis was more widespread than previously been demonstrated. The disease seems to affect virtually the entire African continent, and its epidemiological spectrum of host animals is rich. In a serological screening, Thimm & Wundt (1975) could demonstrate positive *Brucella* antibody serotitres in all African domestic animal species, and 21 of 26 herbivorous respectively 5 of 12 carnivorous wild species were carrying antibodies against brucellosis. Although Africa is badly covered in the world when it comes to science and research on diseases, especially zoonotic (Alonso et al., 2016), there have been studies showing that brucellosis is present or suspected to exist on 82% of the African continent (which represents 40 of the 49 African countries). In 35 of these 40 countries the disease is reported regularly, and in 20 of them the disease infers a major problem to human and animal health and economy (Thimm & Wundt, 1975). The main risks of infection and reservoirs of disease varies between areas, with the different types of animal husbandry, the main kinds of livestock and human consumption habits. For example, in villages where mainly large numbers of cattle are held, the most likely source of human infection is consuming raw milk, although no studies are yet conducted that could prove a strict correlation between those two factors (Ducrotoy, 2015).

In sub-Saharan Africa and particularly Kenya

In Kenya very few studies in this particular subject are conducted why there are difficulties in concluding the ongoing disease state and how the obstacles in preventing, managing and treatment of disease is experienced. Bovine, porcine, ovine and caprine brucellosis are all common, and considered very important in sub-Saharan Africa (McDermott & Arimi, 2002), though there's a great variation in transmission and presence of the different kinds of brucellosis across the sub-continent. Published studies are based on the relative occurrence, by using serological surveys and tests to detect antibodies against the bacteria, showing previous infection. In a study by Paling et al. (1988) the prevalence of antibodies against *Brucella* spp. in wild and domesticated herbivores in Kenya was investigated. Different species of animals were sampled and it was found that antibody titers to *Brucella* spp. were present in oryx (*Oryx beisa),* eland (*Taurotragus oryx*), cattle (*Bos indicus*) and camels (*Camelus dromedarius*). Other authors (Waghela & Karstad, 1986) conducted a serologic survey of African buffalo (*Syncerus caffer)* and blue wildebeest (*Connochaetes taurinus)* within the Maasai Mara region. They found that antibodies against *Brucella* spp. were present in 30 % of the sampled African buffalo, and in 18 % of the blue wildebeest examined. Titers were found in both sexes and in all age groups. Bacterial isolation from the African buffalos were genotyped as biovars 1 and

3, which are the same biovars commonly found in East African cattle. Thus a transmission between these animals and to other species is a possible reason to the disease's highly noticeable prevalence. According to Waghela and Karstad (1986) a distinguishable increase in serological findings of *B. abortus* were clearly noticeable in herds of African buffalo and blue wildebeest that shared watering points and grazing areas with cattle of the Maasai people, and the larger livestock groups or wildlife herds, the higher was the serological prevalence. Furthermore, McDermott & Arimi (2002) are discussing other factors except large herd sizes that may be associated with the high prevalence of bovine brucellosis in livestock, such as the extensive movement that the cattle are exposed to during search for feed and water, and the mingling with other herds while grazing and drinking. Small, confined herds seem to have the lowest prevalence (McDermott & Arimi, 2002). But still, the real time occurrence of brucellosis in animals in sub-Saharan Africa is, like most other infectious diseases, poorly estimated. More scientific studies are still needed for adequate conclusions. In any way, since the main causal organism seems to be *B. abortus*, there is a high potential risk of people getting infected in these areas as well. Human brucellosis is one of the most reported human diseases in sub-Saharan Africa today (OIE, 2009) and in Kenya it tops the list of human infection and illness caused by zoonoses.

Consequences and impact

There are many impacts coming from *Brucella* infection and outbreak. The economic impact is very severe as it is related to the losses *Brucella* spp. causes in animal production. For example, it entails decreases in milk production, fertility losses and trade restrictions. In the US, these costs annually reach 400 million US dollars (Jajere et al., 2016). However, the disease also increases public health costs and decreased productivity in persons that has been affected. The diagnostics and treatment is expensive, and the convalescence may be very long. Studies conducted in Latin America shows an estimated loss of approximately 600 million US dollar a year in this regard, due to bovine brucellosis (Seleem et al., 2010). For farmers and larger livestock industries bovine brucellosis can be crucial as they rely on livestock for their own living. With decreased animal health comes large losses in production and hence also great economic losses (Muktaderul et al., 2011).

MATERIALS AND METHODS

Implementation of fieldwork

Planning and organizing of fieldwork was made in Nairobi before heading out to Maasai Mara. All affected parties were contacted on forehand, so that all were aware and prepared for the visit. Each farmer was informed about which day they would expect sampling and interviewing to take place, so that they could be home and participate in the study.

Study sites

This study was implemented in the Maasai Mara ecosystem in South Western Kenya (Fig. 1). Three villages (Lemek, Mara Rianta and Endoinyo Narasha) were selected based on their proximity to the national reserve and the potential interactions with wildlife. Lemek and Endoinyo Narasha constitutes the areas with less livestock-wildlife overlap as grazing predominately sedentary, unlike Mara Rianta village that has high wildlife-livestock interactions due to pastoralism.

Lemek is a settlement in Kenya’s Narok County. It is located north-east of Maasai Mara, in the south-western part of Kenya. It was established around 1963 and contains approximately 19,000 acres of land, distributed into mainly pasture, grassland and cropland. There are currently 250 households living in the village, measuring up to 3,000 people allocated within these households. Five of the households have access to piped tap water that is used for drinking, cooking and watering the animals. Some households share these as watering points for livestock, others go and seek water from sources within the village. The households usually let their cattle graze within the village, mixed with other livestock, but they never go to the national reserve because of the great distance, which is approximately 55km. There is some engaging in crop farming and many of the locals are participating in extension and education about natural resource management. Due to the relatively mild climate, it is possible to grow both corn, beans and wheat, allowing some of the residents not having to rely on livestock to the same extent as in other villages where the drought is obtrusive. In the past 20 years, the village has experienced annual outbreaks of livestock diseases.

Mara Rianta was established in the 70s and has a population of about 5000 persons spread between approximately 225 households. The village is located in Narok West district, lies within the Mara North conservancy and borders the Trans Mara district. It is approximately 5 km from the Maasai Mara Game Reserve’s Musiara Gate and many farmers has adapted the strategy of grazing their livestock inside the national reserve and conservation areas, due to shrinking pasture sizes and high population and settlements within the village. Since the animals are allowed inside the reserve of Mara North, much human/wildlife conflicts occurs. The community of Mara Rianta does not engage in crop farming or agriculture and depends almost exclusively on livestock. They let their livestock graze both in the reserve and also within the village which means that the animals mix with a lot of other herds, other livestock and different species of wildlife. They usually blend many households together, and in certain areas within the reserve very large groups of more than a thousand animals are let to graze at the same time.

