SERO-PREVALENCE OF BRUCELLOSIS AND ASSOCIATED RISK FACTORS TO *BRUCELLA* PATHOGEN IN TIATY, BARINGO COUNTY, KENYA

BY

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DECLARATION

I declare that this is my original work and has not been presented for the award of a degree in any other university:

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DEDICATION

This research work is dedicated to my parents; Phillip Oloo and Josephine Auma, My wife, Caroline; my daughter Shayna and my sons Rowan and Nillan.

ABSTRACT

Brucellosis is a global zoonotic disease caused by *Brucella* pathogen, which affects man and animals. The disease has been reported across the world including Kenya. Information regarding the sero-prevalence and risk factors of *Brucella* pathogen in camels in the pastoral Tiaty is still scanty despite several reported loss of camels due to zoonotic diseases. The main objective of this study was to investigate sero-prevalence of brucellosis, and the risk factors associated with *Brucella* pathogen infections in Tiaty Sub-County, Baringo County. Thus a cross-sectional study was conducted in the study area whereby a total of 105 sera samples were collected from camels in five study locations, predominantly occupied by camel farmers using a multi-stage sampling technique. First stage involved stratifying the study region into administrative unit (Sub-Locations), followed by calculating the number of households to be sampled within each Sub-Location based on randomly generated geographical coordinates using ArcGIS. The samples were prepared and tested to detect antibodies against *Brucella* pathogen by competitive immunosorbent assay (cELISA) in accordance with the manufacturer's instructions. In addition, data, on location of camels, history of retained placenta or abortion, gender, and age was gathered using a questionnaire. DNA extraction and purification was done on the positive samples using the Norgen bacterial genomic DNA isolation kit, then the quality and quantity of DNA were determined according to the manufacturer's instructions. The data on risk factors was analyzed using chi-squared test (X^2) at 95% confidence interval (CI) to investigate the relationship between brucellosis and the risk factors. The overall sero-prevalence of 20.0% was reported in camels. The percentage sero-prevalence per study location indicated that Ribikwo had the highest seroprevalence of 38.1% while Silale recorded the least 14.3%. Chemolingot, Lolyamorok and Kollowa locations recorded 28.6%, 19.0% and 0.0% respectively. The proportions of seropositivity in the study locations were significantly higher which revealed a significant association between sero-positivity of camel brucellosis with the location, ($p = 0.037$). It was revealed that brucellosis was associated with age and gender of the camels, further logistic regression analysis on gender revealed that there was 4 times more likelihood of females being seropositive as compared to males *(OR = 4.329, 95% CI = 0.971-19.307, P-value 0.050).* Further, logistic regression analysis on age revealed that there was 5.8 times more likelihood of seropositivity of brucellosis occurring in camels < 2 years old compared to those aged 2-3 years and those over 3 years old *(OR = 5.845, 95% CI = 1.340-25.489, P-value 0.019)*. History of abortion was found to have no significant association with camel brucellosis which implies that abortions in camels is mainly not linked to brucellosis. Further logistic regression analysis on the age of the camels found out that there is 0.5 times more likelihood of brucellosis occurring in camels with history of abortion (*OR = 0.522, 95% CI = 0.118-2.305, P-value 0.391).* The high sero-prevalence reported in this study incriminates cattle, sheep and goats as the source of infection since they are reared in close association with camels. The current study revealed endemicity of brucellosis in camels reared in the study area. The determinants of brucellosis seropositivity were found to be gender and age of camels. Therefore, the present study recommends increase of camel brucellosis awareness, one health approach and mass vaccination programs targeting multiple species cattle, goats' sheep, to curb spread of the disease.

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ABREVIATIONS AND ACRONYMNS

ANOVA:	Analysis of Variance
ASAL:	Arid and semi-arid land
BP:	Base pairs
CFT:	Complement Fixation Test
CCLS:	Committee for Clinical Laboratory Standard
DNA :	Deoxyribose nucleic acid
DNTP:	Di Nucleotide Triphosphate
EDTA :	Ethylene diamine tetra acetic acid
ELISA:	Enzyme Linked Immunosorbent Assay
KNBS:	Kenya National Bureau of Statistics
LPS:	Lipopolysaccharide
MRT:	Milk Ring Test
MBP:	Mega Base Pairs
OD:	Optical Density
OIE:	World Organization for Animal Health
OMP:	Outer membrane protein.
PCR	Polymerase Chain Reaction
RBPT:	Rose Bengal Plate Test
RPM:	Revolutions per minute
SPP:	Species
SSAT:	Serum Slow Agglutination Test
WHO:	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Brucella species – are a group of facultative members of the alpha proteobacteria class capable of causing of causing brucellosis in a range of livestock, human and wildlife.

Disease – any deviation from or interruption of the normal structure or function of any part, organ, or system of the body that is manifested by a characteristic set of symptoms and signs and whose etiology, pathology, and prognosis may be known or unknown.

Infectious – ability to transmit a pathogenic agent from an infected individual to another susceptible individual.

Lipopolysaccharide – are large amphipathic glycol conjugates that typically made of a lipid domain attached to a core oligosaccharide ad distal polysaccharide.

Pathogen – is an organism capable of causing a disease to its host upon entering the body.

Risk factors – are characteristics or variables that increases the likelihood of getting a disease or infection.

Sero-prevalence ─The percentage of individuals in a population who have antibodies to an infectious agent based on serology specimens or as measured in blood serum.

Seropositive—showing a positive result of blood test for anti-Brucella antibodies.

Zoonosis ─ disease transmission from animals to humans through consumption of contaminated products e.g. milk, meat or contact with infected animal or discharge.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Brucellosis is a global zoonotic disease with economic, veterinary and public health ramifications posing a threat to livestock and human health. The causative agent of brucellosis is the Gram-negative bacteria of the genus Brucella, and which is comprised of ten Brucella species recognized globally (Njeru *et al.*, 2016). Brucellosis infection in camels is due to cross transmission between sheep, cattle, and goats. In sub-Saharan Africa, brucellosis is still endemic in ASAL, where extensive pastoral system of herding and human-wildlife interaction being implicated for sustainability of livestock diseases when limited surveillance measures are being undertaken (Njeru *et al.*, 2016; Radwan *et al.*, 1992). In Kenya, brucellosis is predominantly reported in pastoral region where the burden constrains livestock production for instance camels (Lamuka *et al.*, 2017; Lokamar *et al.*, 2020; WHO, 2006). Camels have significantly contributed to the economic growth and improvement of food security for the pastoral communities including Kenya (Lamuka *et al.*, 2017; Schwartz, 1992). Camels are reared and grazed together as mobile grazing herds under pastoral production systems in the arid and semi-arid lands (ASALs) of Kenya (Lamuka *et al.*, 2017). These regions are characterized by high levels of poverty, poor infrastructure, extreme weather and a fragile environment and disease spread (Franc *et al.*, 2018; Lamuka *et al.*, 2017). The socio-economic and health challenges on camel productivity such as abortion, stillbirth, herd infertility, retained placenta and comparatively low milk yield have constrained camel species reared in Baringo County (Lokamar *et al.*, 2020). The disease transmission to humans and animals may occur accidentally mainly through consumption of raw milk, contact with fluids from infected animals, or aborted fetuses especially during birth (Holt. *et*

al., 2011). Studies targeting camel brucellosis have reported, that there is a wide spread of the brucellosis in pastoral camels and therefore is linked to infections in cattle, sheep, goat and pigs which are reared together with camels in the same locality. Few study reports available on prevalence of brucellosis in camels are mainly based on surveys studies which without a clear study design and with small sample size suggesting that relying on surveyed data is not sufficient to inform targeted control of brucellosis (Njeru *et al.*, 2016). There are few studies have reports on detection Brucella DNA in an attempt to detect *Brucella* pathogen while in camels Brucella DNA was reported with a prevalence report of 64.7%, (Akoko *et al.*, 2021) and in Samburu and Isiolo at 32%. Thus, these two studies suggested that serological evidence and detection of Brucella DNA places camels in the list of potentials host reservoir for brucellosis. (Muturi *et al.*, 2021a). At least one or more properly validated serological technique can be used to report *Brucella* antibodies in sera samples since it is recommended by OIE for testing brucellosis in camels, For instance, ELISA test is sensitive and specific is recommended prior to its validation in other animals (Gwida *et al.*, 2012). Isolation of *Brucella* bacteria remains to be gold standard for the diagnosis of brucellosis is However, to isolate *Brucella* bacteria is time- and resource demanding; it requires level 3 biocontainment laboratory to handle samples and live bacteria for to enable identification. Handling all live *Brucella* involves risk of laboratory infection and very strict biosafety rules must be observed. In order to evade these demerits associated with laboratory infection, a more sensitive technique can reliably report (Gwida *et al.*, 2012; Yu *et al.*, 2010). Therefore, the objectives of this study were to investigate the sero-prevalence of camel brucellosis and risk factors associated with *Brucella* infection in camel in resource limited, inaccessible pastoral region of Tiaty sub-county. This knowledge is fundamental and provides preliminary information relevant to understanding

epidemiology of brucellosis in camels and creates awareness as well as informs effective control strategies for camel brucellosis in Tiaty Sub-County, Baringo County.

1.2 Problem Statement

Brucellosis has been reported in several livestock species. Efforts have been made to effectively control the disease targeting specific livestock species. In Kenya, camel brucellosis has not been extensively investigated in comparison with those of other livestock species. The disease is endemic and it is highly prevalent in several developing countries where pastoral and agro-pastoral are practised. Cross infection can occur between cattle, sheep, camels and goats. Camel's susceptibility to infection vary depending on sex, age, geographical location of the camels. Adult animals are more prone to brucellosis compared to young animals. This is after attaining sexual maturity and pregnancy stage due to production of erythritol sugar in the foetal tissues than in young camels. Sex of the animal plays a role in influencing brucellosis susceptibility in animals. Female camels, are more susceptible to brucellosis than male owing to the fact that they exposed to more physiological stresses. Geographical location, influences transmission of Brucella bacteria, depending on the ability of the bacteria to survive in different environment. Camels are not known to be primary host for Brucella pathogen but they are susceptible to *B.abortus* and *B melitensis.* In Kenya a few reports are available based on sero-prevalence data on camels, given that a lot of studies have not focused on marginalized and pastoral region in Kenya. Few serological evidence have reported brucellosis is endemicity in camel population reared in Kenya. However, data on sero-prevalence of camel brucellosis and risk factor associated with *Brucella* pathogen in pastoral Tiaty sub-county remains unknown. This gap has been hindering formulation of appropriate control measures and the collaboration between public health and veterinary health

in controlling brucellosis in camels. Therefore, this study has addressed the existing gap in knowledge through identification of Brucella species that are responsible for infection in camels.

