

**SEROPREVALENCE ESTIMATES OF BRUCELLOSIS AND
COXIELLOSIS IN CATTLE, SHEEP AND GOATS: ASSOCIATED
RISK FACTORS AND PERCEPTION IN LIVESTOCK FARMERS
IN NANDI COUNTY, KENYA.**

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**A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN
VETERINARY PUBLIC HEALTH.**

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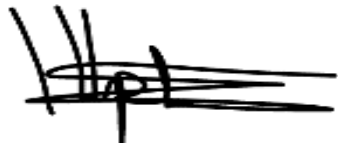
JULY, 2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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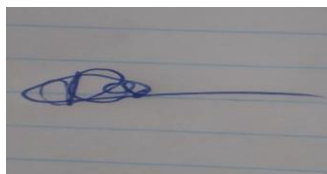
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DEDICATION

I dedicate this thesis to my husband Nicholas Kirwa, daughter Sharon Jephumba and son Felix Kibet for their encouragement and support throughout my study period.

To my mother for her love and prayers and late father for his inspirational advice and encouragement in undertaking this study.

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ABSTRACT

Brucellosis and Coxiellosis are important zoonotic diseases that affect most domestic and wild animals including humans worldwide and have socioeconomic and public health implications. The design was across-sectional study and was conducted to investigate the seroprevalence of these two closely related zoonoses namely brucellosis and coxiellosis in domestic ruminants in all the six sub counties of Nandi County. In addition, risk factors associated with the seroprevalence of the two diseases in animals were assessed as well as knowledge, attitudes and practices (KAPs) towards these diseases.

Total blood samples collected were 1,140 drawn from cattle 63.6 % (n=725), goats 11.6 % (n=132) and sheep 24.8 % (n=283). The samples were collected from 366 households across the county. A multistage sampling technique was used, where wards, herds and individual animal were randomly selected. Screening for Brucella antibodies was done using the Rose Bengal Plate Test (RBPT) and thereafter by indirect Enzyme-Linked Immunosorbent Assay (iELISA) for confirmation. Likewise, sera for Q- Fever analysis were subjected to iELISA. A total of 366 households were interviewed for KAPs assessment.

Results confirmed low seroprevalence of brucella infection in domestic small ruminants in the County, but higher seroprevalence for coxiellosis. Overall seroprevalence in livestock was 0.088% (1/1140) and 5.614% (64/1140) for brucellosis and coxiellosis respectively. In cattle, seroprevalence of brucellosis was 0.138 % (1/725) and 0 % seropositivity in both goats and sheep on iELISA, despite, recording 0.414 % (3/725), 3.180 % (9/283) and 1.515 % (2/132) on screening using RBPT in cattle, goats and sheep respectively. Seroprevalence estimates for coxiellosis on iELISA was 8.138% (59/725) for cattle, 1.413% (4/283) for sheep and 0.758% (1/132) for goats. Three important potential predictors were identified for seropositivity of brucellosis. These were

species (p-value <0.010, CI 95%), age (p-value 0.042, CI 95%) and breed (p-value 0.037, CI 95%). For Coxiellosis, the only significant predictor was animal species where P-value was 0.015, CI 95% and OR 7.260) in the three considered animal species for the presence of *C. burnetii* antibodies. The other variables (breed, sex, age and production system) had no statistically significant association for coxiellosis infection since p-value was > 0.05. Further, the study established livestock farmers in Nandi County were knowledgeable (60%) on brucellosis in animals but low in Coxiellosis (40%). In terms of perception, the participants had negative attitude towards both diseases by reporting 28.05% for brucellosis and 13.9% for coxiella. However, the farmers had good precautionary practices towards control and prevention of these two diseases as shown by 71.58% for brucellosis and 99.55% for coxiellosis.

The data provided valuable information on the status of the two diseases in the Nandi County. It demonstrated presence of circulating brucella and coxiella antibodies in domestic ruminants which may pose a serious zoonoses among the inhabitants. There is therefore, need to sensitize and create awareness among stakeholders in order to minimize misdiagnosis and unnecessary treatment of both animals and humans. From these findings, it was recommended that the County Veterinary services should make deliberate efforts to thoroughly investigate all reported cases of animal abortions, retain placenta, infertility in animals and integrate brucellosis and coxiellosis surveillance in their disease reporting systems. There was also urgent need for the creation of a County Zoonotic Disease Unit (ZDU) to provide a platform that enhances information sharing and joint control and prevention strategies between the Directors of veterinary and medical services under the one health concept.