Endoinyo Narasha is a small village also in Narok County. It was established in the early 1960s and the village is home for approximately 2,500 people. The number of households reaches 230 and the majority is dependent of livestock; keeping and selling livestock, and products that comes from livestock. There is no engaging in any crop farming because of the harsh climatic conditions and extended dry spells. Every year during the wildebeest migration, the community loses large parts of their cattle herds due to livestock disease outbreaks carried by wildlife that grazes on the same pastures. Thus, from 2010 many households have moved to fence their land which have reduced the wildlife-livestock contact. Very few of the households nowadays let their animals graze together, and for many of them the only contact they possibly have with other households is when they cross paths for drinking water. The village is located far away from the Maasai Mara Game Reserve and there is only one rough road connecting it to Narok town. The nearest conservancy is Olkinyei which is about 17 km away.

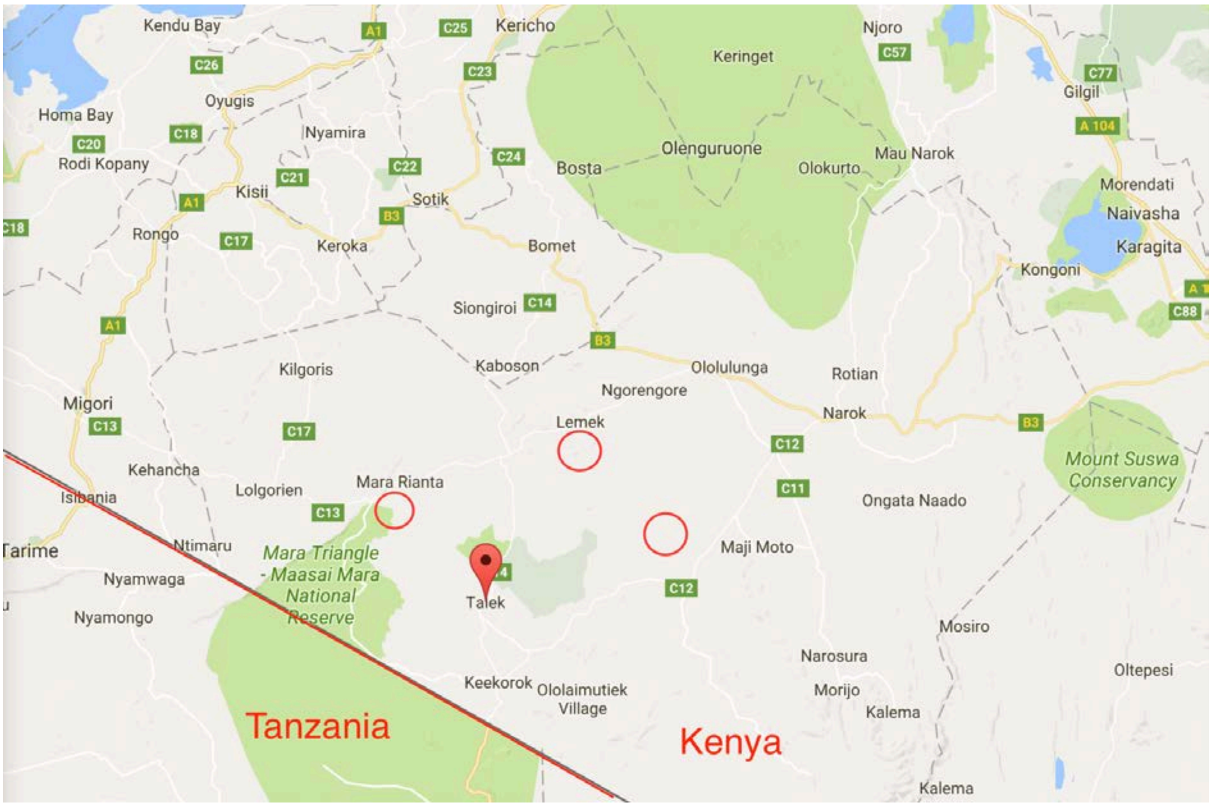

Fig 1: Map showing the sampled villages and their relative location to the national reserve.

Study design and sampling framework

A cross-sectional study design was implemented. Three villages were selected to represent two types of land use. Lemek and Endoinyo Narasha were selected to represent the open areas with low wildlife interactions due to fencing and sedentary grazing while Mara Rianta represented an area with high livestock-wildlife interactions due to nomadic pastoralism.

These three villages have seemingly adopted three different land use strategies in regard to grazing and maintenance of their animals. Livestock are allowed to have different degrees of contact with both wildlife and other animals, which may affect the transmission dynamics of livestock diseases through spill over and contact. The three villages are located within different distances from the national reserve, with Mara Rianta being close to the national reserve while Lemek and Endoinyo Narasha are quite far (Fig. 1). The location of these villages relative to the national reserve can significantly affect the type of land use, grazing patterns and the animal husbandry practices. The proximity of Mara Rianta to the national reserve and Mara North conservancy allow the pastoralists to graze their livestock inside the reserve throughout the year while in Endoinyo Narasha and Lemek, grazing is mainly sedentary in fenced lands, where there is limited contact with other livestock and wildlife. The three villages are dominated by the Maasai tribe, with similar cultural values and traditions in animal keeping.

Questionnaire

Twenty-five households were randomly selected from each village to participate in the study. In each household, a structured questionnaire was administered and data on herd management practices and animal characteristics were collected. Since the study was carried out in collaboration with another study about foot and mouth disease, specific questions in that subject were also included but no analyzed here. The questionnaire was administered during a personal interview with the farmer and covered as many aspects of herd management and the disease situation as was culturally acceptable to ask. It was written in English but translated to Swahili and Maa (the language spoken by Maasai) by native speaker Francis Sopia for easier communication. Farmers were interviewed by Daniel Mutiso, and Francis Sopia were present and available for translation and communicational support through every interview during field work and data collection. The questionnaire included aspects of the annual migration which is called the "transhumance" and contained questions about types of grazing structures, watering points, the number of contacts between herds, other species of livestock that are kept, and the contact with wildlife. The questionnaire took between 30 and 40 minutes to complete.

