SURVIVAL ANALYSIS METHODS TO ESTIMATE INCIDENCE, SURVIVORSHIP AND RISK FACTORS FOR BRUCELLOSIS IN LIVESTOCK IN KAJIADO EAST SUBCOUNTY, KAJIADO COUNTY, KENYA

DORIS BOSIBORI MARWANGA

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Survival analysis methods to estimate incidence, survivorship and risk factors for brucellosis in

livestock in Kajiado East Subcounty, Kajiado County, Kenya.

Doris Bosibori Marwanga

A thesis submitted in partial fulfilment of the requirements for the award of a Master of Science
degree in Medical Statistics of the University of Nairobi.

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DECLARATION

This thesis is my original work and has not been presented in any institution leading to the award of a degree or any other award.

Sign…………………………………… Date…………………………………

   Marwanga Doris Bosibori

I confirm that this work was written by the above-named student and has been submitted with our approval as supervisor.

Signature.. Date ………………………………………

Dr Joseph Mung'atu

Jomo Kenyatta University of Agriculture and Technology

Signature ………………………………… Date …………………………………………

Dr George Agogo

Centers for Disease Control and Prevention, Kenya

DEDICATION

This thesis is dedicated to my husband Daveline, our wonderful children Daveline Jr. and Darla. Thank you for believing in me and for your love, support, encouragement and your unceasing prayers that saw me through this work.

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ACRONYMS AND ABBREVIATIONS

ACUC   Animal Care and Use Committee

AFT   Accelerate Failure time

AHA   Animal Health Assistant

BSL-2   Biological Safety Level 2

CDC   Centers for Disease Control and Prevention

CFT   Complement Fixation Test

ELISA   Enzyme - Linked Immunosorbent Assays

ERC   Ethical Review Committee

GPS   Global Positioning System

HR   Hazard ratio

I-ELISA   Indirect Enzyme -Linked Immunosorbent Assays

IgG   Immunoglobulin G

IgM   Immunoglobulin M

KEMRI   Kenya Medical research Institute

KNBS   Kenya National Bureau of Statistics

OIE   World Organization for Animal Health

Q- Fever   Query Fever

RBT   Rose-Bengal Test

SAS   Statistical Analysis System

SAT   Standard Agglutination Tests

WHO   World Health Organization

ABSTRACT

Brucellosis is a common bacterial zoonotic disease that is caused by various *Brucella* species and mostly affects cattle, goats, sheep, pigs and dogs. It results in significant economic losses and human sufferings. Manifestation in livestock is mainly through abortions, retained placentas, premature births, infertility and reduced milk production. Despite the disease being successfully controlled in many developed regions, it is still of major public health importance in sub-Saharan Africa. In Kenya, there is limited data on incidence of brucellosis in livestock. The main objective of this study was to estimate the incidence rates, disease risk probabilities and the risk factors associated with time to brucellosis infection in different livestock species in Kajiado County. Multistage sampling technique was used whereby in the first stage 4 out of 17 locations were selected randomly, followed by proportionate simple random sampling of herds in the selected locations. Stratified random sampling was used in selected herds to identify animals that were enrolled into the study. A cohort of 1369 sheep, 1711 goats and 709 cattle from 500 compounds were enrolled into the study and followed up for 9 months. At the animal herd level, risk factors that were assessed included production system, mixing with other herds, contact with wildlife and breeding system. At individual animal level, data was collected on breed, age, sex, species, breeding status and breeding system. Blood samples were collected from enrolled animals at enrolment and on each of the two follow-up visits. Sera was tested for antibodies against *Brucella* using competitive Enzyme-Linked Immunosorbent Assays (ELISA). Semiparametric and nonparametric survival analysis techniques were used to explore risk factors associated with time to brucellosis infection in different livestock species. Disease incidence rates were calculated and

survival probabilities compared using the log rank method. Cumulative incidence was 1.7%, 0.7% and 0.3% in cattle, sheep and goats respectively. Incidence rates for infection with brucellosis were highest in cattle with a rate 7 times that of goats, experiencing worse survival that sheep and goats. On bivariate analysis, the hazard was 1.5 times higher in cattle that were more than 2 years old compared to the under 2 years old though the finding was not statistically significant. Similarly, for sheep the hazard was about 2 times for sheep more than 6 months old. On multivariable Cox proportional hazard regression, there was marginal statistically significant association between natural breeding system and brucellosis infection (p=0.05) in cattle, while sheep that were raised under semi-zero grazing system had an increased hazard for brucellosis infection (p =0.0001).

More brucellosis incidence studies for *Brucella* infection are required to aid in the understanding of transmission dynamics and therefore inform prevention and control programs in pastoralist settings.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Brucellosis is transmitted from animals to humans through contact with infected animals and their products directly or indirectly. It is caused by bacteria of the genus *Brucella* and largely affects sheep, goats, camels, cattle, buffaloes, pigs and yaks. It is the most common bacterial zoonotic disease [6].

It manifests as a febrile illness in humans characterized by undulant fever, sweats, malaise, anorexia, headache back pains, chills and joint pains. These symptoms if untreated may persist for several weeks leading to loss of time from normal activities. It affects all age groups [10]. Infections in humans occur through consumption of contaminated dairy and other animal products, transmission through cuts on the skin and inhaling infectious organisms. It may also be transmitted sexually or through blood transfusion but this is rare [11].

Brucellosis in animals is caused by the following species of *Brucella*; *B. abortus, B. melitensis, B. suis, B. ovis, B. canis and B. neotomae.* The major symptoms in infected animals include abortions, retained placentas, premature births, fertility problems and reduced milk production. Infections in animals are caused by consumption of bacteria from sick animals, contaminated animal feeds, natural breeding and inhaling infectious organisms. In goats and sheep, *B. melitensis* is the main cause of brucellosis, *B. suis* causes the disease in pigs and in cattle *B. abortus* is the main cause [1].

In low income countries, there is an average prevalence of 13% in sheep, goats and cattle, 7% in camels, 5% in other species (dogs and pigs) [8]. A recent seroprevalence study in Kenya found a prevalence of 4.1% in cattle, 10.7% in goats and 7.3% in sheep [9]. Risk of transmission is increased by contact with other herds at common feeding and watering grounds, which is a common practice with pastoralists [14]. To control brucellosis in livestock, the most effective strategies include vaccination, testing and slaughtering of infected animals, and enforcement of biosafety management measures.

Survival analysis techniques can be utilized in identifying factors related to survival. These methods are used to study time to event outcomes such as time to diagnosis of brucellosis. They are mainly used to estimate survival distributions, to test hypotheses of equal survival distributions, and to identify risk factors for an outcome of interest. Approaches to survival analysis can be nonparametric, parametric or semiparametric [17].

There are different statistical models that are used to analyze survival time data such a Weibull, Cox Proportional Hazards model, accelerate failure time (AFT), Exponential and Gompertz. The Cox PH model is the most prevalent semiparametric method used to model survival outcomes as it is more general than other parametric models. It makes no assumptions about the probability distribution of survival times [18]. There is limited research on statistical modelling of the burden of brucellosis in livestock in Kenya. Survival methods could provide a better approach to model the burden in terms of time to brucellosis infection in livestock.

1.2 Statement of the problem

Animal brucellosis impacts livestock productivity. It is associated with deaths, abortions and decreased milk production [2]. This in turn has a socioeconomic impact and indirect health effects on people who depend on livestock for their livelihood. In humans, infection usually requires prolonged treatment, is debilitating and therefore can have significant financial and economic consequence in treatment costs as well as loss of time from daily activities [1].

In most developed countries brucellosis has been effectively controlled in livestock populations. However, it still is a key public health concern in Latin America, Middle East, Mediterranean region, parts of Asia and Africa, with estimated human infections above 500,000 per year [3, 4].

In sub-Saharan Africa the disease is prevalent in countries that practice predominantly traditional pastoral production systems and have inadequate programs for disease surveillance and control [4]. This is mainly due to mixing of livestock during their movement in search of pasture, livestock sharing grazing areas with wildlife, and mixing of animals at watering points which is common in pastoral production systems. A systematic review of 244 studies in low income countries [8] showed an average prevalence of 13% in sheep and goats and cattle, 7% in camels and 5% in other species (pigs and dogs). Another seroprevalence study carried out in Kenya found a prevalence of 4.1% in cattle, 10.7% in goats and 7.3% in sheep [9]. These results are an indication that brucellosis is a main concern in developing countries.

There is limited data on brucellosis incidence in livestock in Kenya. A systematic review of brucellosis in Kenya [12] did not find studies on incidence of brucellosis in livestock. Most of the studies that had been carried out were seroprevalence studies which showed high prevalence

of brucellosis in majorly agro-pastoral and pastoral production systems, where Kajiado County is included.

1.3 Justification

Out of 36 priority zoonotic diseases in Kenya, brucellosis is ranked fourth [5]. This shows its importance since its outbreak in livestock was associated with losses in livestock productivity, and losses in market due to trade bans and quarantines. Globally it has consistently been ranked among the most economically important zoonoses [7]. According to the World Health Organization (WHO), brucellosis is classified as one of the top neglected zoonoses [13].

The outcome from this study will be a more precise assessment of survival, incidence rates and risk factors associated with time to brucellosis infection in different livestock species in a majorly pastoral production system in Kenya.

The study findings may inform control methods by the county and national government as well as other stakeholders.

1.4 Research questions

1. What is the incidence of brucellosis in cattle, sheep and goats?
2. What are the differences in time to brucellosis infection in cattle, sheep and goats?
3. What are the factors associated with time to brucellosis infection in cattle, sheep and goats?

1.5 General Objective

To estimate incidence rates, survivorship and risk factors for brucellosis in Kajiado East Sub-County, using survival analysis methods.

1.6 Specific Objectives

1. To estimate incidence rates of brucellosis infection in sheep, cattle and goats.

2. To determine differences in brucellosis infection in sheep, cattle and goats.

3. To determine factors associated with survival times in sheep cattle and goats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of brucellosis

Brucellosis is a zoonotic disease of global importance. The impact of brucellosis to a farmer is economic losses through birth of weak calves, stillbirths, abortions and infertility in livestock [8]. The disease is caused by various bacteria of the species *Brucella*. It mainly affects cattle, pigs, sheep, goats, camels, and dogs but may also affect other ruminants and marine mammals. It is characterized by abortions, infertility, weak calves and stillbirths [17].

2.2 Causative agent

Brucellosis is caused by bacteria of the genus *Brucella*. Of the 12 species of *Brucella* that are known [20], the most common species are *B. abortus* (infects cattle), *B. melitensis* (infects sheep and goats), *B. suis* (infects swine), and *B.Canis* (which infects dogs) [1].

2.3 Mode of transmission

Bacteria from infected animals are shed through the birth fluids, placenta, fetuses and abortion matter of diseased females. These bacteria are able to survive outside for many months, where they can infect other animals. Transmission of *Brucella* in animals is mainly through consumption of food or water that is contaminated with infected secretions. Transmission could also be through infected semen during breeding. The bacteria can also be transmitted to fetuses born of infected mothers or calves could acquire the disease from their mother's milk [18, 19].

2.4 Geographic distribution

Brucellosis is a leading cause of zoonotic infections with a global distribution. However, it is more prevalent in countries with inadequate animal and public health programs. It is a rare disease in many developed nations because of adequate disease control programs, but a major source of zoonotic infections in low income countries [8].