1.3 Justification of the study

Camel keeping play considerable contributions to the economic sector among the pastoral communities marking a significant shift from cattle farming to camel farming which is currently a major source of income and food security in the pastoral region across the globe and in Kenya (Chemuliti *et al.*, 2003; Kagunyu *et al.*, 2014; Njanja *et al.*, 2011). More attention has been accorded to the study of brucellosis in domesticated animal such as cattle, sheep, and goats leaving out camels which has a very important role in the transmission of brucellosis. The seroprevalence and the determinants of brucellosis in the study area has been for a long time remained unknown. A few studies reported brucellosis in counties neighboring Baringo County revealing a wide spread transmission of brucellosis in food animals such as cattle, goats and camels. For instance, studies conducted in pastoral areas of Marsabit and Isiolo counties have shown that camels are reservoirs of brucellosis (Akoko *et al.*, 2021; M. Muturi *et al.*, 2021b). Another study conducted by Lamuka et al, revealed that pastoral farming is characterized by unrestricted mixing of animals in pastures and watering and consumption of raw milk which serve as risk practices for brucellosis transmission (Lamuka *et al.*, 2017). In Baringo County where the current study was conducted, previous studies have shown that brucellosis have serious social and economic implications on productivity of livestock species reared together with camels such as cattle, sheep, goat, sheep. and camels have been affected by abortion, retained placenta, orchitis, and in some cases death of affected animals (Lokamar *et al.*, 2020), which points to the possibility of camels also acting as reservoir for the brucellosis without clinical symptoms. Thus, this study seeks to address the knowledge gap by investigating the sero-prevalence and the drivers of in brucellosis infection in Tiaty that will guide formulation of targeted policies for both the public health and veterinary health departments.

1.4 General objective

To determine sero-prevalence of camel brucellosis and risk factors associated with infection of *Brucella* pathogen in Tiaty Sub-County.

1.5 Specific objectives

i) To determine sero-prevalence of camel Brucellosis in Tiaty Sub-County.

ii).To determine the relationship between camel brucellosis and determinants (gender, age, geographical location, history of abortion and retained placenta) with *Brucella* infection

1.6 Null hypothesis

i).Camels reared in Tiaty area are not infected with brucellosis.

ii). There is no relationship between camel brucellosis and determinants (gender, age, geographical location, history of abortion and retained placenta).

1.7 Significance of the study

The available information regarding camel brucellosis sero-prevalence and determinant of *Brucella* infection has remained unknown for a long period. This study has addressed the gap in knowledge in pastoral camels by generating relevant data. These findings provide a basis for formulation of policies and targeted control programs in all the livestock species geared towards contributing to good health and welfare, of livestock as stipulated in the third sustainable Development Goals (SDGs) of the United Nations. In addition to that these strategies will help to improve health and prevent tropical disease such as brucellosis which affects the rapidly growing camel production sector which is a pillar in sustainability of food security in the big four agenda.

CHAPTER TWO

LITERATURE REVIEW

2.1 Etiology of Brucella species

Brucella are Gram-negative, aerobic, and non- spore forming, facultative intracellular, spore forming, short rods or coccobacilli, non-flagellated, non-capsulated (Abbas *et al.,* 2002a; Holt *et al.,* 2011). Brucella is classified into the genus Brucella which can infect humans, domestic animals and wildlife. (G. Pappas, 2010; Pappas *et al.,* 2006a)Currently the genus Brucella is composed of twelve mono species, however, only six are considered classical species which include *B. abortus, B. melitensis, B. canis, B. suis, B. neotomae*, *B. ceti* and *B. pinnipedialis* from marine animals (Foster *et al.,* 2007). Other species which have also been reported are *B. inopinata* isolated from a human breast implant (Scholz *et al.,* 2008), *B. microti* from the common voles (*Microtus arvalis)* (Scholz *et al.,* 2008), *B. papionis* from baboons (*Papio* spp.), *B. vulpis*, red foxes (*Vulpesvulpes*) and novel Brucella *spp*. in amphibians and fish (J. Godfroid *et al.,* 2011; Whatmore *et al.,* 2016). Camels can be infected by either *B.abortus* or *B. Melitensis (Abbas et al., 2002b)*

2.2 Characteristic of Brucella Pathogen.

Brucella organism grows well under conditions of about 5% -10% of CO_2 and grows faster when the growth medium, is enriched. The bacteria appears smooth or rough when cultured in basal or selective media (Alton *et al.,* 1975). The outer membrane of the cell wall is composed of layers of lipopolysaccharides (LPS) with lipids and proteins interspersed in between. *B. abortus, B .melitensis* and *B. suis* smooth species and made up of two key surface antigens A and M are available in different proportions in the lipopolysaccharides depending on the Brucella species (Alton *et al.,* 1975).

2.3 Ecology, niche and novel reservoir of Brucella

Brucella ecology has evolved rapidly over years and the modern world has provided the pathogen the ability to move from one host to another and has been able to manifest itself in various animal host providing researchers with grounds to trace the manifestations of *Brucella* by utilizing modern techniques (G. Pappas, 2010). Niche is the interrelationship that exists between a species and the way they are connected to each other in a particular ecosystem together and also the way they relate with other things that constitute the ecosystem. The niche of a species in a particular ecosystem confers adaptability and survival to its environment (Polechová *et al.*, 2019). *Brucella* possesses a niche in the intracellular environment of host cells that is specific in a specific cell of the host that is crucial to its sustainability and extensive replication and increase consequently maintains its transmission to other new host cells (Gorvel *et al.*, 2002). On the other hand, reservoir, is an ecologic species that is capable of maintaining and circulating live bacteria through the ecosystem for a period of time. The bacteria can utilize a niche from the host reservoir where it can remain at a low replication rate for a long time, until it finds favorable conditions then starts new replicative cycles and continues to infect other cells (Jacques Godfroid *et al.*, 2013; O'callaghan, 2020). The Brucella preferred and target organs for Brucella include reproductive organs testes, mammary glands and placenta where it has caused reproductive failure, others lungs, spleen Lymph nodes (Dworkin *et al.*, 2006; Poester *et al.*, 2013).

2.4 Prevalence of camel brucellosis globally

Camel Brucellosis is globally distributed and has been reported in all camel-rearing countries whereby the infected may remain asymptomatic carriers of the disease (Wernery, 2014). In some instances, clinical signs, such as abortion, reduced fertility and decreased milk production are manifested in females (Wernery, 2014). In males, brucellosis presents with epididymitis and

orchitis, lesions of lymph nodes and joint capsules, metritis, abortion and reduced fertility (Wernery, 2014). The possibility of cross transmission to other animal species is evident thus, the zoonotic potential of camel's brucellosis should not be underestimated (Zhu *et al.*, 2019). The incidence of brucellosis in increasingly being reported in many developing countries across the world, despite attempts to improve surveillance and control strategies (G. Pappas *et al.*, 2006b). A study reported sero-prevalence of camel brucellosis at 2-5% in countries where pastoralism and extensive husbandry is highly practiced (Khan *et al.*, 2020). In Saud Arabia, *B. abortus* DNA *w*as detected in 8.98%, in which *B. abortus* was detected in camels presenting with diarrhea, and therefore associated with cross border restricted movement of camels in Arabian Peninsula (Alshaikh *et al.*, 2007). Camel brucellosis has been diagnosed in all camel-rearing countries (Sazmand *et al.*, 2012; Wernery, 2014). Studies conducted in Iran from 1987 to 2014 revealed a range of 0.84%–37.83% were positive for *Brucella* (Sazmand *et al.*, 2012), in 2012 32.52% of aborted fetus samples were positive for *Brucella* by conventional PCR (Wernery, 2014).

2.5 Sero-prevalence of camel brucellosis in Kenya

Several studies have reported the frequency of brucellosis in different animal species from various production systems. Studies which have been conducted to investigate brucellosis from pastoral managed camel herds reported sero-prevalence range of 4.6-38 % and 8.0 % from in northern Kenya and a commercial ranch under extensive management in the coast region respectively (Osoro *et al.*, 2015). In another study conducted to investigate brucellosis in milk sampled from camels reported brucellosis infection of 15.36% and 15.22% in Garissa and Wajir counties, respectively (Kagunyu A. *et al.*, 2014). In a study on sero-prevalence of brucellosis in camels is low in extensively kept pastoralist from serum samples collected from camels, reported a prevalence of 2.78% and 1.56% from Garissa and Wajir respectively based on the Rose Bengal

Plate Test for screening. However, all the milk samples examined were negative for Brucella on primary isolation of Brucella on Tryptose Soy agar (TSA), Modified Ziehl-Neelsen's stain as well as under high carbon-dioxide (CO2) concentration (Kagunyu A. *et al.*, 2014). Most recent studies have revealed that camel brucellosis continues to spread in pastoral camels with prevalence report of 64.7% in Narok (Akoko *et al.*, 2021) and 32% in Samburu and Isiolo (Muturi *et al.*, 2021a). Based on the available data camels are at high risk of brucellosis infection since are reared and herded together with other livestock species in the same environment by the pastoral communities. This signals the possibility of brucellosis spill-over from cattle to other animal species living in close proximity and sharing common grazing and/or watering points (Arimi *et al.*, 2005).

Table 2.1: summary of published seroprevalence status of camel brucellosis in Kenya

Study Region / County	Technique	Reference
Coastal Kenya	Detection for presence of Brucella antibodies in serum using RBPT, SAT, c-ELISA reported 8.0 % in a single extensively managed commercial ranch in the coast region	Waghela et al. 1978
Northern Kenya	sero-prevalence study for detection of antibodies against Brucella in serum reported a 4.6 to 38 % from pastoral managed camel herds in northern Kenya, utilizing RBT	Kangunya et al., 1978
Garissa and Wajir	Prevalence of brucellosis and detection antibodies against Brucella in milk from camels utilizing MRT.	Kagunyu *et al.*, 2014)
Isiolo and Samburu	Serological study and detection of Brucella spp. in serum utilizing indirect ELISA and Real time PCR	Muturi *et al* 2021

Marsabit , Kenya	Serological study of brucellosis in serum and Real-time PCR assays with primers specific for IS711 and bcsp31 targets for the detection of Brucella.	Akoko *et. al*, 2020

In Kenya, camel brucellosis has not been extensively investigated in comparison with those of other livestock species. The disease is endemic and it is highly prevalent in several developing countries where pastoral and agro-pastoral are practised. Therefore, the objectives of this study were to investigate the sero-prevalence of camel brucellosis and risk factors associated with camel Brucella infection in selected pastoral area of Tiaty.