For each herd of cattle, the following information was collected for this thesis: herd size (proportions of adults >2yrs, calves <1year); herd management practice (pastoral systems, sedentary or intensive management systems); years of operation; herd vaccination status (history of the herd, dates/time since last vaccination, types of vaccines administered); communal grazing areas/grazing reserves present within the village (yes, no); farmers within the village share these grazing areas (yes, no); farmers from other villages share the communal grazing areas (yes, no); cattle herd graze in the national reserve (yes, no); livestock mix with other herds in the grazing areas (yes, no); farmer sight wildlife near livestock at grazing or when on transhumance (yes, no); cattle has been in contact with wildlife (yes, no); farmer has seen following types of wildlife near livestock (ungulates, predators, monkeys, other); farmers' experience of the contact with wildlife (positive, negative, indifferent); farmer sight following species of other livestock near cattle herd (goats, sheep, pigs, poultry, other cattle); source of water is present within the farm (yes, no); water sources are shared with other herds within the village (yes, no); neighboring villages share watering points (yes, no); number of herds using the same watering point (1-5, 5-10, 10-15, 15-20, >20, none); livestock share trek routes with other herds (yes, no, unsure); herd shares water points on trek with other herds (yes, no, unsure); livestock have been bought from market in the last one year (yes, no); types of husbandry (breeding bull own, breeding bull from other farm, artificial insemination, common use);

personnel working on premises visit other livestock holdings, for instance veterinary officers, milk collectors and artificial inseminators (yes, no); vehicles have free access to the farm (yes, no); following signs of illness have been noticed in cattle (fatigue, loss of pregnancy/abortion/stillbirth, decrease in milk production, mastitis/udder swelling and/or pain, fever); people handling the animals have been experienced following signs of illness (fever, sweats, malaise, headache, pain in muscles, joints and/or back).

For full questionnaire, please see attached document (appendix 1).

Selection of farms

The three villages (Lemek, Mara Rianta and Endoinyo Narasha) chosen for the project were the same as in another project about foot and mouth disease that were carried out in the same area at the same time. Therefor the selection of villages was made by ILRI hence it was possible to use the same blood samples for analyzing *Brucella* spp. as in that other project. In that way the discomfort for the animals was minimized, and since the permission from farmers to sample from their cattle already had been received, the decision of working together was made. The three villages were of different character and had quite different strategies when it comes to grazing and handling the cattle. In Endoinyo Narasha most cattle graze inside fences and have very little contact with other animals. In Lemek the cattle graze freely in the village and usually mix with cattle from other households, and in Mara Rianta most of the cattle herds graze inside the national reserve with close contact to wildlife and other livestock. The households, 25 in each village, were also selected by ILRI in advance through a randomized selection process in order to minimize the risk of disease prevalence bias. Three cattle from each herd were randomly selected by the farmer for blood sampling using a rough method of dividing the group of cattle that were present by three, and then choose one cow from each group after counting a certain number (depending on herd size a specific number was chosen). It was difficult to motivate farmers of letting more cows get sampled if they had a larger herd, why it was decided that three cattle from each would be enough, even though a proportional assortment of cows along the size of herd would have been preferable. That was the original plan when herds usually tend to be greater the closer Maasai Mara they graze. Many households even let their cattle graze together inside the reserve.

Sampling animals

The cows that were selected had to be above one year of age to prevent the risk of remaining maternal antibodies. If a cow that was younger than one year of age happened to be selected, a new one was selected randomly instead, and the first one was released without being sampled. For safety reasons, the largest bulls were excluded because of the inability to captivate and handle them in an appropriate manner. Even cows determined to be heavily pregnant were excluded because of the risks associated with handling them. Sampled animals were without any apparent clinical signs of disease, and got an examination regarding their present health status during sampling. Any abnormalities and deviations detected during clinical examination were recorded.

The original plan was to get samples from 50 households in each village, but when difficulties in capturing the cows arose, and the sampling took longer than excepted and what was planned for, the decision of settle for taking samples from 25 of the households was made during the time. The sampling was carried out for three days in each village during the early morning preferably before the cows were let out to graze. To help with the bleeding of the cattle, two local animal health workers trained by veterinarians named William Kububuk and John Kisurkat was hired, to make it easier to get permission from local tribes to sample their animals, and to achieve compliance.

The selected cows were first caught by one person putting a knot around one of the hind limbs using a rope. Then another person grabbed the cows’ head or horns, twisting its neck softly at one side at the same time as a third person held its tail and dragged the hind to the opposite side, putting the cow off balance and getting it uncoordinated until it tilted and fell to the ground, laying on its side. From there, the local animal worker could manage to sample blood from either the jugular vein or the carotid artery. If somehow the animal worker didn’t get any blood from either the vein or artery on that side, the cow was rolled over its back to the other side and a new attempt was made. As a back-up plan milk from lactating cows could be sampled if no blood could be retrieved, since the analysis kit function as well with milk as with serum. This was not necessary though, when every selected cow in the study could be bled in sufficient quantity.

The sampled cattle were ear tagged and labelled with a unique identification code capturing the household and the animal identification number and using a combination of letters and numbers that were decided in advance. Hence none of the villages’ capital letter were the same, the first symbol of the ear tag become the first letter in every villages’ names – “L” for Lemek, “M” for Mara Rianta and “E” for Endoinyo Narasha. Then a number was put for each household in the order that they were visited. The first household in Lemek was written “L1”, the second “L2” and so on. At last, each individual cow got its own number in the order that they were sampled, ex. 001, 002, 003 etc. This meant that one individual cow could be named L1 001 if it was the first cow from a herd in the first household that was visited in Lemek. The individual number was then thoroughly noted on the vacutainer tube after bleeding the cattle so that no blood sample would be mixed with another. The decision of labelling every individual cow was made to make possible that new samples could be collected if necessary in the future, for further science or easier follow-up if positive analyze results were obtained. Further, all the sampled households were georeferenced for easier access if they were to be visited again in the future.

The extraction of blood was made by using an 18G 1.2x40mm needle, and a 10 ml syringe with the aim of getting 9 ml of blood to fill up the vacutainer tubes. Every vacutainer tube was marked with a certain number that the sampled cow was given during clinical examination, as described above. After each day of sampling, the blood samples were transported to a laboratory owned by KWS (Kenya Wildlife Services) in Sekenani inside Maasai Mara game park. There, the blood samples were centrifuged in 7000 RPM for 10 minutes to separate blood cells from serum. The extracted serum was aliquoted into two cryovials and stored at -20 ^{o}C till further screening. The serum was later on analyzed in a laboratory in ILRI, Nairobi, by using a special ELISA method developed to detect antibodies against *Brucella* spp.