Globally, majority of the cases of brucellosis in humans and animals occur in Sub-Saharan Africa with regions that practice pastoral production having high incidence. The disease has been observed to be persistent in most countries such as Kenya, Tanzania, Ethiopia, Nigeria, Somalia, Uganda, Chad and Zimbabwe [21].

2.5 Clinical presentation in animals

Abortion is a major clinical sign in pregnant cows, usually from the fifth to the eight month of gestation. Still births, weak calves and retained placentas are also evident in infected females [22]. In bulls, brucellosis is characterized by swollen testis and arthritis.

In sheep and goats, abortion experienced in the last 2 months of pregnancy is the main clinical sign. Other signs include weak offspring and retained placenta. In rams it presents as swollen testis. The flock generally experiences decreased fertility, increased lamb/kid mortality and decreased milk production as a result of the disease [1].

2.6 Identification of Brucella

For diagnosis of brucellosis, the gold standard is isolation of *Brucella spp* from blood cultures. This is however time consuming and complicated. Serological tests which are fast, easy to conduct, less expensive, with high sensitivity and fair specificity are still frequently used as diagnostic methods. These include;

2.6.1 Acidified antigen agglutination such as Rose-Bengal Test (RBT)

These have the highest specificity of 99.1%-99.4%. [23]. According to Nielson [24], RBT tests are low-cost, easy to perform and appropriate for screening individual animals.

2.6.2 Standard Agglutination Tests (SAT)

They comprise the sero-agglutination test and the standard tube agglutination test. The SAT is said to be of low sensitivity and specificity than any other standard [24], and therefore the SAT is no longer recommended, within the European Union, as an official screening test for brucellosis.

2.6.3 The Complement Fixation Test (CFT)

It is often used as a confirmatory test for RBT-positive serum. It is the test approved by OIE as the best for international trade [24].

2.6.4 Indirect enzyme immunoassay (ELISA)

These are highly sensitive, with arrange of 76.3%-81.4% [23], but are expensive to perform. These can be carried out with minimum equipment and are simple but robust [1, 24].

2.7 Treatment

Currently, brucellosis in animals has no reliable treatment [1].

2.8 Control and prevention

Control of brucellosis can be effected by identifying, testing and removing infected animals from the herd. There should be proper disposal of placentas and fetuses as well as thorough disinfection of contaminated areas. This will help in removing the sources of infection and therefore protecting susceptible livestock. To prevent transmission to non-infected animal herds, movement of diseased animals should be barred and concerned authorities should issue import permits only to areas and farms that are brucellosis-free [25]. Vaccinating cattle with S19 or RB 51 and sheep and goats with *B melitensis* Rev 1 will significantly reduce vulnerability to *Brucella* infection [1].

2.9 Brucellosis in livestock

In a study by Rahman *et al* [31] to determine the prevalence of brucellosis in ruminants in Bangladesh, sera samples were collected from 188 cattle, 127 goats and 130 sheep and tested with RBT and I-ELISA. Prevalence for brucellosis was established as 2.66% in cattle, 3.15% in goats and 2.31% in sheep. In the three species, prevalence was higher in females compared to males.

In a serosurvey conducted in Togo in 2011[32] to understand the epidemiology of brucellosis and Q-Fever, a total of 596 cattle, 465 sheep and 221 goats were sampled. Prevalence in cattle

was 9.1% while that for goats and sheep was 0%, indicating the disease was more prevalent in cattle than in the small ruminants. The study also showed that brucellosis seropositivity was much higher in cows than in bulls (OR 5.3, 95% CI :1.5-18.7).

A cross-sectional study carried out in the urban and peri-urban areas of Kampala, Uganda in 2011[30] to establish prevalence of brucellosis and its risk factors in cattle, 423 cattle were sampled. In the study, animal level risk factors such as breeding system, history of abortion, history of vaccination and breeding status were not significantly associated with brucellosis infection.

In their study on risk factors for *Brucella* infection in Niger, Abdou *et al* [28] found out that animals that were aged 1-4 years were more susceptible to infections than animals that were less than 1 year old (OR 2.7, 95% CI :1.43-5.28). They also associated nomadic pastoralism with an increased risk of transmission (OR 5.4, 95% CI: 2.84-10.41).

In Kajiado County, a cross-sectional study was carried out by Osoro *et al* [9] to assess the association between human and animal *Brucella* seropositivity. Blood samples were collected from cattle, sheep and goats. In the study, goats showed a higher prevalence (3.6%) compared to cattle (3.3%) and sheep (3.4%). The study did not investigate individual animal level factors associated with brucellosis infection.

Another study in Kajiado County by Nakeel *et al* [35] set out to investigate risk factors for brucellosis, Q- Fever and Leptospirosis in livestock and humans in 3 sub-counties in Kajiado County. They sampled 89 cattle, 75 sheep and 72 goats in high risk areas. The study observed a prevalence of 21.92% in cattle, 8.6% in sheep and 7.3% in goats, showing a difference in prevalence in the three species, with higher prevalence observed in cattle.

In a systematic review of brucellosis in Kenya by Njeru, *et al* [12], 36 national and international publications spanning the years 1916-2016 were reviewed to establish associations between risk factors for brucellosis and seropositivity in Kenya. The review showed there was scanty data on the disease burden estimates. Twenty-one studies on brucellosis in livestock were mainly prevalence studies that reported frequency estimates. Seroprevalence was highest in pastoral and agropastoral systems (9.9%-15%). Low prevalence was seen in small holder farms in Kiambu county. The review did not find studies on disease incidence estimates, giving rationale for this study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

Kajiado County borders Nairobi County and Tanzania border to the south. Its population is 1,117,840 (2019 census) and an area of 21,292.7 km². The county capital is Kajiado but the largest town is Ngong. Most of Kajiado County is an arid rangeland, occupied mainly by the Maasai pastoralist community. The major economic activity is livestock keeping which acts as a major source of livelihood for many inhabitants.

Mashuru Sub-county covers 2903km^2 with a total human population of 50,245 with a human population density of 17.3 persons/km^2 based on the Kenya 2009 census. Most parts of Mashuru are semi-arid receiving an average annual rainfall of 400-500 mm. Livestock keeping and subsistence crop farming are the main agricultural activities in the county.

The longitudinal study was conducted in Mashuru Sub-county of Kajiado County between February 2015 and November 2015. In 2013, brucellosis sero-prevalence was estimated at 15% in humans and 3% in livestock (cattle, sheep and goats) in Kajiado County. Mashuru Sub county reported the highest sero-prevalence, which informed the selection of the study site for the incidence study.

3.2 Study Design

The study design was a longitudinal study. Selected cattle, goats and sheep that were negative for brucellosis were enrolled in the study. They were sampled at three time points during the study period and tested for brucellosis infection at each time point.

3.3 Study Population

3.3.1 Selection of study locations

Four locations were randomly selected for this study. These were Mashuru, Ilmukutani, Arroi and Nkama. In each location, a number of compounds (that consists of one or more households) were enrolled for longitudinal follow up. The number of compounds targeted in each location were weighted by population density based on the 2009 Kenya population census data. Animals in the selected compounds were eligible for enrolment into the follow-up study.

3.3.2 Selection of enrolled compounds

For each selected location, a number of geographical coordinates were generated using ArcGIS mapping software. Coordinates were loaded into Global Positioning System (GPS) receivers and used to identify compounds to recruit into the study. Each consenting compound that owned animals was assigned a unique identifier. All animals in a recruited compound were eligible for enrollment into the study. A baseline questionnaire was administered to the head of the compound to collect demographic, animal ownership, herd characteristics and individual animal information.

3.3.3 Longitudinal follow up in animals

Sheep, cattle and goats in selected compounds, that were free from brucellosis were enrolled in the study. Information on recruited animals was collected at baseline and each enrolled animal assigned a unique identification number that was printed on an ear tag. A follow up visit for sampling was done at month 4 and 9.

3.3.4 The inclusion criteria

Only those compounds that owned animals and agreed to take part in the study were enrolled. In selected herds, only the animals that were free from brucellosis at screening were enrolled. All age categories of animals were eligible for enrolment. Animals that showed signs of sickness were excluded from the study.

3.4 Sample size determination

Due to costs of testing animal samples, only a proportion of animals in selected compounds were enrolled. To estimate the proportion of compounds that would give sufficient data to estimate the contribution of positive animal cases, a sample size calculation assuming a prevalence of 30% in animal herd [9], a precision of 5% and at 95% confidence gave a minimum sample of 322 herds using the formula;

$$n = Z^2_{\alpha/2} * p * (1-p) / E^2, \text{ where}$$

n = minimum sample

p = prevalence of brucellosis (30%)

E^2 = margin of error (5%)

$Z_{\alpha/2}$= Critical value for the distribution

The minimum sample required was 322 herds.

From these livestock herds, samples were collected from up to 6 animals of each species and tested for brucellosis.

3.5 Sampling method

A three-stage sampling method was used. Selected locations were the clusters. Herds of cattle, sheep and goats within a selected location were randomly selected in the second stage. Finally, within a herd, individual animals were selected as the sampling units.

From a list of 17 locations in the study site, 4 (20%) of the locations were selected randomly. To randomly select the locations for the study, a list of all the locations in the study site were drawn as per the 2009 KNBS census. Four locations were randomly selected. In each selected location, compounds were selected using a simple random sampling method, proportionate to the population density of the location by using geo-coordinates generated randomly using ArcGIS mapping software. In selected compounds, animals were stratified into age groups (calves, young adults and adults) and enrolled animals were randomly selected from each age strata.

The multistage sampling that was employed comprised of;
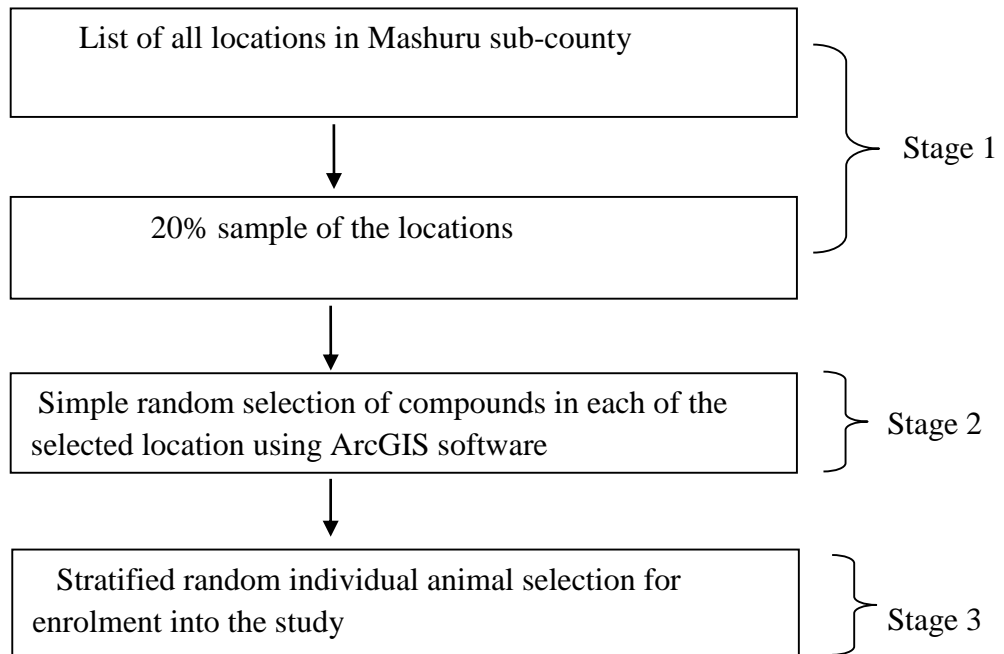
Figure 1: Sampling design.