2.6 Prevalent Brucella species reported from camels across the world

Camels are susceptible to *B. abortus* and *B. melitensis* (Cooper, 1992). *B. melitensis* have been isolated from milk samples from camels indicating that Brucella contaminates milk (Radwan *et al.*, 1992). A study, reported isolation of *B. melitensis* from aborted fetuses, genital discharge, urine and milk (Radwan *et al.*, 1992). *B. melitensis* biovar 1 and *B. melitensis* biovar 3, was also isolated from lymph nodes samples obtained from camels, in Iran and Kuwait, respectively (Zowghi *et al.*, 1988). In Jordan *B. melitensis* biovar 3 was recovered in milk samples and aborted foetus (Hawari, 2008)while in Sudan *B. melitensis* biovar 3 and *B. abortus* biovar 6 was recovered from lymph nodes in different studies (M. T. Musa, Eisa, El Sanousi, *et al.*, 2008). In Libya, *B. abortus* biovar 1 was isolated from fetal stomach contents (Gameel *et al.*, 1993) in Saudi Arabia, *B. melitensis* biovar 1 was isolated in milk samples, vaginal swab and aborted foetus (Gameel *et al.*, 1993); *B. melitensis* biovar 1, 2 were also isolated from camels' milk in the same country; *B. melitensis* biovar 1, 2, 3 was also isolated in milk (Radwan *et al.*, 1992).

In Egypt, *B. abortus* biovar 1 was isolated in lymph nodes, vaginal swab (Abbas *et al.*, 2002b) while a different study, *B melitensis* biovar 3 was isolated from milk of two she-camels and results

suggested that camels were infected from cattle, goat, where *Brucella biovar* 3, is prevalent among ruminants in Egypt. This further suggested that camel brucellosis transmission is further aggravated by the fact that camels are reared in close contact with sheep in the same household. In Sudan, *B. melitensis* without biovar designation was isolated in carpal hygroma tissues, while in another study *B. melitensis* biovar 3 was also isolated in milk (Agab *et al.*, 1994)**.**

Table 2.2: Published DNA probes for genus in camel brucellosis

Region / County	**Brucella antibody or species**	**Technique**	**References**
Marsabit , Kenya	*B. abortus*	Real-time PCR assays with primers specific for IS711 and bcsp31 targets for the detection of Brucella.	Akoko *et. al,* 2020
Isiolo and Samburu	*B. melitensis*	Serological and Real time PCR techniques for detection of Brucella spp.	Muturi *et al* 2021

2.7 Risk factors to brucellosis in animals.

2.7.1 Age of camels

The animal's age has been reported as one of the key inborn factors associated with animal brucellosis. For many years the disease has been associated with adult animals since they are more prone to brucellosis as the animal ages particularly after sexual maturity and pregnancy stage (Bekele *et al.*, 2011), this is partly linked to the production of erythritol sugar in the foetal tissues

and also to frequent interactions with other sick animals (Bekele *et al.*, 2011; Megersa *et al.*, 2012). In young camels, latent infections do occur even though younger camels possess have high ability to resist disease infection whereas in adults and sexually mature animals, susceptibility to infection increases irrespective of the sex of the camels (Bekele *et al.*, 2011; Megersa *et al.*, 2012).

2.7.2 Gender of camels

Previously studies have reported that the sex of the animal plays a role in influencing the brucellosis susceptibility in domestic and wild animals (Muñoz *et al.*, 2010). Other studies have revealed that female camels, are more susceptible to brucellosis than male. This relatively higher susceptibility of female camels than male camels, could be owing to the fact that they exposed to more physiological stresses and partly due to lack of erythritol sugar, males don't interact in herds for long as they are sold once they have reached marketing weight (Salisu *et al.*, 2018)

2.7.3 Geographical location

The transmission of Brucella bacteria, is greatly infection by the ability to survive in different environment. This survival coupled with seasons which are drivers to interaction and contact where the animals meet at pasture and watering points are sparsely distributed increases disease transmission (McDermott *et al.*, 2002; Radostits *et al.*, 2007; H. Schwartz *et al.*, 1992). Brucellosis spread to susceptible camels is equally dependent on the ability to survive in wet environment, which a study reported higher brucellosis positivity during wet season than dry season (Corbel, 1997).

2.8 Diagnosis of brucellosis

2.8.1 Serological diagnosis of brucellosis

Serological tests have been widely utilized in diagnosis, however should not be used alone to provide evidence of brucellosis since the antibodies may be produced at low levels that they can't

be detected in body fluids such as virginal discharge, milk serum, semen or uterine discharge Ghanem *et al.* (2009) Serological tests serve a great step in the routine diagnosis of brucellosis (Alton *et al.*, 1975). The presence of antibodies in serum, milk, vaginal mucus or seminal plasma may be used as a presumptive test (Khan *et al.*, 2020; Njeru *et al.*, 2016) with high practicability, acceptability and low cost implications in brucellosis surveillance studies. Enzyme linked Immunosorbent Assay (ELISA) is an acceptable alternative to blood culture for the diagnosis of brucellosis and it been utilized test for serological detection of anti-Brucella antibodies since it shows high sensitivity, fast, and convenient to detect Brucellosis in suspected animals (Xu *et al.*, 2020a). ELISA assay is available commercially from many sources in kit form and has been applied conventionally in testing for detection of brucellosis in animals. Competitive ELISA (c-ELISA) and Indirect ELISA (i-ELISA) tests are both recommended for brucellosis screening and confirmation for international trade (Perrett *et al.*, 2010; Vanzini *et al.*, 1998). It can discriminate acute infection from chronic infections (Hajia M. *et al.*, 2013). It provides highly sensitive and specific test results hence having advantages over other serological tests (Bundle *et al.*, 2017). Competitive ELISA is simple and readily standardized to differentiate vaccine antibodies of *B.abortus* S19 from natural infection antibodies (Bardenstein *et al.*, 2002).

2.8.2 Techniques for diagnosing of *Brucella* pathogen infectious in camels

In vitro amplification of DNA or RNA, during diagnosing of infectious disease (Bricker, 2002; Sreevatsan *et al.*, 2000). The assay can be utilized to detect Brucella DNA in pure cultures specimens and clinical samples such as blood, urine and serum (Bricker, 2002; Sreevatsan *et al.*, 2000). This has been made possible since the extraction of Brucella DNA can be done directly from the clinical specimen (Bounaadja *et al.*, 2009; Probert *et al.*, 2004). It provides a reliable, sensitive, fast, safe handling and processing of class III fastidious bacteria like Brucella (Doern,

2000). Real -time PCR can perform in identification and characterization of prevalent pathogen in particular region both at genus and specific level when it is modified and designed to include probes, primers which target and performs amplification of regions of the genome where the number of repetitive copies of genetic elements for each species or biovar are located enabling the differentiation of the *Brucella species*. *Brucella* species exhibit high genetic relationship with DNA sequence homology of about 98% (Bounaadja *et al.*, 2009; Cloeckaert *et al.*, 2001). Some of the primers used for the detection of genes such as Insertion Sequence 711 (IS711) from Brucella genome or Insertion Sequence 6501 (IS6501) or 16S rRNA sequence (Bounaadja *et al.*, 2009; Bricker, 2002)16S–23S rRNA spacer region, 1996),31 kDa outer membrane protein (Omp), *bcsp*31 (Probert *et al.*, 2004), 43 kDa outer membrane protein and *omp*2 gene(Fekete *et al.*, 1992; Fekete *et al.*, 1990). The genetic relationship has not hindered discrimination of the various species from each other by high resolution molecular typing methods and techniques.

2.9 Control and Prevention of camel brucellosis

The control of brucellosis in camels involves the use of multiple approaches through proper disposal of dead or carcasses and vaccination of healthy animals (Nicoletti, 2010). The brucellosis control measures should be done in line with methods that are suitable to the parameters prevailing in the regions where the camels are kept. Recommendations for the whole-herd testing and vaccination with appropriate vaccines such as S19 or Rev 1 vaccine for young camels, full dose vaccination should be vaccinated at 4–8 months of age, using a full adult dose of vaccine (Abbas *et al.*, 2002b). At adulthood, camels are vaccinated by live attenuated *B. melitensis* Rev-1 and *B. abortus* S19 which have been proven to be effective against the disease in camels and other

ruminants. However, the two vaccines are feared to be associated with abortion and may also cause infections to human (Abbas *et al.*, 2002b; Wernery, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the Tiaty in Baringo County**.** The area is situated in the pastoral region of Pokot East of Baringo County. It is mainly inhabited by Turgen community. It borders eight other counties namely, west Pokot to the North West, Turkana to the North, Samburu to the North East, Laikipia to the East. It is located on latitudes of 00 degrees 13" South and 1 degree 40" North and Longitudes 35 degrees 36" and 36" degrees 30" East. It covers an area of 11,075.3 square km, approximately 140.5 Km^2 is under water (Fig. 3.1). Tiaty, covers 4540.48 Sq. km and has a population of 133,189 (KNBS, 2009). It is characterized by prolonged drought and seasonal migration of livestock. Communal herding of livestock is highly practiced where animals interact in grazing or watering points. Camels are kept in the same homestead with cattle, sheep and goat. These coupled with uncontrolled mating and lack of policies for vaccination of livestock makes livestock in the region highly predisposed brucellosis.