Laboratory analysis

The sampled serum was transported frozen from KWS in Sekenani to ILRI in Nairobi, and thawed before analysis. The method used for analyzing the sampled material was an indirect Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of antibodies against *Brucella*, called the PrioCHECK® *Brucella* Antibody 2.0 ELISA (Thermo Fisher Scientific). According to the distributor, the test meets EU requirements (directive 64/432) and OIE standards. The test is designed to detect antibodies against *B.abortus* and *B.melitensis* in serum and milk from cattle, sheep and goats, and the test procedure consisted of 4 steps. First, the serum samples were dispensed in the coated wells of a microtiter plate. If there were antibodies directed against *B. abortus* and *B. melitensis* present in the test sample they were attached to the antigen during incubation. The antibodies that had bound were detected using an anti-Ig monoclonal antibody, conjugated to an enzyme that generates a color signal. If color development occurred, it indicated the presence of antibodies against *B. abortus* or *B. melitensis* in the tested sample. After infection with *B. abortus*, IgM-type antibodies appear, followed by antibodies in the IgG class (IgG1 and IgG2). This specific test kit detects antibodies of the IgG1-type in serum and milk. PrioCHECK® *Brucella* Antibody 2.0 ELISA Kit is suitable for surveillance and monitoring programs in both individuals and herd diagnosis, according to the distributor.

In Kenya it is not usual to vaccinate animals against *Brucella spp*. None of the surveyed farmers indicated that they had vaccinated their livestock against brucellosis. Thus, there was no risk of pre-existing antibodies circulating in the blood stream of sampled animals due to vaccination, which could complicate the diagnosis. However, one should still be aware of the risk of cross-reaction with other gram-negative bacteria when using different kinds of serological test kits. According to the distributor, Thermo Fisher Scientific, cross-reaction with for example *Campylobacter, Escherlichia coli* O:117 and O:156, *Francisella tularensis, Pasteurella, Salmonella* and *Yersinia enterocolitica* may occur when using PrioCHECK® *Brucella* Antibody 2.0 ELISA Kit. This is important to have in mind when interpreting the analysis results.

The assay was conducted according to instructions supplied by the manufacturer. Sampled sera and the positive and negative controls were run in duplicates. To measure the optical density (OD) of the wells, a microplate photometer (Synergy™ HT Multi-Detection Microplate Reader (Biotek®, Winooski, VT, USA) was used to measure at 450nm within 15 minutes after color development had been stopped. The OD (OD_{450}) of all samples were recorded and expressed as percent positivity (PP) relative to the mean OD_{450} of the Positive Control, and defined by the ELISA kit supplier as

$$PP = \left(\frac{OD_{450}\ test\ sample}{Mean\ OD_{450}\ Positive\ Control} \right) x\ 100$$

The threshold for determining if *Brucella* antibodies were present in the test sample was according to the manufacturer's recommendations (≥40%). Animals were considered to be positive if it tested positive on the ELISA-plate, and individuals with results near the threshold (40% +/- 2%) were re-analyzed in order to avoid measurement bias.

Data analysis

The data was established in Excel (Microsoft), and cleaning of data was conducted using the same program before transferring to Stata 14 for Windows (STATA 14, StataCorp LP, USA). Collected information was compiled in a database, and included biodata about each sampled animal as well as some specific knowledge about each herd or farm.

With individual *Brucella* test results as the outcome, the possible effect of sex was assessed using the command for a Pearson's chi^2-test in Stata 14 for survey data. The history of abortions, in proportion to infected herds were also considered. A model restricted to females was developed, where the history of abortions was included as an additional predictive factor contributing to *Brucella* infection. Model assumptions were tested using standard procedures including logistic regression for calculating odds ratio, for example when investigating whether there were any differences in seroprevalence between the villages. Pearson's chi^2-test was used for calculating categorical variables and the student's t-test for continuous variables. The proportion of herds with abortions in total and in each area, in correlation to the proportion of seropositive cattle were determined by using a command in excel for comparing two proportions. The method used to calculate a confidence interval for the difference between two proportions was the Newcombe-Wilson method without continuity correction (as described by Newcombe RG, 1998).

A risk score was calculated by adding all factors from the questionnaire believed to contribute to increased risk of transmission. Factors that was taken into account were; whether cattle are let to graze inside the national reserve; whether cattle mix with other herds while grazing; whether cattle mix with other herds at watering points; whether cattle share trek route with other herds; whether herds share water point on trek; whether farmer has bought livestock from other farms; whether working personnel visit other livestock holdings; and whether vehicles have access to farm. The more of these questions answered with "yes" by the farmers, the higher the risk score.

Separate estimates were obtained for each study site, risk score and sex. The influence of risk score on seropositivity in a herd was tested using a student's t-test. The aim was to investigate whether farms with seropositive animals had a higher risk score. First, the standard deviation was calculated with following equation

$$SD = \sqrt{\frac{\Sigma(x_i - m)^2}{n-1}}$$

where "x_i" is the risk score in a farm and "m" is the mean value of all risk scores in all farms. "n" is the number of sampled farms. Furthermore, "t" was calculated as follows

$$t = \frac{m - \mu}{SD/\sqrt{n}}$$

where "SD" is the standard deviation in farms that are positive for *Brucella*, "m" is the mean value of risk score in farms with infected cattle, and "μ" is the mean value of risk score in all farms. "n" is the number of farms with infected animals.

Table 1. *Cattle sample distribution by study site, planned and obtained number of samples included, from a study on cattle in Maasai Mara national reserve in Kenya.*

Study site	Target herds	Herds sampled	Target number of animals	Animals sampled	Sex	Number of animals
Lemek	50	25	150	75	Male	16
					Female	59
Mara Rianta	50	25	150	75	Male	28
					Female	47
Endoinyo Narasha	50	25	150	75	Male	15
					Female	60

Ethical considerations

The sampling of animals was approved by ILRI institutional animal care and use committee, approval number 2016.20. All participating farmers were informed about the study and asked for their informed consent before initiating the study. Permission was obtained prior to study entry. Farmers were unable to remain anonymous since family names, location of the farm and household information would be collected in the survey for follow up and feedback purposes, but farmers were informed in advance of this and that participation was voluntary. Their consent was documented on a separate paper which was signed by the responsible farmer from each household at the time of the visit. All farmers have approved that photos taken of themselves, their animals and their farms are being published.

RESULTS

None of the selected farmers refused to participate in the study. A total of 225 animals were sampled, and 28 of them (12.4%) tested positive for antibodies against *Brucella* spp. in serum. In the different villages, 75 animals were sampled from each, and the number of positive results varied significantly. In Mara Rianta, a total of 17 animals (22.7%) were positive to brucellosis, while in Endoinyo Narasha only 3 animals (4%) had antibodies that was detectable in serum. Lemek had a total of 8 seropositive animals (10.67%). Table 2 shows the individual *Brucella* seroprevalence in cattle by the three study sites and by sex.