The head of the selected compound was consented for the animals to be sampled. At baseline sampling, blood samples were collected from up to 12 of cattle, sheep and goats present at the selected compounds and tested for prior exposure to *Brucella* by RBT. Of each species up to 6 sero-negative animals were enrolled and sampled at two time points; four and nine months during the follow-up period. A baseline questionnaire was administered to the head of each compound to collect demographic, animal ownership, husbandry practices and individual animal information. At follow-up a similar questionnaire on the animals' characteristics was administered to collect information about age, sex, breeding status, and a blood sample was collected from enrolled animals.

3.6 Laboratory Procedures

3.6.1 Specimen collection

Blood samples were collected from enrolled animals. The animal health practitioners bled the enrolled animals (cattle, sheep, and goats) from the jugular vein obtaining 9 cc of blood in a serum separator tube. During collection a bar coding labeling system was utilized. Sample labels consisting of unique identifiers were placed on the serum stock vials. A pre-prepared label was immediately placed on each of the samples as soon as it was collected.

The serum separator tube was centrifuged at 600 rpm for 10 minutes. The tubes were spun in a sealed double containment bucket. The serum was removed from the tube and placed in a labeled plastic tube. Each sample was tested for *Brucella* using Rose Bengal (RBT) and c-ELISA test.

3.6.2 ELISA testing

At the laboratory, the samples collected were tested for *Brucella* IgM antibodies using competitive (SVANOVIR® Brucella -Ab C-ELISA, Sweden) enzyme-linked immunosorbent assay (ELISA) kits. Presence of IgM antibodies in serum signified new infections.

3.6.3 Biosafety measures

Diagnostic specimens were handled in BSL-2 conditions. Work was performed in accordance with BSL-2 conditions, under a licensed BSL-2 safety cabinet. The blood tubes would not leave the hood unless contained in a vessel. The tubes were placed inside of double sealed

buckets if centrifuged. The laboratory personnel wore BSL-2 attire, including the following: lab coats, bonnets, booties, double gloves, and N95 masks.

3.7 Statistical analysis

The following statistical methods were used to analyze the data obtained from the study;

3.7.1 Calculation of incidence rates

a) Crude incidence rates

Incidence rate is a measure of the number of new cases per unit of time. The time units can be in days, months, or years.

$$\text{Incidence rate} = \frac{\text{number of new cases}}{\text{Sum of person-time at risk}}$$

The numerator is the number of new cases that develop during the follow-up period while the denominator is the sum of person/animal-time for each individual in the cohort. It is an estimate of the actual time-at-risk in years, months, or days that all individuals contributed to the study. The study start date can be same or different for each participant. The study end date is usually variable for each participant as some may be lost to follow-up, experience the event of interest during the study or come to end of study without experiencing the outcome.

For this study, incidence rates were calculated as the total number of new brucellosis infections divided by the total animal-time at risk during the follow-up period for each species.

3.7.2 Survival analysis methods

Survival analysis methods are used to analyze data in which we are interested in measuring time to the outcome of interest. The outcome is known as a failure time, event time or survival time. The survival time is always continuous, may be incomplete for some subjects and is always more than or equal to zero. Incomplete survival times are censored.

The aim of survival analysis is to obtain a measure of effect that describes the association between an independent variable and the outcome after adjusting for the other variables. The hazard ratio (HR) is the measure of effect. It is the ratio of the risk of outcome in the exposed group in relation to the unexposed group. It is given by the formula;

HR= (Oa/Ea)/(Ob/Eb); where;

      Oa = observed events in the exposed group

      Ea = expected events in the exposed group

      Ob= observed events in the unexposed group

      Eb= expected events in the unexposed group

A HR of 1 indicates no association. A HR of more than 1 means that one group has greater hazard while a HR of less than 1 means one group has less hazard or risk compared to the other. On the other hand, in terms of survival, if HR is less than 1 then the ratio of resultant survival probabilities will be greater than one, thus one group will have a higher likelihood of survival at any given time period t, after controlling for other explanatory variables.

This study entailed enrolling animals and following them up for a period of nine months to determine when they acquire brucellosis infection. Some animals were lost to follow-up during the study period thus their survival times were censored. This made survival statistical methods appropriate in studying disease risk probabilities.

The following approaches are used in estimating the survival function:

a) **Kaplan Meier curves**

Kaplan-Meier curves are useful in describing survival when the data includes censored observations. For each time interval, we estimate the probability that those who have survived to the beginning of the time interval will survive to the end thus obtaining conditional probabilities. We calculate survival to any time point as the product of the probabilities of surviving each time interval using the formula ;

$$\hat{S}(t) = \left(1 - \frac{d_1}{n_1}\right)\left(1 - \frac{d_2}{n_2}\right) \cdots \left(1 - \frac{d_j}{n_j}\right) = \prod_{i=1}^{j}\left(1 - \frac{d_i}{n_i}\right).$$

d_i = number of events at each time interval

n_i = number at risk in the time interval

Probability of surviving
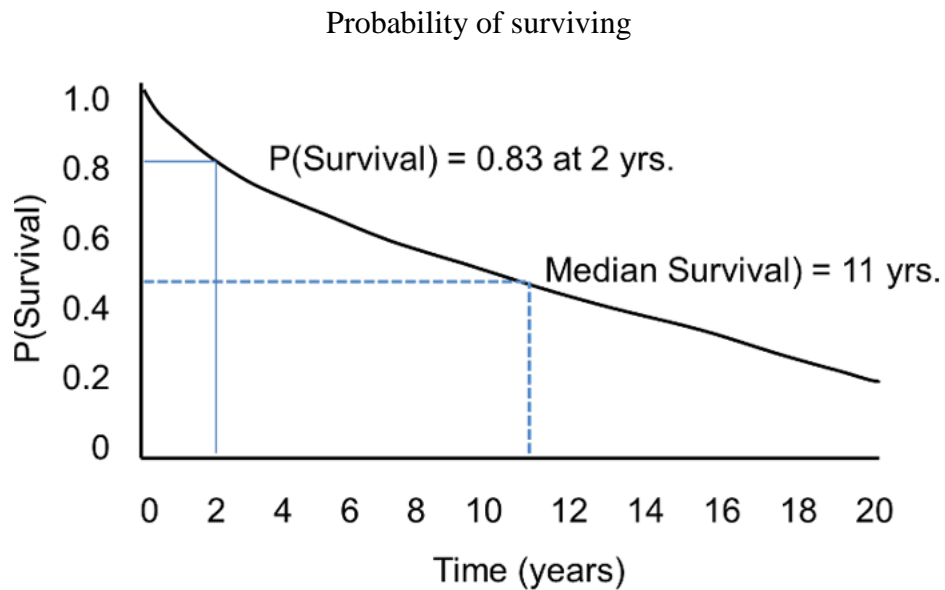
Figure 2: Sample survival curve.

The Kaplan–Meier technique and the log rank test are the most common methods used to estimate survival probabilities and to compare survival between different groups. However, if one wants to know the size of the difference and adjust for other confounding variables, more will need to be done than just KM curves. Moreover, the size of the differences in survival between two or more groups and the associated confidence intervals cannot be obtained from a log rank test. The Kaplan–Meier method and the log-rank test can only be used to assess the effect of one predictor variable at a time, and thus they cannot be used for multivariable analysis, in which case Cox proportional hazards model becomes handy.

To describe survivorship in each species as well as compare survival in different groups, KM curves were plotted. Survival plots for cattle , sheep and goats were compared using log rank test to establish if there were differences in survival across the three livestock species.

b) Nelson Aalen estimator

The Nelson Aalen estimator is a nonparametric estimator useful for the estimation of the cumulative hazard rate function involving censored survival data. No distributional assumptions are needed.

Let the times when events are observed be denoted by $t_1 < t_2 < \ldots$ and let d_j be the number of individuals who die at $t_{j..}$

The Nelson Aalen estimator for cumulative hazard is given by

$$\hat{A}(t) = \sum_{t_j \leq t} d_j / r_j,$$

where r_j is the number of individuals at risk just prior to time t_j. Thus, the Nelson Aalen estimator is a cumulative right continuous step function with increments d_j/r_j at the observed event times.

An example of a Nelson Aalen estimator of cumulative hazard is shown in Figure 3.
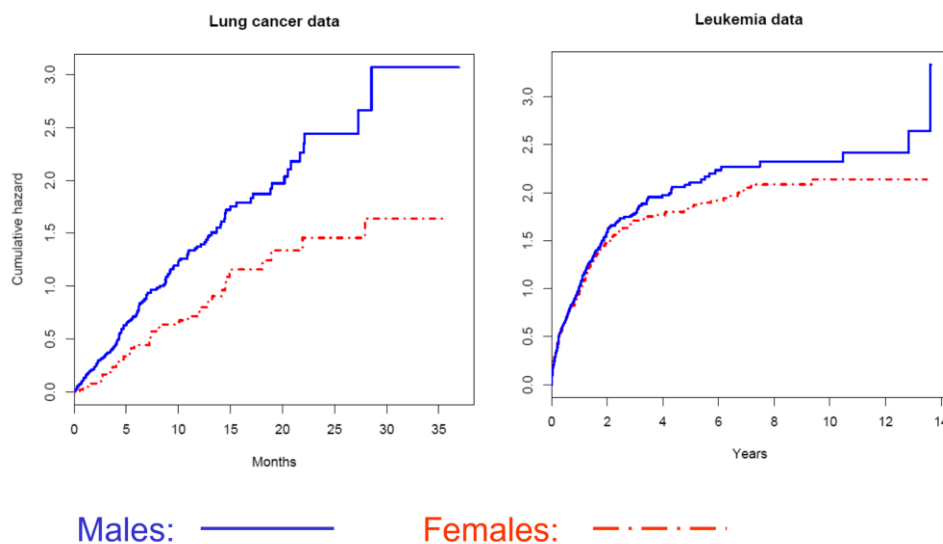
Figure 3. Sample Nelson - Aalen estimator of the cumulative hazard function.

Interpretation of the estimate mainly focuses on the slope of the curve. In the above figure, the cumulative hazard rate is steeper for males than females. We can thus conclude that the risk of experiencing the event of interest for these patients is greater for males than females.

Cumulative hazard functions were used in this study to describe cumulative hazard rates for cattle, sheep and goats.

c) Cox Proportional Hazards regression

This is a survival analysis method that is used to assess the effect of one or many risk factors on survival time. The hazard ratio is the measure of effect in a Cox PH. The hazard can be more than 1 since it is the expected number of events per unit of time.

In comparing hazards in two groups, we use the hazard ratio, which is the total number of observed events in relation to expected events in each of the groups which is given by;

$$HR = \frac{\Sigma O_{Exp,t}/\Sigma E_{Exp,t}}{\Sigma O_{Unex,t}/\Sigma E_{Unex,t}} = \frac{\Sigma O_{treated,t}/\Sigma E_{treated,t}}{\Sigma O_{control,t}/\Sigma E_{control,t}}$$

Cox proportional hazards assumptions include;

    i.   independence of survival times among different individuals in the study sample

    ii.   a multiplicative association between the independent variables and the hazard

    iii.   a constant hazard ratio over time

The Cox proportional hazards model can be expressed as;

$$h(t) = h_0(t) \exp(b_1 X_1 + b_2 X_2 + \dots + b_p X_p)$$

where ;

       $h(t)$ – is the expected hazard at time t

       $h_0(t)$ - is the baseline hazard when all the predictors are equal to zero

From the formula above, the expected hazard ($h(t)$), is the product of the baseline hazard ($h_0(t)$) and the exponential function of the linear combination of the predictors making them assume a proportional effect on the expected hazard.