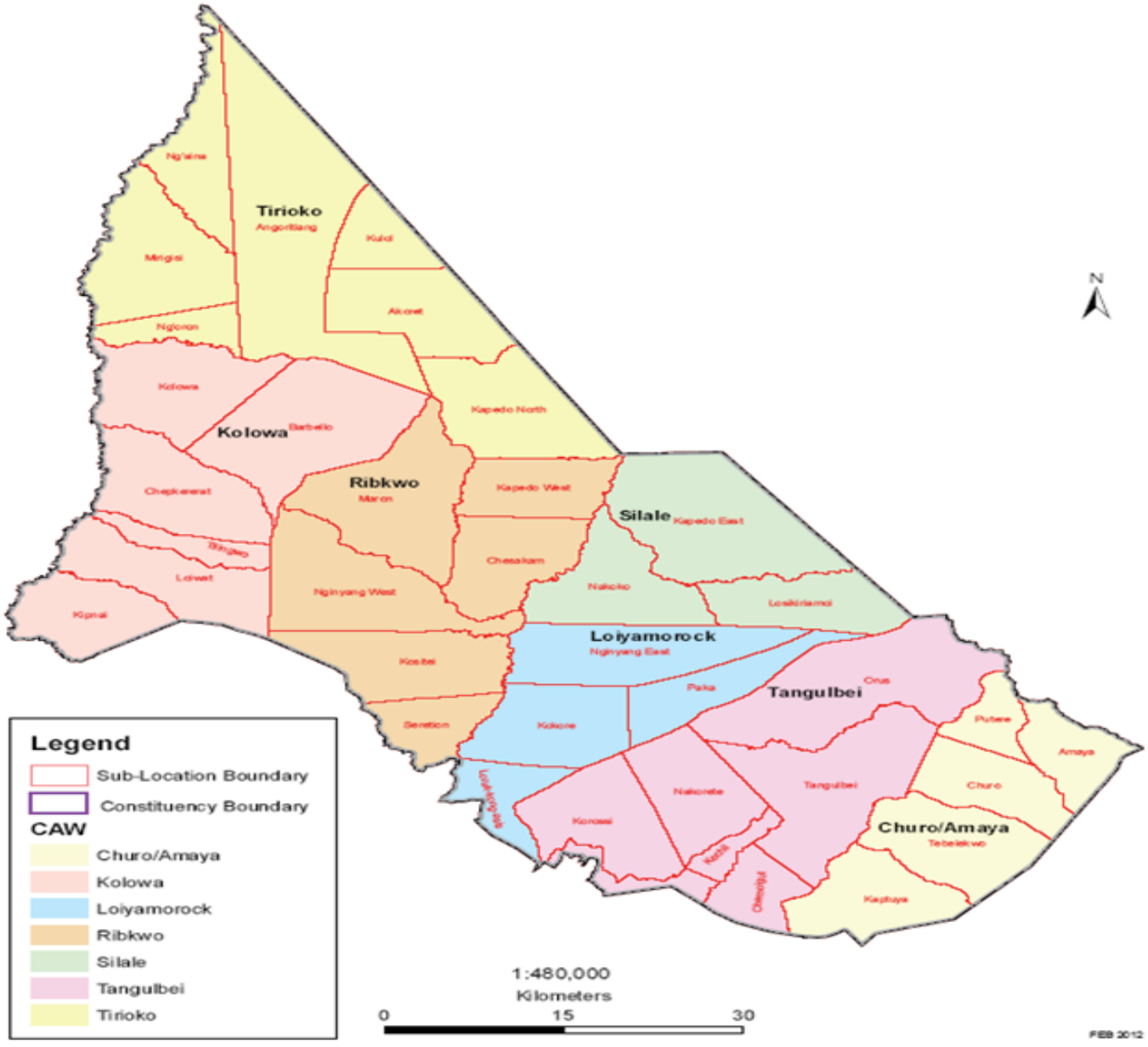

Figure 3.1: Location of Tiaty, Baringo County, Kenya (Source: Kenya Maps, 2022).

3.2 Study Population

The approximate number of camel population in Tiaty Sub-County is 10,500 (KNBS, 2019). The target population for the current study comprised of herds of camels kept in the pastoralist and agro pastoralist and which had not received vaccination against brucellosis for the last 6 month. Camels eligible for sampling were those older than six months of age at the time of sampling and were apparently healthy without symptoms of brucellosis. Camels less than 6 months of age have been reported to have low susceptibility to brucellosis. Camels' were categorized into age groups of older than 3 years, aged between 2 to 3 years and less than 2 years (Gizaw *et al.*, 2017).

3.3 Study design

A cross-sectional study design was utilized to investigate the seroprevalence of brucellosis in camels and the species of *Brucella* spp in selected study regions in Tiaty Baringo County. The study villages (smallest administrative units) were identified putting into consideration the accessibility and availability or population of camels. The farmers who gave consent to participate in the study were given the questionnaire to fill. Data relating to age, sex, history of abortion, history of retained placenta and geographical location of camels were considered as vital determinants of brucellosis infection within and between herds and were assessed in accordance with (Omer *et al.*, 2000) using a pre-tested questionnaire**.** Appendix v

3.4 Sampling method

Sampling was conducted proportionally based on the camel population in the study area. A multi stage sampling technique was utilized. The first stage involved stratifying the study region into administrative unit (Sub-Locations). In the second stage, the number of households to be sampled was calculated within each Sub-Location based on randomly generated geographical coordinates using ArcGIS. The households to be sampled were identified when the team was within the geocode using the "spin bottle method " whereby enumerators spun the bottle until the bottle settles, in the direction facing the mouth of the bottle shows the household keeping camels. The first household in the direction facing the mouth of the bottle was selected. The list of locations where camels are reared was obtained from the nearest Department of animal and veterinary office within the Sub-county.

3.5 Sample size determination

Previously reported data on sero-prevalence was not available, hence an assumed prevalence of 7.4 % was considered for the study area within 95% Confidence Interval (CI) at 5%, absolute precision. Consequently, the sample size (n) was calculated using a formula by Dohoo *et al.,* 2012.

$$n = Z^2 \times P_{ex} \times Q / L^2$$

Where: n = sample size, Z - Confidence Interval = 95% (standard value of 1.96)

Q -1-P_{ex} = (0.926)

P_{ex} Prevalence estimate (expected) = 7.4 %,

L- Absolute precision = 0.05 (5%).

$(1.96)^2 \times 0.074 \times 0.926 / (0.05)^2 = 105$

3.6 Collection, labeling and transportation of samples

The households where the sampling was assigned a unique identification numbers. One hundred and five (105) blood samples aseptically drawn from jugular vein of eligible camels into a plain 10 ml vacutainer tube. Each camel sample was then labelled signed a barcode that also contained three digits that linked them to that of the household. Samples were stored upright in cool box containing ice packs awaiting transportation to Maseno microbiology laboratory. The samples were processed in laboratory, by centrifugation of blood in plain vacutainer tubes at 3000 rpm for 5-10 minutes to obtain serum and transferred into two cryovials with barcode labels. Then stored at -20°C until tested.

3.7 Assessing relationship between brucellosis seroprevalence and risk factors for transmission of brucellosis

This involved administration of a semi-structured questionnaire to each household level alongside camel sampling to determine known risk factors for transmission of brucellosis by interviewing the household head to gather data about sex, age, history of abortion, retained after birth and geographical location of camels. The information on the herd was useful in assessing the Known risk factors brucellosis transmission. The questionnaire was translated from English to the local language to minimize language barrier.

3.8 Laboratory Procedures

3.8.1 Serological detection of anti-Brucella antibodies

Sera samples were examined for the antibodies against Brucella by use of competitive ELISA kit, (Prio CHECK Brucella Ab 2.0 strip Kit) recommended for in vitro detection of antibodies against *B. abortus* in serum and milk. All tests was conducted in strict adherence to the test procedure protocol by the manufacturers; briefly, all reagents and samples were first equilibrated to room

temperature before their use. Serum samples were pre-diluted in dummy plates 1:10, by dispensing 90uL of dilution buffer to the dummy plates and 20 µl of serum sample were dispensed to each of microtiter well plates that are pre-coated with purified standard LPS of Brucella isolates and mixed with 100 µl of the freshly prepared conjugate. 100 µl of both positive and negative controls were dispensed in each test run, sealed and shaken gently and incubated at 25^{o}C for 30 minutes. After incubation the plates were then washed with the wash solution. Then 100 µl of chromogen (TMB) substrate solution was added to each of the wells, and then incubated at 25^{o}C for 15 min and then 100 µl of stop solution was added to each well to stop the reaction. The optical density (OD) of the tests was determined within 15min after colour development using an ELISA reader (Thermo scientific Multiscan FC 3.1). The mean optical density the samples were calculated at a wavelength of 450 nm. The OD of the samples was expressed as percent positivity relative to the mean OD of the mean OD of the positive control. The validation criteria of the test runs was as follows;the mean OD at 450nm of the negative control must be < 0.2 and positive control must be greater than or equal to 1.000 (APHA, 2014). Appendix 1

3.8.2 DNA Extraction and Purification

Genomic DNA was extracted from blood using the Norgen bacterial genomic DNA isolation kit (DNeasy Blood and Tissue Kit, (QIAGEN, Germany, 69504 and 69506) in accordance with the manufacturer's instructions. Briefly, 20 µl, proteinase K and 100 µl of blood was added into a 1.5 ml or 2ml micro centrifuge tube. The volume was adjusted to 220 µl using Phosphate buffered saline (PBS). 200 µl of buffer AL was added and mixed thoroughly by vortexing and then incubated at 56^{o}C, for 10 minutes. 200 µl of 100% ethanol and mix thoroughly vortexing. The mixture were pippeted into a DNeasy mini spin column placed in a 2ml collection tube and centrifuged at ≥ 6000 x g (8000rpm) for 1 minute. The flow and collection tubes was discarded

and the spin column placed into a new 2 ml collection tube, then 500 µl of buffer AW1, and centrifuged for 1 min at ≥ 6000 x g. Then the flow and collection tube was discarded again and the spin column placed into a new 2 ml collection tube, and then 500 µl of buffer AW2 and centrifuged for 3min at 20,000 x g (14,000 rpm). The flow and collection tube were discarded again and spin column transferred into 2 ml micro centrifuge tube, to elute the DNA, 200 µl of buffer AE will be added to the centre of the spin column membrane and then incubated for 1 min at room temperature (25^{o}C) then centrifugation at ≥ 6000 x g for 1min. DNA elution process was repeated twice to increase DNA yield. The quality and quantity of the extracted DNA was determined using a Nano Drop™ 2000c Spectrophotometer (Thermo Fisher Scientific, USA). The extracted DNA were stored at −20°C until further analysis. Appendix IV

3.9 Data management and statistical analysis

The data captured in Microsoft Excel 2010 version. The percentage positivity was calculated by taking the positive samples for brucellosis by ELISA test divided by the total number of samples tested. The coded data was later transferred to SPSS Version for statistical analysis. The non-parametric test, chi-squared test (X^2), was done to analyze for the association between camel sero-positivity and the variables/ risk factors. The degree of association was tested using logistic regression analysis and computed by Odds ratio (OR) at 95% confidence interval (CI). For statistical inference, a test value was considered to be statistically significant when p-value is less than 0.05.