Table 2. *Distribution of Brucella infected cattle in Maasai Mara national reserve in Kenya, divided into study sites and sex.*

	Total	Positive	% Positive	95% CI
Total	225	28	12.44	7.71 – 15.41
Female	166	25	15.06	10.41 – 21.29
Male	59	3	5.08	1.74 – 13.92
Lemek	75	8	10.67	5.50 – 19.66
Mara Rianta	75	17	22.67	14.66 – 33.34
Endoinyo Narasha	75	3	4.00	1.37 – 11.11

As shown in table 2, there seems to be a difference in what areas cattle belong to, with regard to the risk of *Brucella* infection. It is a significantly greater risk of being infected in Mara Rianta compared to Endoinyo Narasha, with cattle from Mara Rianta having an odds ratio 7.03 higher than cattle from Endoinyo Narasha (p=0.003). Compared to Lemek, the odds ratio of being infected is 2.45 times higher in Mara Rianta (p=0.053).

There also seems to be a slightly significant difference between males and females when it comes to *Brucella* infection (p=0.046 with Pearson's chi2-test), with females having an odds ratio 3.98 higher than males. There is a possibility that this distribution occurred by chance, however, the confidence intervals do overlap each other, and calculating the odds ratio using logistic regression results in no clearly significant differences, even though females in this study were infected in larger extent.

Table 3. *The results of a study on cattle from Maasai Mara national reserve in Kenya; Balance between sexes in relation to Brucella infection.*

Sex	Negative (%)	Positive (%)	Total (%)
Female	141	25	166
	(84.9)	(15.1)	(100.0)
Male	56	3	59
	(94.9)	(5.1)	(100.0)
Total	197	28	225
	(87.5)	(12.4)	(100.0)

Farms in Mara Rianta are generally larger compared to the two other villages. Livestock husbandry is illustrated in table 4. All of the participating farmers claims that their animals have regular contact with wild ungulates and other cattle. The most common symptom in cattle observed by farmers is loss of pregnancy/abortion/stillbirth, and in humans, the most common experienced symptom is headache and pain in muscles, joints and/or back.

A total of 20 farms had one or more *Brucella*-infected cattle. In 13 of the infected farms, the farmer claims that they had experienced abortion/loss of pregnancy and/or stillbirth in their cows sometime during the last one year. The number of infected females from these farms were 14. 38 of all 75 farms stated that abortion/loss of pregnancy and/or stillbirth had occurred during the last one year. The odds ratio of having had abortion/loss of pregnancy and/or stillbirth if *Brucella* is present in a farm is 2.2 higher than if *Brucella* is not present (CI: 0.7-6.4). As shown in table 5, cows tend to have higher risk of losing pregnancy/abortion/stillbirth if they live in a farm where *Brucella* is present, but no significant difference could be observed in this case based on this limited selection of animals. Between the villages, no clear significant difference could be found either because of the small number of sampled individuals, but in 34.2% of the farms that have experienced abortion in cattle the last one year, antibodies against *Brucella* was present in serum in at least one individual animal (illustrated in table 6).

Table 4. *Questionnaire summary. Results from a study on cattle in Maasai Mara national reserve in Kenya.*

	Overall	Lemek	Mara Rianta	Endoinyo Narasha
Cattle herd size	109 (5-400)	97 (15-300)	144 (14-400)	85 (5-300)
Years of operation	34.6 (5-70)	35.32 (15-60)	37.08 (15-70)	31.44 (5-70)
% Grazing in reserve	60	32	100	48
% Recently bought livestock (during last one year)	78.7	88	64	84
% Contact with wildlife				
Ungulates	100	100	100	100
Predators	76	52	100	76
Monkeys	34.7	0	36	68
% Contact with other livestock				
Cattle	100	100	100	100
Goats	80	92	48	100
Sheep	80	92	48	100
Pigs	0	0	0	0
Poultry	0	0	0	0
% Signs of illness noticed in cattle				
Fever	4	8	4	0
Fatigue	0	0	0	0
Loss of pregnancy/abortion and/or stillbirth	50.7	48	68	36
Decrease in milk production	9.3	12	4	12
Mastitis/udder swelling and/or pain	22.7	32	24	12
% Self-reported signs of illness experienced by people handling the animals				
Fever	45.3	44	48	40
Sweats	14.7	16	16	12
Malaise	30.7	20	20	52
Headache	57.3	60	48	64
Pain in muscles, joints and/or back	57.3	56	52	64

Table 5. *The results of a study on cattle in Maasai Mara national reserve in Kenya; Relation between Brucella positive females and occurrence of abortions.*

	Farm with abortion	Farm without abortion	Total
Positive female	14 (16.5%)	11 (13.3%)	25 (14.9%)
Non positive female	71 (83.5%)	72 (86.7%)	143 (85.1%)
Total	85 (100.0%)	83 (100.0%)	168 (100.0%)

Table 6. *The results of a study on cattle in Maasai Mara national reserve in Kenya; Relation between Brucella positive farms and occurrence of abortions.*

	Farm with abortion	Farm without abortion	Total
Positive farm	13 (34.2%)	7 (18.9%)	20 (26.7%)
Non positive farm	25 (65.8%)	30 (81.1%)	55 (73.3%)
Total	38 (100.0%)	37 (100.0%)	75 (100.0%)

There was no significant evidence, based on this limited selection, that a higher risk score had influence on *Brucella* infected cattle (t=1.794, critical values -2.093 to 2.093 with significance level 99.5%). Seropositive farms generally do have a higher risk score, but the differences are too small to draw relevant conclusions. In table 7, the risk scores are presented, both in the different study sites, and in infected and non-infected farms. None of the farms had a risk score lower than 5. Farms with at least one seropositive animal have risk score 6-8. One of the infected farms have risk score 6, 10 infected farms have risk score 7 and 8 infected farms have risk score 8. There seems to be no difference in distribution, the differently scored farms are spread between all of the three villages.