The predicted coefficients b_1, b_2, b_p in the model signify the change in the predicted log of the hazard ratio for a unit change in the independent variable, controlling for all the other independent variables.

If the hazard ratio for a predictor is close to 1, then the variable is not associated with survival. If the hazard ratio is less than 1, then the predictor is linked to better survival. If the hazard ratio is more than 1, then the predictor is related to diminished survival.

The Cox proportional hazards model makes no assumptions about the shape of the baseline hazard function and thus it is referred to as a semi-parametric model.

The Cox proportional hazards model was used for bivariate and multivariate regression of factors that were associated with survival time. Hazard ratios were calculated using the Cox proportional hazard model. The hazard ratios were used to measure the magnitude of the associations between the predictor variables and the outcome.

d) Proportionality Assumption

This is a very important assumption, that needs to be met, in order to appropriately apply Cox proportional hazards regression model to the analysis of survival data. It assumes that the hazards are proportional over time implying that the effect of a predictor is constant over time. The proportionality assumption can be assessed statistically or graphically.

In assessing the assumption statistically, we include in the model, predictor by time interactions and test for statistical significance. If the interactions are statistically significant, then the assumption of proportionality is violated.

In assessing the assumption graphically, we use several graphical presentations to evaluate whether the assumption is realistic. We plot graphs of residuals and study trends over time. If statistically or graphically there is no indication of the hazards being proportional over time, then it may not be appropriate to use Cox proportional hazards model. To account for the violation of the assumption, we may need to make some adjustments such as stratifying the data to ensure that that the hazards are proportional within groups.

In this study, test of proportionality assumption was carried out by evaluating the Schoenfeld residuals. Where the assumption was violated, adjustment was made in the Cox PH model to account for this.

3.8 Data Management

3.8.1 Data collection

Data on the enrolled animals was collected by trained Animal Health Assistants (AHA). Structured questionnaires were preloaded into smartphones and used to collect data specific to the herds and individual enrolled animals. Collected data was downloaded from the smartphones and stored in Microsoft Access database in a password protected computer. A back up of the database was created in case of damage and or loss of original data and stored in a password protected computer.

Information on herd demographics and risk factors including animal's age, sex, clinical history, breed, sex, breeding status, breeding system, and contact with other animals was collected at enrolment and at each subsequent follow-up. The study follow-up time was 9 months. Animals were enrolled in February 2015 and followed up until November 2015. At the fourth- and ninth-months, risk factors data and samples were collected from the enrolled animals.

3.8.2 Study variables

The dependent variable was time to brucellosis infection i.e. time to when an animal first tested positive for brucellosis. Survival time was measured in days.

The independent variables were: age, sex, breed, production system, breeding system, contact with other herds and contact with wild animals. These variables were measured at enrolment of the animals into the study.

Once an animal tested positive, it was excluded from the study and therefore was censored at the respective time during data analysis.

Censoring was done for animals that tested negative at the end of the study or those that were lost to follow-up during the study.

3.8.3 Limitations of the Study

The major limitation of this study is that it had a substantial percentage of animals that were lost to follow up. This is likely to affect the size of effect of the estimates.

3.9 Data analysis and presentation

Data was cleaned, coded and analysed using SAS 9.4 and R Studio Version 3.6.1 software.

Frequency analyses were performed to examine the distribution of study data and to consider appropriate stratifications. Preliminary comparisons of the demographics (age group, gender, breed etc.) were made.

Disease incidence rates were obtained by dividing the number of new brucellosis infections observed in the study period, by the total animal-years of follow-up. The total animal-time was calculated as total years under follow-up minus days of missing data. Survival models were fitted to identify the factors associated with time to brucellosis infection in different species of livestock.

Statistically significant differences were identified using Chi-square. Hazard rates were calculated by hazard ratios. 95% confidence intervals and p-values were calculated and used to

assess the significance of the results. Data was fitted into bivariable and multivariable Cox PH survival regression models to assess the association between identified risk factors and the dependent variable, which was time to *Brucella* infection. All the analyses in this study were considered statistically significant at a p-value of <0.05.

3.10 Ethical Considerations

Animal Subjects

Ethical clearance and approval for this study was obtained from the KEMRI Ethical Review Committee (ERC) and Animal Care and Use Committee (ACUC), and CDC Institutional review board.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of the sampled animals

The study enrolled 709 cattle, 1711 goats and 1369 sheep. Majority of the enrolled animals were female: cattle (78%), goats (73.8%) and sheep (79.3%). The dominant breed was indigenous represented by 91.8% of cattle, 94.9% of goats and 81.95% of sheep, with more than 40% of the animals being raised in a nomadic pastoralism production system. About half (44%) of the enrolled cattle were adults (greater than 3 years) while adult (> 1 year) goats and sheep were 59% and 63% respectively. Majority of the animals (87%) had contact with wild animals and nearly all (98.6%) had contact with other herds and were bred naturally, as shown in Table 1.

Table 1. Characteristics of followed up animals in the brucellosis incidence study in Kajiado East Sub-County, 2015

Characteristic	Cattle			Goats			Sheep		
	Number (%)	Infected(%)	Censored(%)	Number (%)	Infected(%)	Censored(%)	Number(%)	Infected(%)	Censored (%)
Sex									
Male	156(26.0)	2(1.28)	154(98.72)	448(26.2)	1(1.22)	447(99.78)	284(20.7)	2(0.7)	282(99.3)
Female	553(78.0)	10(1.81)	543(98.19)	1263(73.8)	4(0.32)	1259(99.68)	1085(79.3)	8(0.74)	1077(99.26)
Age(cattle)									
< 2 years	284(40.1)	3(1.06)	281(98.94)						
2-3 years	113(15.9)	3(2.65)	110(97.35)						
>3 years	312(44.0)	6(1.92)	306(99.08)						
Age (Shoats)									
< 6 months				269(15.7)	1(0.37)	268(99.63)	232(16.9)	1(0.43)	231(99.57)
6-12 months				422(24.7)	1(0.24)	421(99.76)	274(20.0)	2(0.73)	272(99.27)
>1 year				1020(59.6)	3(0.29)	1017(99.71)	863(63.0)	7(0.81)	856(99.19)
Breed									
Indigenous	651(91.8)	12(1.84)	639(98.16)	1623(94.9)	5(0.31)	1618(99.69)	1122(81.95)	9(0.8)	1113(99.2)
Exotic	4(0.6)	0(0)	4(100)	8(0.47)	0(0)	8(100)	12(0.88)	0(0)	12(100)
Cross	54(7.6)	0(0)	54(100)	80(4.7)	0(0)	80(100)	235(17.17)	1(0.43)	234(99.57)
Production system									
Settled pastoralist	284(40.2)	6(2.11)	278(97.88)	720(42.2)	2(0.28)	718(99.72)	570(41.6)	2(0.35)	568(99.65)
Agro pastoralist	123(17.4)	2(1.63)	121(98.37)	378(22.1)	1(0.26)	377(99.74)	298(21.8)	1(0.34)	297(99.66)
Mixed farming	17(2.4)	0(0)	17(100)	49(2.87)	0(0)	49(100)	36(2.6)	0(0)	36(100)
Commercial ranch				6(0.35)	0(0)	6(100)			
Peri-urban				5(0.29)	0(0)	5(100)			
Semi-zero grazing	13(1.84)	0(0)	13(100)				8(0.6)	1(12.5)	7(87.5)
Nomadic pastroralist	269(38.1)	4(1.49)	265(98.51)	550(32.2)	2(0.36)	548(99.64)	457(33.4)	6(1.31)	451(98.69)
Contact with wild animals									
No	96(13.5)	2(2.08)	94(97.92)	227(13.3)	0(0)	227(100)	168(12.3)	2(1.19)	166(98.81)
Yes	613(86.5)	10(1.63)	603(98.37)	1473(86.2)	5(0.34)	1468(99.66)	1198(87.5)	8(0.67)	1190(99.33)
Don't know				8(0.47)	0(0)	8(100)	3(0.22)	0(0)	3(100)
Contact with other herds									
No	15(2.1)	0(0)	15(100)	45(2.6)	0(0)	45(100)	27(2.0)	1(3.7)	26(96.3)
Yes	694(97.9)	12(1.73)	682(98.27)	1663(97.4)	5(0.3)	1658(99.7)	1342(98.0)	9(0.67)	1333(99.33)
Breeding system									
Artificial insemination	10(1.4)	1(10)	9(90)						
Natural breeding	690(97.9)	10(1.45)	680(98.55)	1687(98.8)	5(0.3)	1682(99.7)	1361(99.4)	10(0.73)	1351(99.27)
Both	4(0.6)	0(0)	4(100)	21(1.2)	0(100)	21(100)	4(0.3)	0(0)	4(100)
Don't breed	2(0.3)	1(50)	1(50)				4(0.3)	0(0)	4(100)

4.2 Incidence rates of brucellosis in cattle, sheep and goats

For each followed up animal, the time at risk was calculated in years, from the day they were enrolled to the time they first turned positive for brucellosis or were lost to follow up or were still uninfected at end of the study. The follow-up time in animal years was totaled by species. The number of animals enrolled and the new brucellosis cases for the two sampling periods were as shown in Table 2. Brucellosis incidence rates were different across the three species. Cattle had 2.7 (35.6/13.1) times the rate of brucellosis infection compared to sheep and a rate of 6.8 (35.6/5.2) times compared to goats.

Table 2. Sampled animals at 4 months and 9 months follow-up

Species	Follow-up cohort	No. Sampled Round 1	New cases at Round 2	No. Sampled Round 2	New cases at Round 2	Total number of new cases	Total animal years of follow-up	Incidence rate per 1000 animal years
Cattle	709	660	7	264	5	12	337	35.6 new cases
Sheep	1369	1308	8	779	2	10	765.8	13.1 new cases
Goats	1704	1625	4	1014	1	5	970.3	5.2 new cases

4.3 Comparing survival in cattle, sheep and goats

Out of the 709 followed up cattle, 12(1.70) % of them were infected with brucellosis, while 697(98.3%) were censored. Of the 1704 goats, 5(0.29%) tested positive, while 10(0.73%) of the enrolled 1369 sheep tested positive during the follow-up period. The mean survival time was 269.64, 276.44 and 257.67 days for cattle, goats and sheep, respectively. The median survival time was not reached during the study period for the three species, which shows the outcome was rare as shown in Table 3.