3.10 Ethical considerations.

The study was approved by the Ethics Review Committee of the Maseno University Ethics Review Committee (Approval ref. no MSU/DRPI/MUERC/00600/18) (Appendix 1) and permission was sought from the National Commission for Science, Technology, and Innovation (Ref. no.

NACOSTI/P/18/4661/26645) (Appendix II) that enabled this study to be conducted in Baringo County. Academic approval was obtained from the School of Graduate Studies. Appendix III. Verbal consent was sought prior to data collection. Data obtained was handled with utmost confidentiality at all levels.

3.11 Validity and reliability of research findings

Construct reliability test was assessed using Cronbach's Alpha test. The results revealed an alpha value 0.82 (Hair, 2013). The value was considered reliable since it was above 0.7.The pretesting of the field protocols was carried out and optimization of the laboratory protocols was also done to guarantee reliability of the research findings.

CHAPTER FOUR

RESULTS

4.1. Seroprevalence of *Brucella* antibodies.

The overall sero-prevalence of 20.0% was reported in this study. The study revealed that Ribikwo had the highest number of seropositive animals N=8, followed by Chemolingot, Lolyamorok and Silale. None of the camels sampled from Kollowa had brucellosis infection. The seropositivity percentage per study location indicated that Ribikwo had the highest seroprevalence of 38.1% while Silale recorded the least 14.3%. Chemolingot, Lolyamorok and Kollowa locations recorded 28.6%, 19.0% and 0.0% respectively. The proportions of seropositivity in the study locations were significantly higher which revealed a significant association between sero-positivity of camel brucellosis with the location, (p = 0.037). Table 4.1

Table 4.1: Camel brucellosis seropositivity based on study locations

Study Locations	No. of camels sampled (N=105)	c-ELISA positive (N=21)		P- value
		N	F	
Chemolingot	24	6	25.0%	
Kollowa	25	0	0.0%	
Lolyamorok	10	4	40.0%	**0.037**
Ribikwo	37	8	21.6%	
Silale	9	3	33.3%	

Legend: Pearson Chi-square. *Statistically significant at P ≤ 0.05.

4.2 Relationship between brucellosis and gender of the camels

Out of the 21 *Brucella* seropositive camels; males were 10 (48.0%) and females were 11 (52.0%). The Chi-squared test revealed that there's association between Brucella seropositivity and gender of the camels. The 59.5% seropositive females and 40.5% males was comparable (p-value = 0.553). Further Logistic regression analysis revealed that there was 4 times more likelihood of

females being seropositive as compared to males *(OR = 4.329, 95% CI = 0.971-19.307, P-value 0.050).* Table 4.2

Table 4.2: Relationship between brucellosis seropositivity and gender of the camels

Variables (Gender)	Serological detection of antibodies using c-ELISA				X^2	P-value	OR	95% CI	P-value
	Yes		No						
	N	%	N	%					
Male	10	48.0	34	40.5	0.3	0.553	4.329	0.971-19.307	**0.050**
Female	11	52.0	50	59.5					

Legend: Pearson Chi-square. *Statistically significant at P ≤ 0.05. OR odds ratio, 95% CI 95% Confidence Interval. OR generated through logistic regression analyses.

4.3. Relationship between age and brucellosis seropositivity

This study reported that 29.0 % of the seropositive camels were adult (>3 years old), 57.0% were young ranging from 2 years to 3 years and 14.0 % were young < 2 years (14.0%). Chi-square test revealed that the association between seropositivity of brucellosis and age of the camels in years; 2-3 years (57.0%), Over 3 years (29.0%) and < 2 years (14.0%) was comparable (p-value = 0.105). Further logistic regression analysis performed revealed that there was 5.8 times more likelihood of seropositivity of brucellosis occurring in animals < 2 years old compared to those aged 2-3 years and those over 3 years old *(OR = 5.845, 95% CI = 1.340-25.489, P-value 0.019).* Table 4.3

Table 4.3: Relationship between age and brucellosis seropositivity

Variables	Serological detection of antibodies using c-ELISA				X^2	P-value	OR	95% CI	P-value
	Yes		No						
	N	%	N	%					
Age (years)									
< 2 years	3	14.0	12	14.3					
2 -3 years	12	57.0	28	33.3	4.5	0.105	5.845	1.340-25.489	**0.019***
Over 3 years	6	29.0	44	52.4					

Legend: Pearson Chi-square. *Statistically significant at P ≤ 0.05. OR odds ratio, 95% CI 95% Confidence Interval. OR generated through logistic regression analyses.

4.4. Association between history of abortion, retained and brucellosis seropositivity.

Camels which reported history abortion were 71.0% (P > 0.05), and retained placenta 42.9% (P-value = 0.495). These showed no statistical significant association with *Brucella* seropositivity which means abortion in camels is mainly not linked to brucellosis. Further, logistic regression analysis revealed that there was 0.5 times more likelihood of seropositivity of brucellosis occurring in camels experiencing abortion (*OR = 0.522, 95% CI = 0.118-2.305, P-value 0.391).* Table 4.4 However, chi-square test revealed that the relationship between history of abortion and seropositivity of brucellosis was comparable (p-value 0.605). while regression analysis for seropositivity and retained placenta showed that there is 1.6 times more likelihood of seropositivity of brucellosis occurring in camels having retained placenta after birth (*OR = 1.607, 95% CI = 0.342-7.965, P-value 0.562).* However, chi-square test revealed that the association between seropositivity of camel brucellosis and abortion was comparable *(p-value 0.495).* Table 4.5

Table 4.4: Relationship of brucellosis seropositivity and history of abortion

Variables	Serological detection of antibodies using c-ELISA				X^2	P-value	OR	95% CI	P-value
	Yes		No						
	N	%	N	%					
Abortions									
Yes	15	71.0	55	65.5	0.3	0.605	0.522	0.118-2.305	0.391
No	6	29.0	29	34.5					

Legend: Pearson Chi-square. *Statistically significant at P ≤ 0.05. OR odds ratio, 95% CI 95% Confidence Interval. OR generated through logistic regression analyses

Table 4.5: Relationship between seropositivity and history of retained placenta

Variables	Serological detection of antibodies using c-ELISA				X^2	P-value	OR	95% CI	P-value
	Yes		No						
	N	%	N	%					
Retained placenta									
Yes	9	42.9	43	51.2	0.5	0.495	1.607	0.342-7.965	0.562
No	12	57.1	41	48.8					

Legend: Pearson Chi-square. *Statistically significant at P ≤ 0.05. OR odds ratio, 95% CI 95% Confidence Interval. OR generated through logistic regression analyses

CHAPTER FIVE

DISCUSSIONS

5.1 Seroprevalence of brucellosis in camels in Tiaty.

The current study detected brucellosis in camels reared in 4/5 location Tiaty. Many studies have reported have reported camels as reservoirs of brucellosis considered as a top priority zoonosis, yet understudied in Kenya. Investigating camel brucellosis in the study area helps to address the gap in knowledge that existed regarding the status of the disease in camels, that is vital to alleviate the burden of brucellosis transmission to humans as well improving the rapidly growing camel production sector in Kenya (Lamuka *et al.*, 2017). Sero-prevalence of camel brucellosis has been reported in other regions across the world has been linked to several challenges such as vaccination of camel herds, dominated by high frequency of unrestricted movement of camels across the borders, sharing of communal grazing lands and watering points (O'Connor *et al.*, 2016), 26.5% obtained in camel in Yobe State, Nigeria (Adamu *et al.*, 2014). The findings of this study disagree with other studies, 3.9% conducted in southern by c-ELISA and 3.1% by i-ELISA in northern Somalia (Ghanem *et al.*, 2009).

The results is in agreement with 4.6-38.0% range in herds of camel reared under pastoral management in northern Kenya (Osoro *et al.*, 2015; Waghela *et al.*, 1978), 23.8% reported in Darfur, Western Sudan (Musa, Eisa, Sanousi, *et al.*, 2008) and 19.4% in Jordan which was associated with lack of awareness regarding zoonotic diseases coupled with existing habit of raw milk consumption and close contact with animals (Hawari Azmi, 2008). However, this study disagrees with a lower seroprevalence of 9.3% reported in camels in Marsabit County (Akoko *et al.*, 2021). In Ribikwo and Chemolingot locations, animals are reared in confinement since it is dominated by agro-pastoralists, this may be responsible for the high sero-prevalence in the two study regions. This is due to the high spread of brucellosis within the entire heard under this

management. In Silale and Lolyamorok location majority of the farmers are pastoralist and are characterized by constant movement of animals in search of food and water, this may play a vital role in transmission of brucellosis hence the prevalence. In Kollowa, the camels were free from brucellosis this may be associated to the low number of camels reared thus reducing contact of camels in extensive production systems, interaction with other livestock species and proper handling of the aborted foetus has contributed to the reduced spread of zoonotic diseases (Warsame *et al.*, 2012). Seroprevalence of camel brucellosis may be attributable to lack of brucellosis control programs including vaccination, the unrestricted animal movements across the borders in regions. This situation is further constrained by rearing camels with small and/or large ruminant animal's creating the existence of infection in the herd facilitates the transmission of the disease. The specific ELISA assay with high sensitivity and specificity has been used in brucellosis screening in camels (Xu *et al.*, 2020b).