Table 7. *The results of a study on cattle in Maasai Mara national reserve in Kenya; Average risk score (and range) for the villages and divided into Brucella seropositive respectively seronegative farms. The risk score was calculated based on how many risk practices were reported by the farmer.*

Study area	Risk score
Lemek	7 (6-8)
Mara Rianta	7.64 (7-8)
Endoinyo Narasha	6.84 (5-8)
Brucella positive farms	7.4 (6-8)
Brucella negative farms	7.07 (5-8)

DISCUSSION

In present study, *Brucella* was found in 12.44% of the 225 sampled animals. Since tests for antibodies doesn't only show active infection, this is indicative of the animals that have been exposed to the bacteria. However, this might be an overestimation as the test used may cross-react with antibodies against other bacteria. There seems to be a significant difference in which areas the cattle are held and let to graze, since the odds ratio of being infected was 7.03 higher in Mara Rianta, compared to Endoinyo Narasha (p=0.003). In Mara Rianta, the herds are let together in large groups, grazing daily in Maasai Mara national reserve, adjacent to wildlife daily. Farmers in Endoinyo Narasha have put fences around their farms, keeping animals from close contact to others than within the own herd. Females in this study were more likely to be infected, but not enough males were sampled to be able to show any significant differences. All of the seropositive males (3) were found in Mara Rianta, which may be a coincidence, but there is a possibility that it represents the real distribution of infected males, but because of the limited selection of only 3 animals in each herd, it can't be ascertained. The impact of herd size with respect to *Brucella* infection was in this case difficult to analyze since infected animals came from farms with highly variable herd size and it was correlated with the study sites. It is likely that a larger herd size entails a greater risk for *Brucella* infection, since bacteria is then able to transmit between several more animals, but due to a small number of sampled farms, no significant differences could be found. Infected cattle in present study came from farms containing 5 – 300 animals. In 2 farms, one in Mara Rianta and one in Lemek, all 3 sampled cattle had anti-*Brucella* antibodies in serum. These herds consisted of 49 respectively 200 animals. 4 farms, 2 in Mara Rianta and 2 in Lemek, had 2 infected cattle each. Herd sizes in these farms varied between 50 and 270 animals. Previous studies suggest small, confined herds having the lowest prevalence of various infectious diseases (McDermott & Arimi, 2002). To have the ability of drawing significant conclusions from information collected in present study, more herds have to be sampled.

All of the participating farmers had access to at least one own bull that they used for breeding. It has long been recognized that the spreading of sexually transmitted diseases is less extensive in farms that uses artificial insemination, given that the procedure and hygiene routines are strictly controlled, and that semen is examined and tested thoroughly before use. Basit et al., (2015) shows a significantly higher prevalence of *Brucella* infections in naturally mated cattle (8%) compared to artificially inseminated (6%). Since brucellosis in cattle is a venereal disease it's a possibility that the transmission cycle is an ongoing problem in Maasai farms due to natural breeding with infected individuals. Antibodies against *Brucella* spp. were found in samples from both sexes, why bulls may act as a carrier of *Brucella* infection to female cattle through natural breeding practice, since symptoms in males aren't as prominent as in females, and are easily missed. Since breeding practice remain the same around the Maasai Mara area, it is impossible to point it out as a single risk factor for disease because disease outbreak seems to undulate instead of being constant, although brucellosis in general is known to be found in any season of the year (Gul et al., 2007). The experience of abortion seems to vary between farms, and possible reasons may include that farmers doesn't notice if a cow loses pregnancy, females may be infected without having abortion, females may abort of other reasons, high percentage of young individuals in a farm and so on. In present study, *Brucella* seems to circulate in herds where farmers claim not to have experienced abortion. However, the questions are addressed to only inform about events in the past year. Antibodies are usually detectable in 4-10 weeks, depending on size, the route of infection and stage of pregnancy (Godfroid et al., 2002), but may in some cases remain in serum long time past infection, why this study is not applicable for investigating the disease incidence. It is a possibility that cattle in these infected farms have had abortions earlier, but is not just mentioned by the farmer. The presence of antibodies against *Brucella* spp. does not necessarily mean that sampled animals are undergoing an active infection at the moment of sampling, it only indicates exposure to bacteria.

The odds of the farm experiencing abortions if *Brucella* is circulating in a farm observed in this study was 2.2 higher than if *Brucella* couldn't be proven in serum, when measuring the proportion of infected farms that claimed having had bovine abortions during the previous year. However, no significant conclusion can be drawn from this information, and there are many other causes why female cattle may abort. It's also possible for females to have brucellosis without undergoing abortion. The question about whether a farm has experienced abortion in cattle during the last year seems not to be particularly relevant in order to predict *Brucella* infection. Hovingh, (2009) describes reasons to abortions in dairy cattle, mentioning brucellosis as the second most common cause among infectious diseases worldwide (brucellosis-free countries and regions excluded). Infectious agents are still considered the most frequently thought of cause of abortion in cattle, but aside from disease, genetic abnormalities in the fetus, toxic agents and heat stress can all affect the reproductive performance in a herd. The last-mentioned factors are causes that aren't as often diagnosed as infectious diseases, but may still be an explanation to why some of the asked farmers claims to have experienced abortion in their herds, even though *Brucella*-infection couldn't be detected in their cattle. Since diagnostic methods such as ELISA-analysis search for antibodies against bacteria, and not the actual bacterial antigen, it is possible that sampled cattle with *Brucella*-negative results may still be infected, especially if they're newly infected hence antibodies aren't produced during the primary stage of infection. In case of *Brucella* infection, the concentration of antibodies increases in various extent, depending on many factors. The duration of the response can also be affected by different factors, including immune status, sex, species, age, pregnancy and virulence of the bacterial strain (Godfroid et al., 2002). Infected cattle may therefore be missed, why sampling in known affected herds ought to be collected repeatedly under a certain time span for adequate surveillance. More relevant conclusions could also be drawn if specific females that undeniably have had an abortion lately would be sampled for investigation of *Brucella* infection as a cause of abortion. Furthermore, the questionnaire could be expanded and adjusted with aim to gain more information about potentially *Brucella* infected farms. However, there are too many questions that potentially could be relevant, and all cannot be evaluated at once. There is a risk of an expanded questionnaire being too overwhelming for the farmers, who may not want to participate at all in that case.

The scoring system designed in this study was formed mainly to investigate whether there was a possibility to use similar systems to predict the risk for infection in the future. The number of questions answered with “yes”, that founded the risk-scoring system, seems to follow the percentage of positive animals, but *Brucella* positive farms had not significantly higher score. Even the selected areas are divided by the percentage of seropositive animals, but due to the limited number of sampled animals, it is scarcely significant. The difference is minimal. If a scoring system like this should be used in order to predict disease, perhaps it would be possible to develop better and more detailed questions to use as an index of risk factors. At the moment, results only seems to be indicative. In future studies, it is suggested that each risk factor should be analyzed separately in order to obtain improved results. Difficulties in asking more detailed and informative questions may be overcome by motivating farmers to answer more detailed. They are currently under pressure, and all time is valuable, therefore the questions would need to be designed in such a way that they provide adequate information without being more time consuming. Some of the questions could have a different focus point, so that more specific information about the situation of *Brucella* infection was obtained. By establishing a reasonable scoring system with easy observation points for the farmers to keep track of, perhaps it could be used to earlier detect signs of infection, and thereby even prevent disease outbreak. It could even contribute as a part of a surveillance program for brucellosis.