Table 3. Summary statistics of the enrolled livestock and follow-up time

Species	Number	Failed	% Failed	Censored	% censored	Survival time (in days)			
						Mean	Std. dev	Min	Max
Cattle	709	12	1.70	697	98.30	173.86	76.05	90.67	291.22
Goats	1704	5	0.29	1699	99.71	207.49	76.48	91.0	291.43
Sheep	1369	10	0.73	1359	99.27	204.19	77.22	90.67	291.23
Total	3782	27	0.71	3755	99.29	200.00	77.68	90.67	291.43

4.3.1 Test of no difference

On comparison, the survival curves of the three species of livestock were significantly different (p value = <0.0001). The survival curves did not present the expected decreasing trend with follow up because few events. We did not observed events between 150 days and 250 days of follow-up. This is because sampling was only done at two time periods. Cattle had the lowest survival probability relative to sheep and goats as shown in Figure 4.
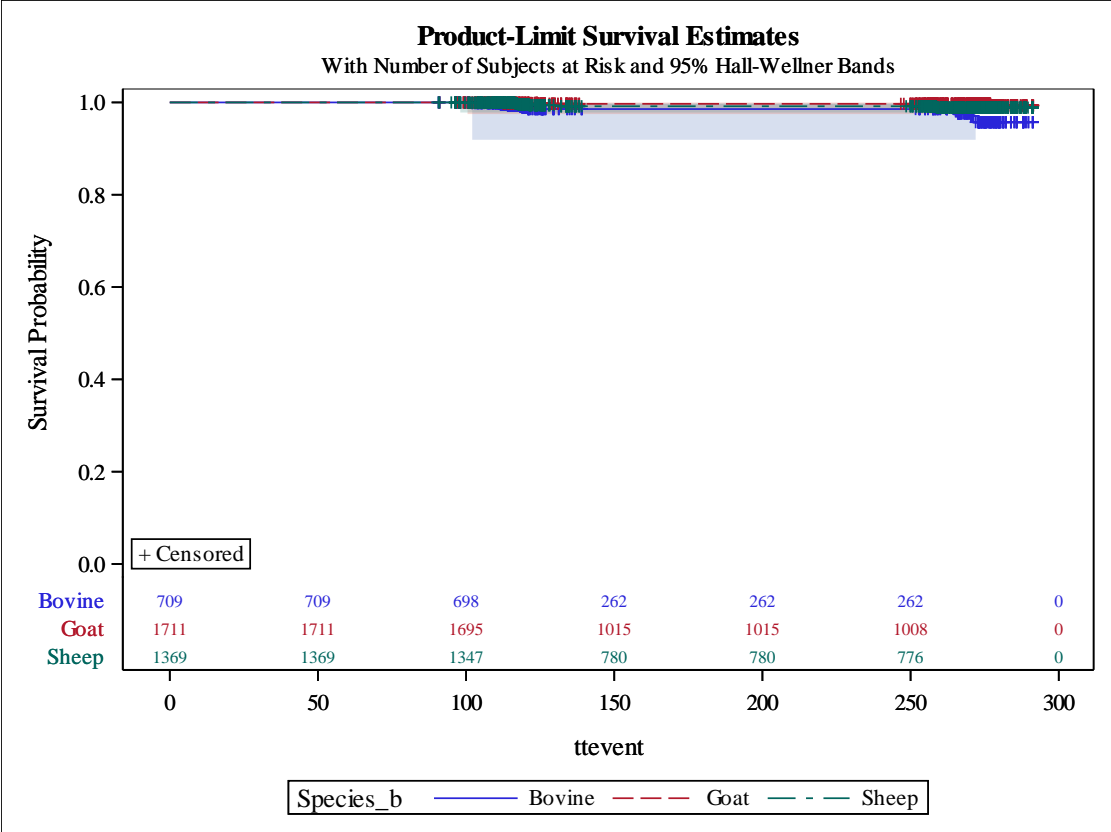
Figure 4: Kaplan Meir curves for comparison of survival in cattle, sheep and goats.

Cattle had the highest hazard of brucellosis infection compared to goats and sheep throughout the study period, as shown by the steeper cumulative hazard line that is maintained throughout the study period.
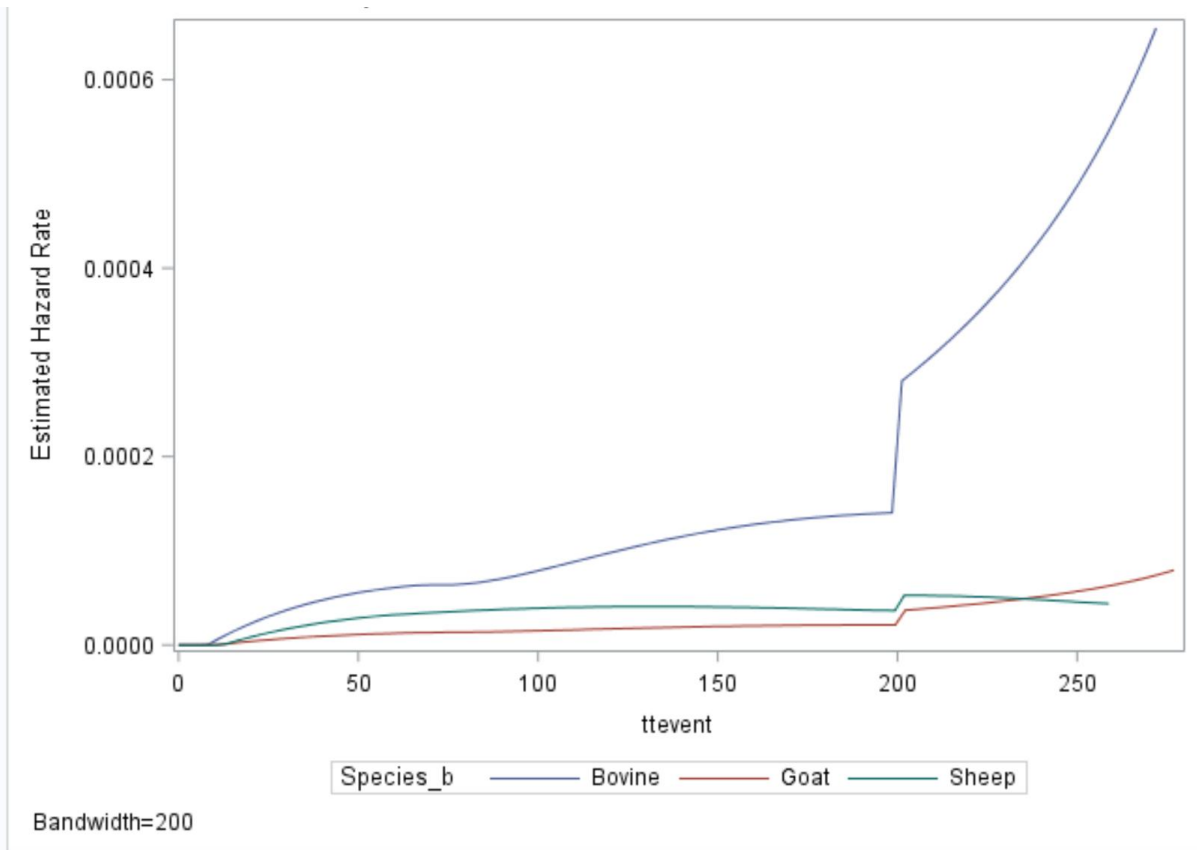
Figure 5: Nelson Aalen estimator for comparison of the hazard rates for cattle, sheep and goats.

4.4 Factors associated with survival times: Using Cox proportional hazards model

4.4.1 Test of proportional hazards assumption

To apply the Cox proportional hazards regression in the analysis of the data, the assumption of proportional hazards had to be satisfied. The proportionality of hazards on the study variables of interest was checked by inspection of the Schoenfeld residuals. The residuals are expected to have a mean of 0 for the proportionality assumption to be satisfied. A plot of these residuals will not show trend over time for the variables of interest when the proportionality assumption is met.

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For sheep and goats, the proportional hazards assumption was met for all the variables of interest. However, for cattle the variable production system violated this assumption as shown in Table 4.

Table 4. Analysis of the Schoenfeld residuals for proportionality assumption. A significant p value indicates a violation of the proportional hazards assumption

Variable	Cattle			Goats			Sheep		
	rho	Chisq.	P value	rho	Chisq.	P value	rho	Chisq.	P value
Sex	-0.727	8.12	0.4	0.637	1.94	0.163	-0.14	0.197	0.657
Age	0.420	2.22	0.136	0.215	0.249	0.618	0.361	1.2	0.274
Breed	-0.489	0	1	-0.182	0	1	0.61	3.95	0.067
Breeding system	-0.308	2.95	0.086	-0.415	0	1	0.762	0	1
Production system	0.727	6.77	0.009	-0.516	1.5	0.22	-0.584	3.33	0.068
Contact other herds	0.105	0	1	-0.764	0	1	-0.08	0.062	0.804
Contact wild animals	0.187	0.322	0.571	-0.821	0.064	0.801	0.300	0.939	0.333

Figure 6 shows some of the plots of the Schoenfeld residuals versus time for variables using cattle data.
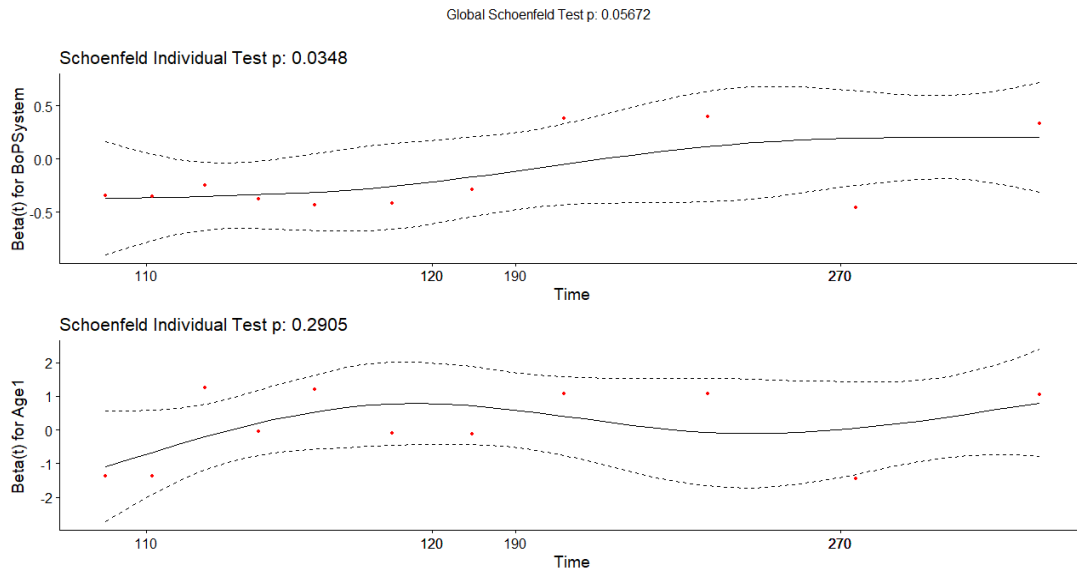
Figure 6. Scaled Schoenfeld residuals versus time for cattle data.

To adjust for non-proportional hazards of the variable production system in cattle, an interaction term between production system and time was added to the Cox PH regression model.

4.4.2 Bivariate analysis

In bivariate analysis (i.e. time to brucellosis infection with each covariate), seven variables were analyzed for each species, to determine the association of each of the variables with time to brucellosis infection. These were age, sex, breed, production system, breeding system, contact with other herds and contact with wild animals. Female cattle had 1.3 times the hazard of infection with brucellosis compared to male cattle. The older cattle (> 2 years) also exhibited a higher risk for brucellosis infection compared to the younger ones (HR >1.0). Cattle that were raised in herds where they practiced natural breeding showed a reduced risk of brucellosis infection compared to those from artificial breeding systems and this association was statistically significant (p=0.017). For goats, the hazard of brucellosis infection was 1.3 times higher in females compared to males. The risk was however lower in older goats compared to the younger ones. The hazard of brucellosis infection in goats raised under the nomadic pastoralist system was 1.256 times that of goats raised in a settled pastoralist system. Male sheep had lower survival than females. The expected hazard rate in sheep raised in nomadic pastoralism was about 4 times higher compared to those in raised in settled pastoralism. Across the three species, breed was not an important risk factor as almost all the animals were predominantly indigenous. The breeding system was predominantly natural, and this did not yield much comparable estimates in association with brucellosis infection. Despite the strength of their associations, most of the factors analyzed were not statistically significant at (p<0.05) as shown in Table 5.