5.2. Relationship between seroprevalence brucellosis and risk factors.

In this study, brucellosis seroprevalence and age was analyzed for camels less than 2 years, 2-3 years and over 3 years**.** The seroprevalence increased with the age of camels. A higher seroprevalence of 29.0% was reported in older camels greater than 3 years in comparison to 14.0% in young camels less than 2 years. This report revealed a significant association of age with seroprevalence (p= 0.09). Studies conducted elsewhere have also reported a higher prevalence in adults as compared to younger camels (Megersa *et al.*, 2012). Brucellosis infection is higher since the growth and multiplication of *Brucella* pathogen is increased by age and sexual maturity and is dependent on the increasing level of sex hormones and erythritol (Poester *et al.*, 2013). Furthermore, with increased age exposure to the animals is enhanced (Dhand *et al.*, 2006). The findings of this study agrees with previous studies which reported 64.8% in adults than 35.2% in

younger camels in Jordan (Hawari, 2008), 6.8% to 9.2% (Musa, Eisa, Sanousi, *et al.*, 2008), similarly, this study is in agreement with another study which reported *Brucella* seropositivity in adult at (13.8%) and in young camels at (0%) in Afar Ethiopia (Ghanem *et al.*, 2009). The trend of higher *Brucella* seropositivity in adult camels is partly associated with sexual maturity and females animals as age factor is considered as a risk factor owing to increased production of erythritol sugar in the uterus during gestation period which enhances the virulence and multiplication of disease causing microorganism in the body (Abebe *et al.*, 2017; Gizaw *et al.*, 2017). A statically significant difference was observed between the camel sexes where a higher number of seropositive was observed in females (52.0%) than males (48.0%) in the current study. This findings are supported by other studies which showed a significantly higher seropositivity in females than males in Kano municipality abattoirs (Adamu *et al.*, 2014) and Sokoto, Nigeria (Junaidu *et al.*, 2006). Similarly, this study is in line with sero-prevalence of 2.4% in females than 1.4% in males (Waktole *et al.*, 2022). In addition, disagrees with other studies which reported 0.45% in female, by cELISA in Oman (Alrawahi *et al.*, 2019). There was statistical difference in seropositivity in study locations (p= 0.037). However, the current study found no statistical association between location with seropositivity (p = 0.109, OR= 0.169, CI=0.019-1.483).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY OF THE STUDY FINDINGS

This study maintains the observation that camels reared in Tiaty sub county, Baringo County are infected with brucellosis. However, no infection of brucellosis was reported in Kollowa, this presents a unique situation in brucellosis transmission. Most seropositive camels were found in Ribikwo and lowest in Silale. The proportions of seropositivity in the study locations were significantly higher which revealed a significant association between sero-positivity of camel brucellosis with the location, ($p < 0.05$). The results were inconsistent with the study observation, which revealed that there's an association between seropositivity of brucellosis among camels and gender; female's 59.5% and 40.5% males was comparable (p-value > 0.05). Further regression model revealed that there was 4 times more likelihood of females being seropositive as compared to males. The results disagree with the study observation, revealing an association between seropositivity of brucellosis with age of the camels with 5.8 times more likelihood of brucellosis infecting camels less than 2 years old in comparison to those aged 2-3 years and those above 3 years of age. Brucellosis seropositivity and history of abortion and retained showed no statistical significant association with brucellosis implying brucellosis is not the main cause of abortion in camels. The study did not establish the species of *Brucella* that is there is no Brucella species circulating in camels in Tiaty Sub-County Baringo County.

6.2 Conclusions

Camels play an important role in the sustainability and transmission of brucellosis. Investigating seroprevalence in camels is a crucial first step towards bridging the gap in knowledge that existed in a rapidly growing economic sector among pastoralists across the globe (Lamuka *et al.*, 2017). The current study revealed the endemicity of brucellosis in camels found in Tiaty, The current evidence of brucellosis infections, in Tiaty supports the previous study reports which have shown wide spread of brucellosis in several livestock species including camels in the neighboring county of Isiolo (Muturi *et al.*, 2021a) and Marsabit county (Akoko *et al.*, 2021) which indicate that the population of camel keepers in the region are at high risk of transmission. This poses a serious public health risk and food safety threat in the rapidly growing camel sector (Lamuka *et al.*, 2017). The high sero-positivity may be associated with cross species transmission which incriminates cattle, sheep and goats as the source of infection since they are reared in close association with camels in "boma". The determinant of brucellosis seropositivity were gender/sex and age of camels. Moreover, the study cannot overlook the risk of brucellosis transmission due to unrestricted movement of animals, lack of hygienic conditions during management and free exchange of unsuspecting infected animals with no prior clinical symptoms in the markets, awareness of brucellosis, routine habit of consumption of raw milk and close contact with infected animals.

6.3 Recommendations from the study

i).Increasing camel brucellosis awareness, developing suitable laboratory facilities, promoting collaboration between veterinary and human healthcare personnel, county government and private institutions.

ii).The high sero-prevalence of brucellosis revealed in this study calls for researchers to design strategies such as “global one health”, approach.

iii). Brucellosis control strategies such as mass vaccinations of potential host species including, sheep, cattle, goats.

6.4 Recommendations for future studies.

i). Future studies should be expanded to include a range of real-time PCR typing options for *Brucella* species in order to reveal the species circulating in the camel population.

ii). A study using longitudinal design should be formulated targeting both human and livestock to determine the transmission dynamics of *Brucella* species between human and livestock.

iii). More targeted studies should be developed for characterization of the Brucella biovars and strains in both livestock and humans.

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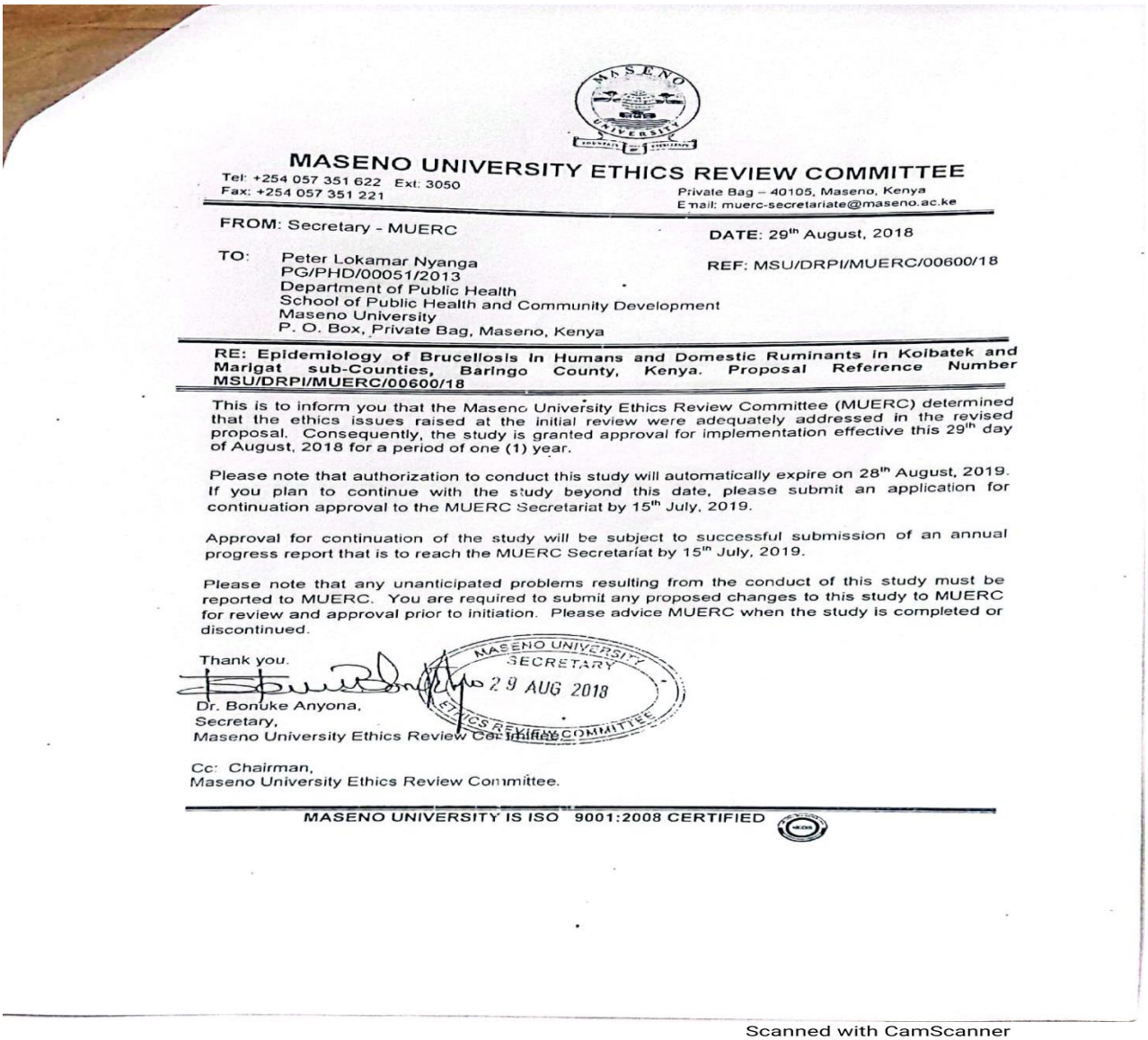
APPENDICES

Appendix I: Ethical Clearance Certificate



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 29th August, 2018

TO: Peter Lokamar Nyanga
PG/PHD/00051/2013
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00600/18

RE: Epidemiology of Brucellosis in Humans and Domestic Ruminants in Kolbatek and Marigat sub-Counties, Baringo County, Kenya. Proposal Reference Number MSU/DRPI/MUERC/00600/18

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 29th day of August, 2018 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 28th August, 2019. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th July, 2019.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th July, 2019.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 29 AUG 2018 ETHICS REVIEW COMMITTEE

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

Scanned with CamScanner

Appendix II: Research Permit from NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/18/4661/26645**

Date: **6th December, 2018**

Peter Lokamar Nyanga
Maseno University
Private Bag
MASENO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Epidemiology of brucellosis in humans and domestic ruminants in Koibatek and Marigat Regions, Baringo County, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Baringo County** for the period ending **6th December, 2019.**

You are advised to report to **the County Commissioner and the County Director of Education, Baringo County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GKalerwa

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Baringo County.

The County Director of Education
Baringo County.