In a similar study conducted by Shirima et al., (2016), the prevalence of brucellosis in a wildlife/livestock interface area were investigated, including samples from humans in close contacts to animals. Infection could at that point not be proven in any of the 82 participating humans, even though a majority of them had experienced symptoms and signs of illness that can be connected to *Brucella* infection. However, samples were collected from a very limited group of people, making the study less reliable since *Brucella* spp. is proven to be transmissible between animals and humans in investigations with larger and more randomly selected study groups (Dean et al., 2012).

Wildlife in the proximity of livestock may constitute a risk to the livelihood of the farmers, both because of the unavoidable human-wildlife conflicts but also because they are a possible source of infectious diseases and thereby a risk to the health of the livestock. A significant difference was found in prevalence of brucellosis between the villages, when cattle that are let to graze inside the national reserve and hence commingling freely with wildlife in a higher degree as in Mara Rianta, were more likely to be infected with *Brucella* spp. than cattle that mostly graze behind fences within the own village, as in Endoinyo Narasha. Brucellosis, that potentially is carried by wildlife, could be a source of disease in livestock, and infection have been reported in both African buffaloes (*Syncerus caffer),* blue wildebeest (*Connochaetes taurinus),* oryx (*Oryx beisa)* and eland (*Taurotragus oryx)*; species that are very common in and around the Maasai Mara area (Paling et al., 1988; Waghela & Karstad, 1986). In present study, only one farmer describes the experience of being in contact with wildlife as “indifferent”. The other 47 participating farmers only have negative experiences of the contact with wildlife. A majority of them describes a fear of diseases transmitting from wildlife to their livestock, which was regarded as the largest threat followed by crop destruction and predation. However, proper fencing and night guarding is nowadays used to keep predators away, minimizing the risk of livestock being attacked and hurt, which usually is not a problem during the day. Still, there are lots of negative experiences from wildlife, but many can also directly benefit from living in proximity to wildlife, by being provided goods and services in protected areas. Further investigations in livestock and human populations in areas adjacent to wildlife and a more thorough characterization of circulating pathogens is still necessary, in order to become more aware of the problems and enlighten the opportunities of producing surveillance and monitoring programs that in the future hopefully may lead to disease freedom.

Smits (2013) discusses the possibilities of introducing surveillance programs in affected areas, suggesting adequate vaccination to be one of the most important factors in successful control. Live, attenuated vaccines are currently available, and many of them are successfully used in cattle worldwide. However, vaccines are being re-evaluated since there's a risk of interference with serologic analysis, which may aggravate diagnosis (Letesson et al., 1997). None of the participating farmers had vaccinated their cattle against brucellosis, which reduced the risk of analytic errors considerably, since there was no risk of pre-existing antibodies in sampled cattle due to vaccination. In Kenya, it's still quite unusual to vaccinate livestock against brucellosis, mostly because of difficulties in handle the vaccines, since many of them are sensitive for temperature change which make them inconvenient to use, and farmers don't have accurate knowledge in benefits that comes from vaccination. Godfroid et al., (2011) concluded that implementation of livestock vaccination campaigns could significantly lower disease incidence in both animals and humans, especially in low-income countries where brucellosis is still considered endemic. But it would require a strict regime where all participating parties have to follow instructions, otherwise efforts would be in vain. Many countries have successfully adopted a surveillance program where screening for *Brucella* is completed regularly. Many serological tests have been developed in order to function as a routine diagnostic test just for this reason. Still, there are certain difficulties in serological testing that have to be taken in mind, since cross-reactions with other gram-negative bacteria do occur, which may cause false positive test results. To confirm diagnosis, it requires isolation in culture of bacteria in blood and/or bone marrow, molecular methods, or strong serological evidence of infection through standard tube agglutination or ELISA. Many of these methods are often difficult to obtain or unavailable in low-income countries, why there may be a risk to underestimate the number of cases in these areas.

Omemo et al. (2012) claims in a study that the awareness of brucellosis is low, and at present, not many farmers in and around Maasai Mara are aware of the problems associated with the disease, other than it causes abortions and other health related issues in cows, decreasing their economic income. Many do not reflect that they themselves and their behavior can be a potential risk of infection. Hygiene and prevention strategies need to be highlighted so that farmers get the opportunity to properly handle suspected infected animals. Proper education and information about what signs are relevant to keep track of should be offered to farmers, giving them a chance to detect diseased animals in time. Both in humans and animals, the participating farmers mentioned that they had experienced symptoms that could be related to brucellosis. The most common self-reported symptoms in humans handling the animals were headache and pain in muscles, joints and/or back, while the most common observation in cattle was abortion and stillbirth, and in many of these farms, *Brucella* was detected in serum in one or more of the sampled cattle. Since abortions, loss of pregnancy and/or stillbirths are a major economic loss for affected farmers, many expresses interest in overcoming these problems. Still, many cases of human brucellosis are mistaken for other diseases with similar symptoms. Physicians should have to be more aware about brucellosis being a possible disease in these cases, and more resources needs to be invested in diagnostics so that treatment can begin in time, before disease transmission becomes overwhelming. In countries that successfully has eradicated the disease, effort has been directed at early detection, elimination and prevention in animals, since no practical treatment is available. This method has made many individual herds, areas and even whole countries brucellosis-free. In low-income countries, where *Brucella* spp. is still present, effort cannot be put in the same extent. Since the method of culling affected herds is unthinkable, resources should instead be put on surveillance, protecting of uninfected animals and prevention. It is an uncertain strategy to let *Brucella* circulate between animals within a farm, but once a herd has received infection, there is currently not much to do but protect non-infected animals and humans in contact. A risk like that may need to be accepted in the development of a functioning control program. Countless obstacles come from having *Brucella* present in livestock herds, why farmers may not always want to admit having diseased animals within the farm. Adversities are often experienced, such as inability to sell animals and animal products, since people don't want to buy from affected farmers. Such events may jeopardize the livelihood of farmers, why it's not a rare occurrence that they may hide diseased animals, unaware of the consequences.

Brucellosis remains an important problem in Kenya since there is currently no possibility of eradicating the disease the same way as in high-income countries. Vaccination campaigns do occur but are still rare, and aren't applied often enough to achieve relevant protection and immunity. The method of culling affected animals is not applicable under any circumstances in these areas where livestock is the basis of farmers' livelihood. As long as the disease is endemically spread and circulating in and between animal and human populations, there will always be a risk of transmission into brucellosis-free areas and across country borders. It ought to be in a common interest for all countries to cooperate for keeping the disease under control, to ever overcome the problem and achieve global brucellosis freedom. In Kenya, a suitable method of controlling disease has to be introduced, where compliance from all affected parties is gained.