Table 5: Results of the bivariate Cox PH test for the predictor variables for cattle, sheep and goats

Covariate	Cattle			Goats			Sheep		
	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	Pvalue
Sex (Female)	1.254	0.274-5.728	0.7705	1.268	0.142-11.353	0.8320	0.884	0.188- 4.165	0.8760
Age									
Kids/Lambs/calves	1		Ref	1		Ref	1		Ref
Young adults	1.979	0.399-9.824	0.4038	0.683	0.043-10.941	0.7873	1.814	0.164-20.004	0.6269
Adults	1.511	0.377-6.048	0.5601	0.802	0.083-7.720	0.8486	1.863	0.229-15.146	0.5605
Breed									
Indigenous	1		Ref	1		Ref	1		Ref
Exotic	0	0	0.9978		0	0.9980	0	0	0.9945
Cross breed	0	0	0.9922		0	0.9955	0.582	0.074-4.596	0.6075
Production system									
Settled pastoralist	1		Ref	1		Ref	1		Ref
Agro-pastoralist	1.039	0.208-5.194	0.9628	1.246	0.110-14.093	0.8587	1.173	0.106-12.963	0.8962
Mixed farming marginal	0	0	0.9945	0	0	0.9962	0	0	0
Commercial ranch	-	-	-	0	0	0.9991	-	-	-
Peri-urban	-	-	-	0	0	0.9989	-	-	-
Semi-zero grazing	0	0	0.9955	-	-	-	118.953	10.421-1357.808	0.0001
Nomadic pastoralist	0.505	0.142-1.802	0.2927	1.256	0.176	0.8202	3.878	0.783-19.217	0.0969
Production system*time	1.006	1.000- 1.012	0.0710	-			-		-
Contact wild animals (Yes)	0.923	0.202- 4.222	0.9179	>999	0	0.9949	0.547	0.116-2.575	0.4451
Contact other herds (Yes)	>999	0	0.9923	>999	0	0.9947	0.189	0.024-1.490	0.1138
Breeding system									
Artificial insemination	1	-	Ref	-	-	-	-	-	-
Natural	0.076	0.009- 0.636	0.0174	1		Ref	1		Ref
Both	0	0	0.9950	0	0	0.9954	0	0	0.9964
Don't breed	1.898	0.107- 33.820	0.6627	-	-	-	0	0	0.9950

For cattle, breeding system was significantly associated with brucellosis infection as shown in Figure 7. There was a statistically significant difference in survival of cattle from the different breeding systems using the log rank test (p<0.0001). The median survival time for those animals that were not bred was 110 days.
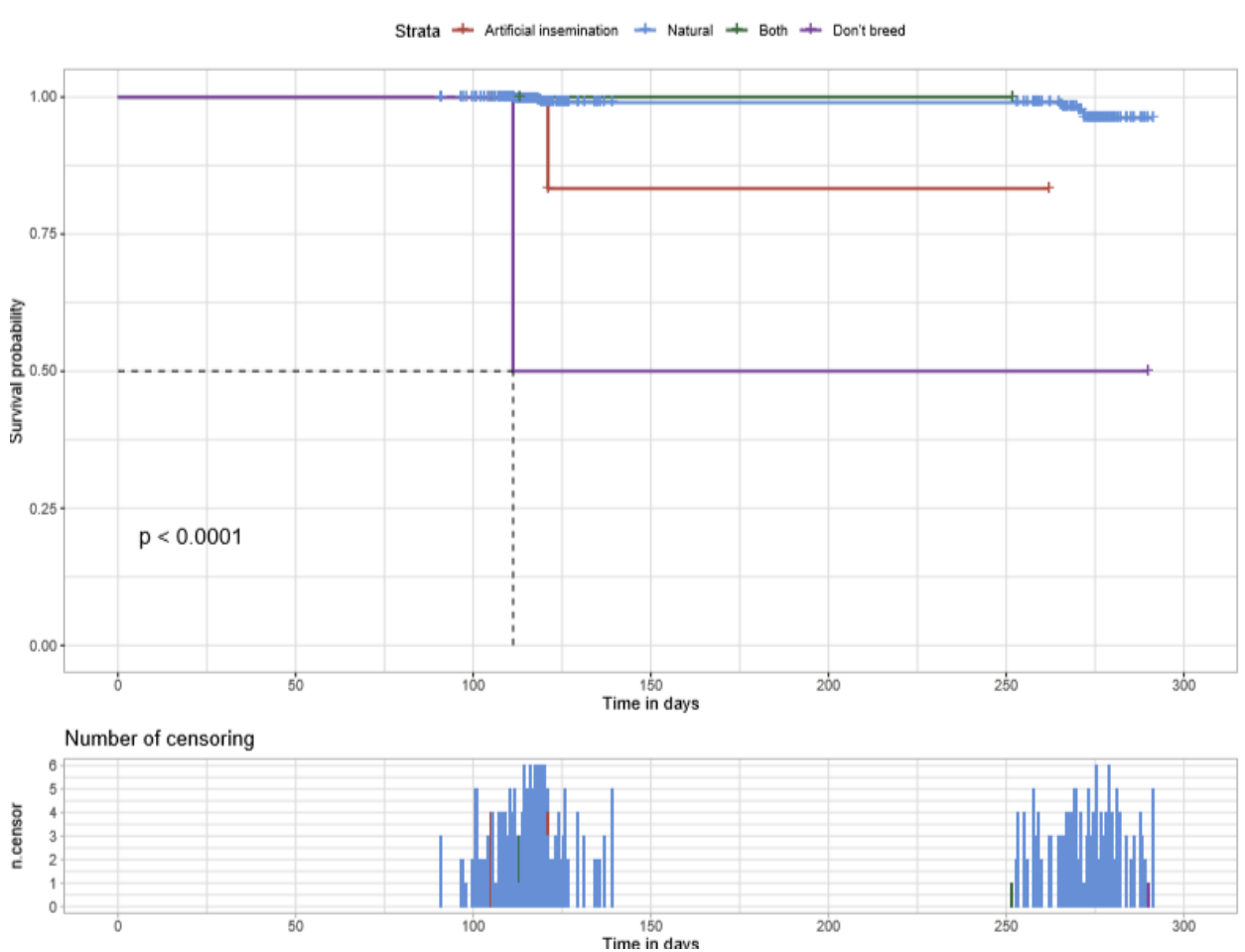
Figure 7. Comparison of Kaplan Meir survival curves in cattle by breeding system.

Survival probabilities were also significantly different (p<0.0001) for sheep across the different production systems as shown in Figure 8.
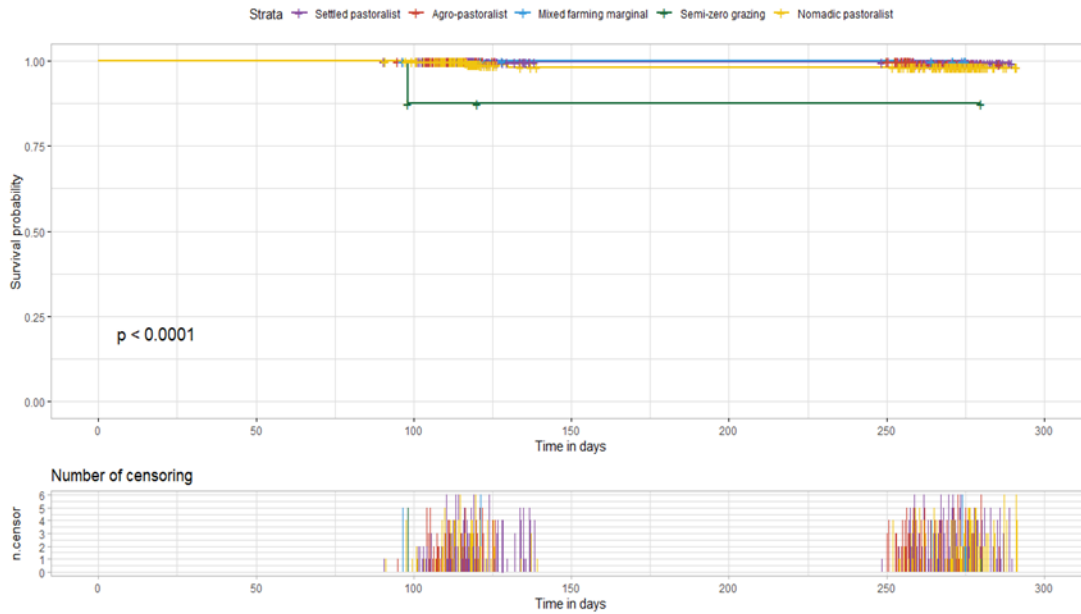
Figure 8. Comparison of Kaplan Meir survival curves in sheep by production system.

4.4.3 Multivariable Cox proportional hazards model

For each species, the variables that were significant ($p \leq 0.1$) in bivariate analysis were entered into a Cox PH regression multivariable model. For cattle the variables were breeding system, production system and the interaction between production system and time. For sheep, the variables that were significant in bivariate analysis ($p \leq 0.1$) were contact with other herds and production system. The p values were relaxed for the variables entered in the multivariable model due to their importance in brucellosis infection.

On multivariable analysis, for cattle there was borderline statistically significant association between natural breeding system and brucellosis infection (p=0.05), whereby animals that were from natural breeding system had a reduced hazard compared to those from artificial

insemination systems, after adjusting for the production system (HR=0.06). There was no statistically significant association between the production system and brucellosis infection, after controlling for the breeding system. These results are shown in Table 6.

Table 6: Results of the multivariate Cox PH regression for cattle

Covariate	Cattle Adjusted HR	95%CI	P value
Breeding system			
Artificial insemination	1		Ref
Natural	0.06	0.004 - 1.029	0.0524
Both	0	-	0.9972
Don't breed	1.56	0.044 - 55.575	0.8081
Production system			
Settled pastoralist	1		Ref
Agro-pastoralist	0.20	0.016-2.75	0.235
Mixed farming marginal	0	0	0.995
Semi-zero grazing	0	0	0.993
Nomadic pastoralist	0	0 - 573.709	0.244
Production system * Time	1.0	0.996-1.017	0.220

Sheep that were raised under semi-zero grazing system showed an increased hazard for brucellosis (p =0.0001), after adjusting for having contact with other herds. There was no statistically significant association between contact with other herds and brucellosis infection after controlling for the production system. These results are shown in Table 7.

Table 7: Results of the multivariate Cox PH regression for sheep

Covariate	Sheep Adjusted HR	95%CI	P value
Contact with other herds (Yes)	0.253	0.031-2.094	0.2025
Production system			
Settled pastoralist	1		Ref
Agro-pastoralist	1.222	0.110-13.535	0.870
Semi-zero grazing	123	10.81-1413.15	0.0001
Nomadic pastoralist	3.589	0.715-18.015	0.1205

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Brucellosis incidence rates in cattle, sheep and goats

The incidence for brucellosis over the 9 months follow-up period was 1.7%, 0.7% and 0.3% in cattle, sheep and goats respectively. Brucellosis incidence rates were highest in cattle, followed by sheep and lowest in goats. Cattle had about 7 times the rate of brucellosis infection compared to goats and about 3 times compared to sheep.