Appendix III: Research Proposal Approval Letter

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSC/SC/00056/2016

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 16th August, 2021

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR OLOO DICKENS OUMA — MSC/SC/00056/2016

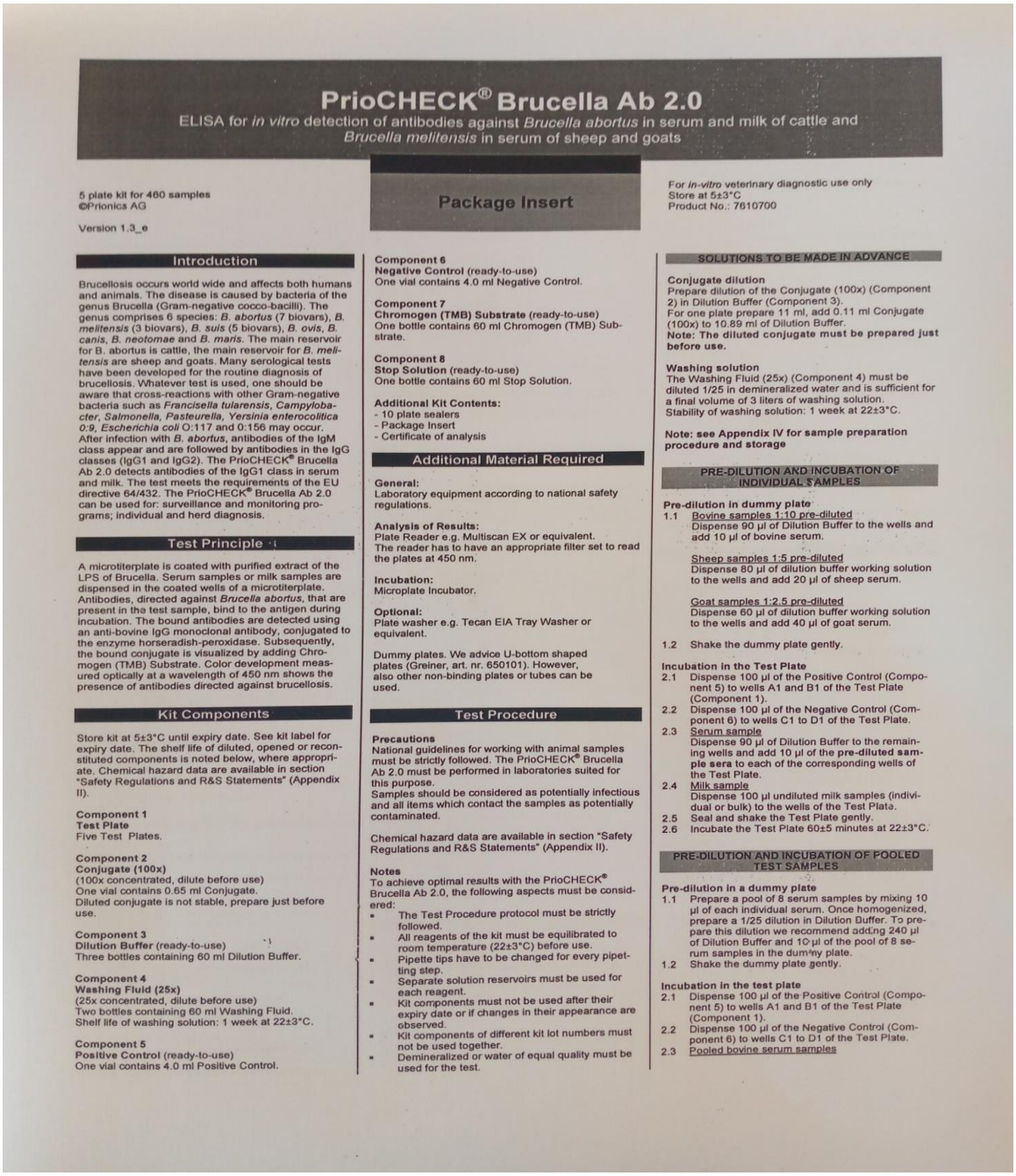
The above named is registered in the Master of Science in Molecular Microbiology and Biotechnology in the School of Physical and Biological Sciences, Maseno University. This is to confirm that his research proposal titled "Prevalence and Molecular Characterization of Brucella Species in Cattle Raw Milk in Busia and Transzoia County." has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

16 AUG 2021

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*

Appendix IV: Prio CHECK™ Brucella Antibody ELISA for in vitro detection of antibodies Protocol (Adapted: From PrioCHECK™ handbook).



PrioCHECK® Brucella Ab 2.0

ELISA for *in vitro* detection of antibodies against *Brucella abortus* in serum and milk of cattle and *Brucella melitensis* in serum of sheep and goats

5 plate kit for 460 samples
©Prionics AG

Version 1.3_e

Package Insert

For *in-vitro* veterinary diagnostic use only
Store at 5±3°C
Product No.: 7610700

Introduction

Brucellosis occurs world wide and affects both humans and animals. The disease is caused by bacteria of the genus Brucella (Gram-negative cocco-bacilli). The genus comprises 6 species: *B. abortus* (7 biovars), *B. melitensis* (3 biovars), *B. suis* (5 biovars), *B. ovis*, *B. canis*, *B. neotomae* and *B. maris*. The main reservoir for B. abortus is cattle, the main reservoir for *B. melitensis* are sheep and goats. Many serological tests have been developed for the routine diagnosis of brucellosis. Whatever test is used, one should be aware that cross-reactions with other Gram-negative bacteria such as *Francisella tularensis*, *Campylobacter*, *Salmonella*, *Pasteurella*, *Yersinia enterocolitica 0:9*, *Escherichia coli* O:117 and 0:156 may occur. After infection with *B. abortus*, antibodies of the IgM class appear and are followed by antibodies in the IgG classes (IgG1 and IgG2). The PrioCHECK® Brucella Ab 2.0 detects antibodies of the IgG1 class in serum and milk. The test meets the requirements of the EU directive 64/432. The PrioCHECK® Brucella Ab 2.0 can be used for: surveillance and monitoring programs; individual and herd diagnosis.

Test Principle

A microtiterplate is coated with purified extract of the LPS of Brucella. Serum samples or milk samples are dispensed in the coated wells of a microtiterplate. Antibodies, directed against *Brucella abortus*, that are present in the test sample, bind to the antigen during incubation. The bound antibodies are detected using an anti-bovine IgG monoclonal antibody, conjugated to the enzyme horseradish-peroxidase. Subsequently, the bound conjugate is visualized by adding Chromogen (TMB) Substrate. Color development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against brucellosis.

Kit Components

Store kit at 5±3°C until expiry date. See kit label for expiry date. The shelf life of diluted, opened or reconstituted components is noted below, where appropriate. Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix II).

Component 1
Test Plate
Five Test Plates.

Component 2
Conjugate (100x)
(100x concentrated, dilute before use)
One vial contains 0.65 ml Conjugate.
Diluted conjugate is not stable, prepare just before use.

Component 3
Dilution Buffer (ready-to-use)
Three bottles containing 60 ml Dilution Buffer.

Component 4
Washing Fluid (25x)
(25x concentrated, dilute before use)
Two bottles containing 60 ml Washing Fluid.
Shelf life of washing solution: 1 week at 22±3°C.

Component 5
Positive Control (ready-to-use)
One vial contains 4.0 ml Positive Control.

Component 6
Negative Control (ready-to-use)
One vial contains 4.0 ml Negative Control.

Component 7
Chromogen (TMB) Substrate (ready-to-use)
One bottle contains 60 ml Chromogen (TMB) Substrate.

Component 8
Stop Solution (ready-to-use)
One bottle contains 60 ml Stop Solution.

Additional Kit Contents:
- 10 plate sealers
- Package Insert
- Certificate of analysis

Additional Material Required

General:
Laboratory equipment according to national safety regulations.

Analysis of Results:
Plate Reader e.g. Multiscan EX or equivalent.
The reader has to have an appropriate filter set to read the plates at 450 nm.

Incubation:
Microplate Incubator.

Optional:
Plate washer e.g. Tecan EIA Tray Washer or equivalent.

Dummy plates. We advice U-bottom shaped plates (Greiner, art. nr. 650101). However, also other non-binding plates or tubes can be used.

Test Procedure

Precautions
National guidelines for working with animal samples must be strictly followed. The PrioCHECK® Brucella Ab 2.0 must be performed in laboratories suited for this purpose.
Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix II).

Notes
To achieve optimal results with the PrioCHECK® Brucella Ab 2.0, the following aspects must be considered:
- The Test Procedure protocol must be strictly followed.
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

SOLUTIONS TO BE MADE IN ADVANCE

Conjugate dilution
Prepare dilution of the Conjugate (100x) (Component 2) in Dilution Buffer (Component 3).
For one plate prepare 11 ml, add 0.11 ml Conjugate (100x) to 10.89 ml of Dilution Buffer.
Note: The diluted conjugate must be prepared just before use.

Washing solution
The Washing Fluid (25x) (Component 4) must be diluted 1/25 in demineralized water and is sufficient for a final volume of 3 liters of washing solution.
Stability of washing solution: 1 week at 22±3°C.

Note: see Appendix IV for sample preparation procedure and storage

PRE-DILUTION AND INCUBATION OF INDIVIDUAL SAMPLES

Pre-dilution in dummy plate
1.1 Bovine samples 1:10 pre-diluted
Dispense 90 µl of Dilution Buffer to the wells and add 10 µl of bovine serum.

Sheep samples 1:5 pre-diluted
Dispense 80 µl of dilution buffer working solution to the wells and add 20 µl of sheep serum.

Goat samples 1:2.5 pre-diluted
Dispense 60 µl of dilution buffer working solution to the wells and add 40 µl of goat serum.

1.2 Shake the dummy plate gently.

Incubation in the Test Plate
2.1 Dispense 100 µl of the Positive Control (Component 5) to wells A1 and B1 of the Test Plate (Component 1).
2.2 Dispense 100 µl of the Negative Control (Component 6) to wells C1 to D1 of the Test Plate.
2.3 Serum sample
Dispense 90 µl of Dilution Buffer to the remaining wells and add 10 µl of the **pre-diluted sample sera** to each of the corresponding wells of the Test Plate.
2.4 Milk sample
Dispense 100 µl undiluted milk samples (individual or bulk) to the wells of the Test Plate.
2.5 Seal and shake the Test Plate gently.
2.6 Incubate the Test Plate 60±5 minutes at 22±3°C.

PRE-DILUTION AND INCUBATION OF POOLED TEST SAMPLES

Pre-dilution in a dummy plate
1.1 Prepare a pool of 8 serum samples by mixing 10 µl of each individual serum. Once homogenized, prepare a 1/25 dilution in Dilution Buffer. To prepare this dilution we recommend adding 240 µl of Dilution Buffer and 10 µl of the pool of 8 serum samples in the dummy plate.
1.2 Shake the dummy plate gently.