Disease surveillance, mapping of transmission routes, vaccination campaigns, isolation of exposed animals and thorough examination before introducing new individuals to a healthy animal herd ought to be fundamental since all mentioned factors are important cornerstones in successful disease control. Since there are no vaccines available for humans, prevention of brucellosis in humans relies fully in controlling the animal reservoir.

CONCLUSIONS

Bovine brucellosis is a remaining obstacle in Kenya. Antibodies against *Brucella abortus* and *Brucella melitensis* were present in cattle in and around the Maasai Mara national reserve, suggesting that the infection is very likely to circulate within the ecosystem. Present study reinforces the theory that brucellosis may spread from wildlife since livestock with more wildlife contact also were the ones with the highest prevalence of antibodies in serum. But one should not forget that the disease is easily transmitted between livestock among themselves as well, and to and from the people handling them. Through this study, one cannot say that wildlife is the main cause of infection, but it suggests that they are one important factor that keep the infectious cycle ongoing.

In the present study, bovine brucellosis was found to be present in all three participating villages, suggesting that constant surveillance is still required in case the situation and prevalence rates were about to change. This seroprevalence study was conducted in order to elucidate the disease distribution and extent in livestock in villages located in the wildlife–livestock interface area in the Maasai Mara region so as to invent control and preventive strategies. Thus, further studies in this particular topic, and education of transmission pathways and risk factors in farming households where brucellosis remains an important infectious disease, is required if the prevalence of bovine brucellosis in Kenya, and in particular the Maasai Mara, is to be lowered.

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Appendix 1

Questionnaire

<table>
<tr><td colspan="3">Outbreak investigation form</td></tr>
<tr><td colspan="3">Index case details/ epidemiological unit</td></tr>
<tr><td>Date of form entry:</td><td>Farm owner:

Years of operation:</td><td>Farm name:</td></tr>
<tr><td>Farmer’s Telephone Number: (optional)</td><td>Cattle herd size: (Proportions of adults >2 yrs, calves <1 years)

Goats/sheep nos.</td><td>Herd vaccination status/history of the herd,(dates/time since the last vaccination, vaccines administered prior to outbreaks)</td></tr>
<tr><td>Location of the farm: (Village, Sub-location and Location)</td><td colspan="2">GPS coordinates of the farm:

Herd management practice (pastoral systems, sedentary or intensive management systems)</td></tr>
<tr><td>Proportion of infected cattle in the farm.</td><td colspan="2">The date of onset of clinical signs of the first case in the farm</td></tr>
<tr><td colspan="3">Clinical details of the cases:

Eg Lameness, depression, salivation, staring coat, abortions, anorexia, heat intolerance, decreased milk, typical lesions on the tongue, foot or teat.</td></tr>
<tr><td colspan="3">Type of sample collected: a) Blood……… b) Oral pharyngeal / throat or buccal cavity swabs or epithelium samples/lesions ……… c) Milk………….
Sample reference number (should match the ear tag on the sampled animal)……………</td></tr>
<tr><td colspan="3">The cattle breed sampled:
a) Local breed eg Zebu or Borana………………………………………………………
b) Others (specify)…………………………………………………………………………
c) Sex of the sampled cattle…………………………………………………………….
d) Age (approximate)……………………………………………………………………...</td></tr>
</table>

Exposure factors' trace back: Farmers' interviews

1. Which areas did you graze prior the outbreak?
2. Do you have communal grazing areas/ grazing reserves within the village?

    a. Yes b. No

3. Do farmers within the village share these grazing areas?

    a. Yes b. No

4. Do farmers from other villages share the communal grazing areas?

    a. Yes b. No

    If yes, when? ____

5. Do your cattle graze in the national park?

    a. Yes b. No

6. Does your livestock mix with other herds in the grazing areas?

    a. Yes b. No

7. How many herds do they mix with while grazing?

    a. Daily: _______ b. Weekly: _______ c. Monthly: _______

8. Did any of the herds your livestock mixed with while grazing have FMD in the last month?

    a. Yes b. No c. Unsure

9. Do you sight wildlife near your livestock at grazing or when on transhumance?

    a. Yes b. No

10. Which types of wildlife do you see near your livestock?

    a. Ungulates b. Predators c. Monkeys d. Other.

11. How do you experience the contact with wildlife?

    a. Positive b. Negative c. Indifferent.

12. Which species of livestock do you see?

    a. Goats   b. Sheep   c. Pigs   d. Poultry   e. Other

13. Do you have a water source for your herd within the farm?

    a. Yes   b. No

14. Are these water sources shared with other herds within the village?

    a. Yes   b. No

15. Do neighboring villages share these watering points?

    a. Yes   b. No

16. How many other herds use this watering point for their livestock?

    a. 1-5   b. 5-10   c. 10-15   d. 15-20   e. >20

    f. None

17. Do your cattle mix with other herds at watering point? How many herds do they mix with when they drink?

    a. Yes   b. No

    If yes, how many?: ____

18. Does your livestock share trek routes with herds?

    a. Yes   b. No   c. Unsure

19. Do your herd share water points on the trek with other herds?

    a. Yes   b. No   c. Unsure

20. Did the neighbouring farmers have FMD in the last one year?

    a. Yes   b. No   c. Unsure

21. Are there herds within the village that you know have FMD?

    a. Yes   b. No

22. Did you buy any livestock in the last one year? How many, species and which market?

    a. Yes   b. No

    If yes, please specify: ____

23. Type of husbandry eg breeding bull, AI, breeding bull own, common use, breeding bull from another farm etc

    Please specify: ____

24. Are there dipping points common for the locals? {as areas where cattle mix freely hence are foci of FMD}

    a. Yes   b. No

25. Do the personnel working on the premises visit other livestock holdings, for instance the veterinary officers, milk collectors and artificial inseminators?

    a. Yes   b. No

26. Do vehicles have free access to the farm?

    a. Yes   b. No

27. Have you noticed any of the following signs of illness in your cattle? (Choose as many as needed):

    a. Fatigue
    b. Loss of pregnancy/abortion/stillbirth
    c. Decrease in milk production
    d. Mastitis/udder swelling and/or pain
    e. Unwillingness to walk/stand
    f. Fever
    g. Blisters in mouth, teats or hooves?

28. Have any of the people handling the animals experienced:

a. Fever
b. Sweat
c. Malaise
d. Headache
e. Pain in muscles, joints and/or back

(Choose as many as needed)