This result is consistent with a multicenter study carried out by Hassan *et al* [27] in Egypt where a prevalence of brucellosis was 4.98% in cattle, 4.8% in sheep and 2.19% in goats. Another study of the 3 species by Anna *et al* [32] found seropositivity in cattle at (9%) while the small ruminants (sheep and goats) had 0% positive for *Brucella*. Recent studies in Malaysia [34] showed the same differences in prevalence (2.5% cattle, 1.5% sheep, 0.91% goats). However, a seroprevalence study carried out in Kajiado county in 2012 [9], showed a comparable prevalence of brucellosis in cattle (3.3%), sheep (3.4%) and goats (3.6%).

Brucella abortus is the main causal agent for brucellosis in cattle. In this study set up, characterized by majority of the herds coming into contact with wild animals, it could be important in the transmission of the disease thus yielding high incidence rates in cattle.

One of the objectives of this study was to compare incidence of brucellosis in cattle, sheep and goats in Kajiado East sub-county. The results showed that brucellosis occurred at a much higher prevalence in cattle than in sheep and goats.

There is scanty data on longitudinal studies for brucellosis. However, the results from this study agree with the prevalence studies, showing that the disease is more prevalent in cattle than it is in goats and sheep. And since incidence rates and prevalence rates are somehow related, we can make the above conclusions.

5.1.2 Factors associated with survival times in sheep cattle and goats

From this study, older livestock had a higher hazard rate compared to younger ones and the males had better survival than female species, though the associations were not statistically significant. Cattle from natural breeding systems showed a reduced hazard rate compared to those from artificial insemination systems. Nomadic pastoralism seemed to favor transmission infection, even though this association was not statistically significant. Animal breed and contact with wild animals did not show increased risk of infection.

Results from other similar studies [26, 31, 32] found incidence rates to be higher in female animals than in male animals and the prevalence rates to be higher in cattle than in goats and sheep. In their study in Togo, Anna *et al* [32] found 5.3 times higher odds of brucella seropositivity in female cattle compared to male. From our study, male sheep may have had worse survival because sexually mature rams are more prone to infections than younger ones [33]. Non-pregnant animals may not show signs such as abortions, hence may not be easily identified and curled; this could be the reason for high prevalence in female cattle.

In other brucellosis studies [28, 32], older animals that were more than 1 year old were more prone to brucellosis than younger animals, whereas those raised under nomadic pastoralist systems had 5.4 times higher odds for infection. This could be because older animals have had longer exposure with infected animals compared to the younger ones and susceptibility increasing with sexual maturity and pregnancy. A study by Walubengo *et al* [29] also found that animal level seroprevalence was higher in cattle raised in the pastoral systems compared to those in the zero-grazing system. Cattle over 2 years old had a higher risk of being infected that those less than 2 years [29, 32].

In our study, there were cattle that were positive for brucellosis from a herd bred using artificial insemination breeding system. This is similar to findings from a study on risk factors for bovine brucellosis in Uganda [30]. This study suggests use of artificial insemination with contaminated semen as a potential source for brucellosis infection. Similar to findings by Makita *et al* [30], our study showed no significant association of breeding system and age with brucellosis infection.

Similar to Boukary *et al* [28], our study did not find a significant association between contacts with other herds and wild animals and the risk of infection. This could be because greater than 98% of the study animals had contact with other herds as well as contact with wild animals. All animals infected with brucellosis had contact with wild animals and other herds.

A cross sectional study carried out in Uganda in 2011 [30] also found no statistically significant risk factors for brucellosis infection in cattle at the animal level characteristics.

In this study, we experienced a big number of non-significant effects for the association between study variables and brucellosis infection. The outcome was rare and this is potential contributor to non-significant associations. Out of the enrolled 709 cattle, only 12 (1.7%) of them became

infected with brucellosis during the study. For the enrolled 1369 sheep, only 10 (0.7%) were infected and for the enrolled 1704 goats, only 5 (0.3%) turned positive.

5.2 Limitations of the study

The number of events observed during the study period were few. This may have contributed to non-significant associations in modelling the hazard.

The study was carried out within 9 months. This was not long enough to observe a significant number of events and loss to follow-up was experienced during animal movements in search of pasture.

5.3 Conclusion

This study showed that cattle had higher incidence rates compared to goats and sheep, thereby having worse survival in relation to brucellosis infection. This study also indicated a relationship between time to brucellosis infection and cattle gender, age and production system though the associations were not statistically significant. For sheep, contact with other herds and production system was shown to be related to time to brucellosis infection although the association was not statistically significant.

The above results may have lacked statistical significance, due to the fact that the animals were observed for a shorter period of time (less than a year) giving not enough time to observe more events. For rare outcomes subjects need to be observed over a longer period of time which a limitation of cohort studies. There was also a significant loss to follow up which may have affected the results.

5.4 Recommendations for the current study

The study concluded with a relatively small number of events. This could be because the animals were observed for a shorter time period (9 months) and the outcome was rare. To observe more events the follow-up time could be increased.

Machine learning for survival data could also be explored to boost the data.

5.5 Recommendations for further research

There are limited longitudinal studies of brucellosis in livestock in Kenya. More studies should be carried out to better understand transmission dynamics of brucellosis in pastoralist communities. There should be more research into design and analysis of longitudinal studies with rare events.

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APPENDICES

Appendix 1 : CONSENT FORMS

Consent form for the compound head

Epidemiologic and Laboratory Assessment of the Burden of Brucellosis in Kenya Introduction:

We are visiting your household as part of a research project to investigate the links between human and animal health. This study is administered by researchers from Kenya Medical Research Institute (KEMRI), Centers for Disease Control and Prevention (CDC), Ministry of Public health and Sanitation and the Ministry of Livestock Development. The goal of this project is to look at the diseases that can be transmitted from animals to humans and to design new ways of carrying out surveillance and control of infectious diseases in this part of Africa. A total of 17,000 animals and 2500 human beings will participate in the study.

Purpose:

Some human illnesses may be caused by germs that are carried by animals, including domestic livestock. We are doing a research study to see if the animals in this area are carrying these germs, and if they are passing them to people. To do this, we would like to collect samples from part or all the animals that are owned by your household as well as from three people within your household, and test them for some of the germs that may possibly cause illness among humans and animals. The samples that we would like to collect include some blood. We would also like to ask you some questions about how the animals are managed. All this will take from one to a few hours, depending on the number of animals. The facts about you and your family from this study will be kept private as much as allowed by the law. No names will be used on any of the study reports.

Handling of specimens:

We will test the samples collected from your animals and members of your household at the KEMRI/CDC laboratory in Kenya, Central Veterinary Laboratory(CVL) and at other laboratories

abroad, as not all tests can be carried out in Kenya. We would also like to ask if we can store these samples to do more tests at a later time.

Benefits from being in the study:

Participants will be in this study will get free advice on management and animal health, including for those illnesses which are diagnosed in this study. Any information obtained from these tests that might be important for your family's health, or for your animals' health and welfare, will be communicated to you through project staff. Identification of diseases affecting your animals will help improve their health and welfare, as well as that of your family.

In addition, information obtained from this study may help the Ministry of Health decide when and where brucellosis disease may occur. SSC 2193 Version 4_11102012 Page 111.

Risks from being in the study:

If you are comfortable with it, we may ask you or members of your household to help with restraining the animals. This may expose you or your family members to risk of injury from the animals. Handling and restraining animals for sample collection can be slightly stressful for the animals and for people from the household who are participating. Every care will be taken to minimize this stress. Drawing blood can cause brief pain to the animals, and may result in brief bleeding. Sampling the animals may take some time, as will answering the questions about the animals.

Except for minor pain, bruising and bleeding that may be a part of taking blood, there are minimal risks from being in this study. In rare cases, an infection can result from drawing blood. If such infection occurs, the project will assume costs of treatment of the infection. In addition, it is possible that other people will find out that you participated in this study.

Voluntary participation:

Deciding whether or not to be in the study today is your choice. You can choose not to join, or

to drop out at any stage. This will not adversely affect you in any way. Should any more questions arise or if you feel like you, your family or your animals might have been harmed by being in the study, please contact Dr Stellah Kiambi or Dr Eric Osoro. Should you have any questions about your rights as research participants, please contact the secretary, KEMRI/NERC (tel. 0202722541 or 0722205901 or 0733400003). You will receive a copy of this signed consent form to take away with you The consent form has been explained to me and I agree for my family and animals to take part in the study. I have been told that I am free to choose not to take part in this study at any time and that saying "NO" will have no effect on my family or me.

Head of family	Name:	Signature/Thumb	date☐☐/☐☐/☐☐
	....	print: ..	
Witness*	Name:	Signature:	date☐☐/☐☐/☐☐
	....	....	
Interviewer	Name:	Signature:	date☐☐/☐☐/☐☐
	....	....	

Animal sampling Consent Forms

Brucellosis in livestock

We are visiting your household as part of a research project to investigate the links between human and animal health. This study is administered by the Kenya Medical Research Institute (KEMRI) and the Centers for Disease Control and Prevention (CDC). The goal of this project is to look at the diseases that can be transmitted from animals to humans and to design new ways of carrying out surveillance and control of infectious diseases in this part of Africa.

Some human illnesses may be caused by germs that are carried by animals, including domestic livestock. We are doing a research study to see if the animals in this area are carrying these germs, and if they are passing them to people. To do this, we would like to collect samples from part or all the animals that are owned by your household, and test them for some of the germs that may possibly cause illness in people. The samples that we would like to collect include some blood. We would also like to ask you some questions about how the animals are managed.

All this will take from one to a few hours, depending on the number of animals.

The facts about you and your family from this study will be kept private as much as allowed by the law. No names will be used on any of the study reports.

Storage and exportation of specimens:

We will test the samples collected from your animals and members of your household at the KEMRI/CDC laboratory in Kenya and at other laboratories abroad, as not all tests can be carried out in Kenya. We would also like to ask if we can store these samples to do more tests at a later time.

Benefits from being in the study

People who agree to be in this study will get free advice on management and veterinary care of their animals, including for those illnesses which are diagnosed in this study. Any information obtained from these tests that might be important for your family's health, or for your animals' health and

welfare, will be communicated to you through project staff. Identification of diseases affecting your animals will help improve their health and welfare, as well as that of your family.

Risks from being in the study

If you are comfortable with it, we may ask you or members of your household to help with restraining the animals. This may expose you or your family members to risk of injury from the animals. Handling and restraining animals for sample collection can be slightly stressful for the animals and for people from the household who are participating. Every care will be taken to minimize this stress. Drawing blood can cause brief pain to the animals, and may result in brief bleeding. Sampling the animals may take some time, as will answering the questions about the animals.

Deciding whether or not to be in the study today is your choice. You can choose not to join, or to drop out at any stage. This will not adversely affect you in any way. Should any more questions arise or if you feel like you, your family or your animals might have been harmed by being in the study, please contact Dr Stellah Kiambi. Should you have any questions about your rights as research participants, please contact **the secretary, KEMRI/NERC (tel. 0202722541 or 0722205901 or 0733400003).**

You will receive a copy of this signed consent form to take away with you SSC 2193 Version 4_11102012 Page 93 The consent form has been explained to me and I agree for my family and animals to take part in the study. I have been told that I am free to choose not to take part in this study at any time and that saying "NO" will have no effect on my family or me.