Incubation in the test plate
2.1 Dispense 100 µl of the Positive Control (Component 5) to wells A1 and B1 of the Test Plate (Component 1).
2.2 Dispense 100 µl of the Negative Control (Component 6) to wells C1 to D1 of the Test Plate.
2.3 Pooled bovine serum samples

PrioCHECK® Brucella Ab 2.0

Dispense 100 µl of the 1/25 diluted pooled serum samples to the remaining wells of the Test Plate.

2.4 Milk sample
Dispense 100 µl undiluted milk samples (individual or bulk) to the wells of the Test Plate.

2.5 Seal and shake the Test Plate gently.

2.6 Incubate the Test Plate 60±5 minutes at 22±5°C.

Continue with the following procedure for both individual and pooled samples.

INCUBATION WITH CONJUGATE

3.1 Empty the Test Plate and wash the plate 6 times with 200-300 µl washing solution. Tap the plate firmly after the last wash cycle.

3.2 Dispense 100 µl of the diluted conjugate to all wells.

3.3 Seal the Test Plate and incubate 30±5 minutes at 22±3°C.

INCUBATION WITH CHROMOGEN (TMB) SUBSTRATE

4.1 Empty the Test Plate and wash the plate 6 times with 200-300 µl washing solution. Tap the plate firmly after the last wash cycle.

4.2 Dispense 100 µl of the Chromogen (TMB) Substrate (Component 7) to all wells.

4.3 Incubate the Test Plate (in the dark) for 10 minutes at 22±3°C.

4.4 Add 100 µl of the Stop Solution (Component 8) to all wells.

4.5 Mix the content of the wells of the Test Plate.

Note: Start the addition of Stop Solution 10 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and the same pace as the Chromogen (TMB) Substrate solution was dispensed.

READING OF THE TEST AND CALCULATING THE RESULTS

5.1 Measure the optical density (OD) of the wells at 450nm within 15 minutes after color development has been stopped.

5.2 Calculate the mean OD_{450} of the Positive Control (wells A1 and B1).

5.3 Calculate the mean OD_{450} of the Negative Control (wells C1 and D1).

5.4 Calculate the percent positivity (PP) of all the test samples according to the formula below.

The OD_{450} of all samples are expressed as percent positivity (PP) relative to the mean OD_{450} of the Positive Control (wells A1 and B1).

$$PP = \left[\frac{OD_{450} \text{ test sample}}{\text{Mean } OD_{450} \text{ Positive Control}} \right] \times 100$$

RESULT INTERPRETATION

Validation criteria

7.1 The mean OD_{450} of the Negative Control must be < 0.2.

7.2 The mean OD_{450} of the Positive Control must be ≥1.000.

Note: Not meeting any of these criteria is reason to discard the results of that specific test run.

Note: If the mean OD_{450} of the Positive Control is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percent positivity

Bovine serum (individual) samples

PP < 40% (negative)
Brucella antibodies are absent in the test sample.
PP > 40% (positive)
Brucella antibodies are present in the test sample.

Bovine milk (individual or bulk) samples

PP < 25% (negative)
Brucella antibodies are absent in the test sample.
PP > 25% (positive)
Brucella antibodies are present in the test sample.

Bovine serum (pooled) samples

PP < 35% (negative)
Brucella antibodies are absent in the test sample.
PP > 35% (positive)
Brucella antibodies are present in the test sample.

Sheep serum (individual) samples

PP <25% (negative)
Brucella antibodies are absent in the test sample.
PP >25% (positive)
Brucella antibodies are present in the test sample.

Goat serum (individual) samples

PP <25% (negative)
Brucella antibodies are absent in the test sample.
PP >25% (positive)
Brucella antibodies are present in the test sample.

Serum samples in a positive pool need to be retested individually.

Appendix I

Notice
This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

Liability
Prionics AG warrants its products will meet their applicable published specification when used in accordance with their applicable instructions and within the declared products life time. Prionics AG makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of Prionics AG products appearing in Prionics AG published catalogues and product literature may not be altered except by express written agreement signed by an officer of Prionics AG. Representation, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.
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Prionics AG and Prionics Lelystad B.V. are ISO 9001:2000 certified companies.

Appendix II

Safety Regulations and R&S Statements

National Safety Regulations must be strictly followed.

R&S Statements

Component 1
Test Plate
Hazard Code: This product is not classified according to EU regulations.

Component 2
Conjugate (100x)
Hazard Code: This product is not classified according to EU regulations.

Component 3
Dilution Buffer (ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 4
Washing Fluid (25x)
Hazard Code: This product is not classified according to EU regulations.

Component 5
Positive Control (ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 6
Negative Control (ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 7
Chromogen (TMB) Substrate (ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 8  *C Corrosive*
Stop Solution (ready-to-use)
Hazard Code: R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if feel unwell, seek medical advice immediately (show the label on vial).

Appendix III

Sample preparation procedure and storage

Serum
- Samples can be stored at -20°C before testing.
- Samples can be tested individually or in a pool of 8 samples.
- Serum samples have to be pre-diluted in a dummy plate first.
- When titrating serum samples, two-fold serial dilutions should be prepared in Dilution Buffer.

Milk
- Milk samples can be stored at 5±3°C before testing. If milk samples are not tested within 3 days after collection it is recommended to add sodium azide (0.02%) as a preservative.
- The fluid of the milk sample to be tested should be collected from underneath the creamy layer.
- Individual and bulk milk samples are tested undiluted.

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Appendix V: DNA extraction protocol using Qiagen blood and tissue DNA extraction Protocol (Adapted: From Qiagen DNeasy handbook).

Procedure:

1. Pippet 20ul Qiagen proteinase K into the bottom of 1.5ml micro centrifuge tube.

2. Add 200ul sample to the micro centrifuge tube containing 20ul of proteinase K

3. Add 200ul buffer AL

4. Incubate at 56 C for 1 hour.

5. Briefly centrifuge the 15ml micro centrifuge tube to remove the drops from the inside of the lid

6. Add 200ul ethanol (96 – 100%) to the sample, and mix by pulse vortexing for 15 seconds. After mixing, briefly centrifuge to remove drops from inside.

7. Carefully apply the mixture from step six to the QIamp mini spin column 2ml collection tube without wetting the rim. Close the cap and let it stand for 30 minutes before centrifuging at 8000rpm for 1 minute.

8. Place the Qiamp minim spin column in a clean 2ml collection tube and add 500ul of buffer AW1 without wetting the rim. Close and centrifuge at 8000rpm for 1 minute. Then place the spin column in another clean 2ml collection tube.

9. Carefully open the spin column and add 500 ul of buffer AW2 and spin at full speed of 14000rpm for 3minute, then transfer the spin column to another clean 2ml collection tube for a further centrifugation at 14000rpm for 1 minute.

10. Place the Qiamp mini spin column in a clean 1.5 micro centrifuge tube. Carefully open the spin column and add 30ul buffer AE or distilled water. Incubate at room temperature (15-25°C) for at least 2hours, then centrifuge at 8000rpm for 2 minutes.

Appendix VI: Questionnaire used to collect data in Tiaty Sub-County.

	PREVALENCE OF BRUCELLOSIS IN CAMELS IN TIATY SUB-COUNTY, BARINGO COUNTY, KENYA	
	Brucellosis Baseline Compound Questionnaire	
	The following should be read to every potential respondent; May I have a couple of minutes of your time, please? We are researchers affiliated to Maseno University seeking to determine the sero-prevalence of brucellosis and the species of Brucella circulating in camel keeping communities in Tiaty sub- county Baringo County. The questionnaire will be carried out on potential risk of spread of brucellosis. In addition, blood samples (10ml) will be collected from the camels to assess the serological and molecular types of Brucella circulating in the population. Participation is at your own free-will. Although it is not likely that you will benefit directly, the information from this study is expected to benefit your community by enabling the different stakeholders involved in the study to recommend and design appropriate interventions to minimize the prevalence and spread of brucellosis. Any information given will be kept confidential but it can be used in other forums or published in research journals. Moreover, your name will be kept anonymous. Thank you for your assistance in this important endeavor.	
	Do you agree to participate in this survey? The Eligibility Criteria is that the participant must own camel	**1. Yes; Proceed with survey**
		2. No; End the survey
	Date of the survey	
	Start time of the survey	
	Geo point	
Q	**QUESTIONS**	**OPTIONS**
A	**COMPOUND INFORMATION** *[To be answered by the compound Head]*	
	Questionnaire Number	
A.1	Compound ID	
A.2	County	Baringo
A.3	Sub-County	Tiaty
A.4	Sub-Location	1.
		2.
		3.
A.5	Village	1.
		2.
		3.
A.6	Enter the number households in the compound	
B	**ANIMAL DEMOGRAPHICS**	
B.1	**Do you own any livestock (Cattle, Goats, Sheep, Camels)**	**1. Yes(Move to B2)**

		2. No (End the survey)
B.2	**If yes, how many animals in the following categories do you own?**	
	B.21 Cattle	
	B.22 Goats	
	B.23 Sheep	
	B.24 Camels	
C.5	**Have you experienced any of the following signs in your livestock in the last 1 year?**	
	C.51 Abortions	1. Yes
		2. No
	C.52 Still births	1. Yes
		2. No
	C.53 Retained placenta	1. Yes
		2. No
	C.54 Swollen testes	1. Yes
		2. No
	C.55 Weak calf/kid/lamb	1. Yes
		2. No
	C.56 Repeat breeder	1. Yes
		2. No
	C.57 Swollen joints	1. Yes
		2. No
C.6	**Have you ever found aborted fetuses on the grazing pastures and watering points in the last 3 months?**	1. Yes
		2. No
		3. Don't know
D	**INDIVIDUAL ANIMAL** *[To be answered by compound head and the person taking care of the animals. To be filled for each individual animal recruited]*	**Repeat this section for all the individual animals in the compound of study. (sheep, goat and camel)**
D.4	**Maturity status**	
	D.41 Young	1. Yes
		2. No
	D.42 Young adult	1. Yes
		2. No
	D.43 Adult	1. Yes
		2. No
D.5	**What is the gender of the animal?**	**1. Female (Move to D.6)**

		2. Male (Skip to D.13)
D.13	**What is the breeding status of the animal?**	**1. Male (Proceed)**
D.16	**Have you experienced the following symptoms in this animal?**	
	Swollen testes	1. Yes
		2. No
	Swollen joints	1. Yes
		2. No
	Apparent infertility	1. Yes
		2. No
END THE SURVEY BY APPRECIATING THE RESPONDENT FOR HIS/HER TIME AND ACCEPTANCE TO PARTICIPATE IN THE STUDY		