Appendix 2: QUESTIONNAIRE

PART I: COMPOUND INFORMATION

*To be answered by the **compound head.***

A. General Information

Date (dd/mm/yyyy):		Interviewer's Name:	
Sub-location:		Compound ID:	
Compound Geo-codes:			
Compound Head's Name (*at least two names*):			
Telephone:			
Number of households in compound:			

B. Animal demographics

B1   Do you own any livestock (Cattle, Sheep, Goats and Camels)?

  ○ Yes *(Go to B2)*   ○ No *(Move to Baseline Household questionnaire)*

  How many animals of each species do you own?

Livestock	Maturity status	B2. Number Owned	B3. Breeds	B4. Production system	B5. Usual calving season	B6. Breeding system	B7. Source of semen

Livestock	Maturity status	B2. Number Owned	B3. Breeds	B4. Production system	B5. Usual calving season	B6. Breeding system	B7. Source of semen
Cattle	Calves						
	Young adults						
	Adults						
	Total						
Goats	Kids						
	Young adults						
	Adults						
	Total						
Sheep	Lambs						
	Young adults						
	Adults						
	Total						
Camels	Calves						
	Young adults						
	Adults						
	Total						

Breeds: 1 = Indigenous   2 = Exotic   3 = Cross breed

Production system: 1 = Settled pastoralist   2 = Agro-pastoralist   3 = Mixed farming marginal   4 = Commercial ranch   5 = Peri-urban   6 = Semi-zero grazing 7=Nomadic pastoralist

Livestock	Maturity status	B2. Number Owned	B3. Breeds	B4. Production system	B5. Usual calving season	B6. Breeding system	B7. Source of semen

Usual calving period: 1 = Anytime   2 = Jan - Mar   3 = Apr – June   4 = July – Sept   5 = Oct - Dec

Breeding system: 1 = Artificial insemination   2 = Natural   3 = Both   4 = Don't breed

Source of semen;   1 = Own bull   2 = Other bull

B8   What is the main source of drinking water for your livestock?   *(Go to Part II)*

    ○ Pan/pond water   ○ Borehole   ○ River   ○ Communal trough   ○ Tap

    ○ Other (Specify) _____

PART II: HERD INFORMATION

*Part to be answered by the **compound head***

C.	***Risk factor information***

C1   Who else owns animals in the herd other than those in this compound??

☐ Relatives   ☐ Friends

☐ Neighbour   ☐ No one else

☐ Other (Specify)   _____

C2   Has your herd come in contact with other herds during grazing or watering in the past 3 months?

               ○ No   ○ Don't know

  ○ Yes

C3   Has your herd come in contact with wild animals during grazing or watering in the past 3 months?

○ Yes   *(Go to C4)*   ○ No *(Skip to C5)*   ○ Don't know *(Skip to C5)*

C4   If yes, which wild animals? (*Tick all that apply*)

☐ Zebra   ☐ Buffalo   ☐ Antelope   ☐ Waterbuck

☐ Wildebeest   ☐ Other (Specify)

C5   Have you experienced any of the following signs in your livestock in the last one year?

(*Prompt and tick all that apply*)

☐ Abortions   ☐ Still births   ☐ Retained placenta

☐ Swollen testes   ☐ Weak calf/kid/lamb   ☐ Repeat breeder

☐ Swollen joints   ☐ No *(Skip to C11)*

If you have experienced abortions, stillbirths and/or weak calf/kid/lamb in the last one year, fill this table:

	C6. No. of abortions	C7. No. of stillbirths	C8. No. of weak calf/kid/lamb	C9. No. of animals with retained placenta	C10. Number of Live births in the last one year
Cattle					
Goat					
Sheep					
Camel					

C11   Do you own cattle?

○ Yes   ○ No   *(Go to C16)*

C12   Have your cows been bred by bulls belonging to another herd in the last 1 year?

○ Yes   ○ No   ○ I do not own cows   ○ Don't know

C13   Have your bulls bred cows belonging to another herd in the last 1 year?

○ Yes   ○ No   ○ I do not own bulls   ○ Don't know

C14   Do you use designated areas when your cows give birth?

○ Yes   ○ No   ○ Sometimes   ○ Don't know

C15   Have you ever vaccinated your cattle herd against Brucellosis?

○ No   ○ Don't know

○ Yes

C16   Do you own sheep?

○ Yes   ○ No (*Go to C20)*

C17   Have your female sheep been bred by male sheep belonging to another herd in the last 1 year?

○ No   ○ I do not own female sheep   ○ Don't know

○ Yes

C18   Have your male sheep bred female sheep belonging to another herd in the last 1 year?

○ No   ○ I do not own male sheep   ○ Don't know

○ Yes

C19   Do you use designated areas when your female sheep give birth?

○ Don't know

○ Yes   ○ No   ○ Sometimes

C20   Do you own goats?

○ Yes   ○ No (*Go to C25)*

C21   Have your male goats bred female goats belonging to another herd in the last 1 year?

○ No   ○ I do not own male goats   ○ Don't know

○ Yes

C22   Have your female goats been bred by male goats belonging to another herd in the last 1 year?

○ No   ○ I do not own female goats   ○ Don't know

○ Yes

C23   Do you use designated areas when your female goats give birth?

○ Yes   ○ No   ○ Sometimes   ○ Don't know

C24   Have you ever vaccinated your goat herd against Brucellosis?

      ○ No   ○ Don't know
○ Yes

C25   Do you own camels?

      ○ Yes   ○ No *(Go to C29)*

C26   Have your female camels been bred by male camels belonging to another herd in the last 1 year?

      ○ No   ○ I do not own female camels   ○ Don't know
○ Yes

C27   Have your male camels bred female camels belonging to another herd in the last 1 year?

      ○ No   ○ I do not own male camels   ○ Don't know
○ Yes

C28   Do you use designated areas when your female camels give birth?

      ○ Don't know
○ Yes   ○ No   ○ Sometimes

C29   Have you ever found aborted fetuses on the grazing pastures and watering points in the last 3 months?

      ○ No   ○ Don't know
○ Yes

C30   How do you usually dispose aborted fetuses/still births?   *(Go to Part III:D)*

○ Bury   ○ I do not dispose   ○ Burn

○ Throw in the bin   ○ Throw in the bush   ○ Don't know

○ Feed to dogs   ○ Other (Specify)

PART III: INDIVIDUAL ANIMAL INFORMATION

*To be answered by **compound head** and the person taking care of the animals*
To be filled for each individual animal recruited

Number of animals
recruited: **_____** animals

	D. Individual Animal Details

D1 **Tag number:** _____

D2 **Species:**
- ○ Cattle *(Skip to D3)*
- ○ Sheep *(Skip to D4)*
- ○ Goat *(Skip to D4)*
- ○ Camel *(Skip to D5)*

D3 **Age (Cattle):** *(Skip to D6)*
- ○ Less than 2 years
- ○ 2 – 3 years
- ○ Over 3 years

D4 **Age (Shoats):** *(Skip to D6)*
- ○ Less than 6 months
- ○ 6 – 12 months
- ○ Over 1 year

D5 **Age (Camels):** *(Go to D6)*
- ○ Less than 4 years
- ○ 4 – 6 years
- ○ Over 6 years

D6 **Breed:**
- ○ Indigenous
- ○ Exotic
- ○ Cross breed

D7 **Maturity status:**
- ○ Young
- ○ Young adult
- ○ Adult

D8 **Sex:**
- ○ Female *(Go to D9)*
- ○ Male *(Skip to D16)*

D9 **Breeding status:**
- ○ Nulliparous *(Skip to D20)*
- ○ Post-partum *(Skip to D11)*
- ○ Pregnant *(Go to D10)*
- ○ Not pregnant *(Skip to D12)*

D10 If pregnant, how far along is the pregnancy?
(If unknown, do pregnancy diagnosis if possible)
_____ Months *(Skip to D13)*

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D11   If post-partum, when was the last parturition?

                                           _____ Days ago *(Skip to D14)*   ○ Don't know

D12   If not pregnant, when was the last parturition?

                                           _____ Months ago*(Skip to D14)*   ○ Don't know

D13   If pregnant, how was pregnancy achieved? :   *(Skip to D15)*

○ Artificial Insemination   ○ Used own bull   ○ Used other bull   ○ Don't know

D14   If post partum or not pregnant, how was the most recent pregnancy achieved?   *(Go to D16)*

○ Artificial Insemination   ○ Used own bull   ○ Used other bull   ○ Don't know

D15   How many times has this animal been pregnant in its life time?   *(Go to D16)*

○ Once   ○ Twice   ○ Three times   ○ More than three times

○ Don't know

D16   **Breeding status:**   ○ Young *(Skip to D20)*   ○ Breeding *(Go to D17)*   ○ Castrated *(Skip to D20)*

D17   If used for breeding, has this male been used for breeding within the herd in the last 1 year?   *(Go to D18)*

                              ○ No   ○ Don't know

                   ○ Yes

D18   Has the male been used for breeding outside the herd in the last 1 year?   *(Go to D19)*

                              ○ No   ○ Don't know

                   ○ Yes

D19   Have you ever experienced any of the following in this animal? *(Prompt and tick all that apply)* *(Go to D20)*

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☐ Swollen testes   ☐ Swollen joints   ☐ Apparent Infertility

D20	**Weight in Kgs:**	_____ Kgs	*(Go to D21)*
D21	**Girth measurement:**	_____ cm	*(Go to D22)*
D22	**Temperature:**	_____ °C	*(If Male, Go to D25)*

D23   **Vaginal discharge:**   ☐ Clear   ☐ Colored (Specify)   _____

(Go to D25)   ☐ Smelly

D24   **Samples collected:**   ○ Serum   ○ Whole blood

Appendix 3: ANIMAL SAMPLE TRACKING SHEET

INDIVIDUAL ANIMAL DATA

Breed: A = Indigenous   B = Exotic   C = Cross breed

Sex: M = Male   F = Female

Age (Cattle): A = < 2 years   B = 2-3 years   C = > 3 years

(Shoats): A = < 6 months   B = 6-12 months   C = > 1 year

(Camel): A = < 4 years   B = 4-6 years   C = > 6 years

Bred/ Breed within herd? Y = Yes   N = No   DN = Don't know

Bred/ Breed outside herd? Y = Yes   N = No   DN = Don't know

Females:

Breeding Status: A = Nulliparous   B = Pregnant   C = Post-partum   D = Not pregnant

Breeding system: A = AI   B = Served by bull   C = Don't know

History: 1 = Abortions   2 = Still births   3 = Weak young   4 = Metritis   5 = Retained placenta   6 = Swollen joints   7 = Reduced milk   8 = Infertility

Discharge: A = Clear   B = Colored _____   C = Smelly   D = No discharge

Males:

Breeding status: A = Young   B = Breeding   C = Castrated

Breeding system: A = Serves cows   B = Not used for breeding

History: 1 = Swollen joints   2 = Swollen testicles   3 = Apparent infertility

Animal number	Animal Tag No.	Breed	Sex	Age	Breeding status	Pregnancy status ___ months pregnant	Last parturition was ___ months ago	Ever been pregnant ___ number of times	Breeding system	Breed/ Bred within herd?	Breed/ Bred outside herd?	History	Weight (Kgs)	Girth (cms)	Temp (°C)	Discharge
1																
2																
3																
4																
5																