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LIST OF ABBREVIATIONS AND ACRONYMS

BSC	Biological Safety Cabinet
CIDP	County Integrated Development Plan
DVS	Director of Veterinary Services
ELISA	Enzyme- Linked Immunosorbent Assay
GDP	Gross Domestic Product
ILRI	International Livestock Research Institute
KAPs	Knowledge, Attitudes and Practices
KNBS	Kenya National Bureau of Statistics
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RVIL	Regional Veterinary Investigation Laboratories
TNZD	Tropical Neglected Zoonotic Diseases
WOAH	World Organization of Animal Health
WHO	World Health Organization

CHAPTER ONE

1.0. INTRODUCTION

Brucellosis and Coxiellosis (Q- fever) are among the leading tropical neglected zoonotic diseases (TNZD) (Franc *et al.*, 2018). They are caused by *Brucella species* and *Coxiella burnetii* organisms respectively (Njeru *et al.*, 2016). *Brucella* and *Coxiella* infection in ruminants are characterized by abortion, delivery of weak young ones or still births, hence causing serious reproductive losses (Porter *et al.*,2011; Coelho *et al.*,2015). Both infections cause chronic disease in the uterus/mammary gland of infected animals (Vaidya *et al.*, 2012). The bacteria are mostly shed in placenta and birth fluids as well as milk and feaces (Burns *et al.*, 2018). The diseases cause severe fever and long-lasting infection in human beings (Kanani *et al.*,2018). *Coxiella* organisms are resistant within the environment and may be transmitted through aerosols which may cause widespread outbreaks due to low infectious doses (Burns *et al.*,2018). The *coxiella* organisms may also be spread by tick bites to livestock. The control of these pathogens poses a great challenge because the infection is latent in nature. Control of brucellosis through culling of infectious animals within a herd are more difficult and complicated (Porter *et al.*,2011).

According to Njeru *et al.*, 2016, data on the true status of brucellosis and Coxiellosis in Kenya are limited. Previously done studies appeared to have been biased to arid/semi arid lands, since the information in highland areas are either scanty or completely lacking. No data was available on ruminants in Nandi County.

1.1. Background information on animal brucellosis

Brucellosis is primarily a reproductive disease. The disease is regarded among one of the most significant world's widespread zoonoses (Poester *et al.*, 2002). The causative agent is brucella species that comprised of seven terrestrial and two marine species. The terrestrial Brucella species include *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, *B. canis*, *B. neotomae* and *B. microti*. The two brucella species that were isolated from aquatic mammals are *B. pinnipedialis* and *B. ceti* (Chota *et al.*, 2016). The first 3 terrestrial brucella species have several biovariants (OIE, 2008). Species of concern for the region are *B. abortus* which primarily affect cattle and causes undulating fever in man and *B. melitensis* in goats and sheep causing Malta Fever in man. Brucellosis also occurs in pigs, camels, equine, dogs and several species of wildlife (Borreillo *et al.*, 2006). *B. abortus* is principal cause of brucellosis in cattle, resulting in loss of income arising from low milk production, declined calving percentage, delayed calving interval, culling due to infertility, treatment cost, abortions, still birth, retained placenta, weak calves at birth as well as loss of man hours in affected people. In bulls, infection causes epididymitis, hygroma, orchitis and seminal vesiculitis (McDermont *et al.*, (2002).

Exposure in livestock is primarily through contacts with uterine materials such as, aborted fetus, uterine fluids and placentas. It is also transmitted via milk from the infected dams and vaginal discharges. Other routes include contaminated water or feed, inhalation and also through the conjunctiva (Nicoletti., 2002). Young animals may be infected *in utero* or infected colostrum and milk. Documented reports of venereal transmission of brucellosis have also been described. In naive susceptible herd, rate of abortions varies from 30.0 to 70.0 % (Bercovich., 1998). Brucella infection can be life- long, and during following pregnancies, there may be infection of the gravid uterus and allanto-chorion. Thereafter, abortion hardly recurs but cases of uterine or

mammary infection may recur. Normally the reproductive performance of carrier animals are not affected and are usually retained in herd making effective control programs extremely difficult because they are chronic carriers (Pappas, 2005). Human exposure occurs primarily through the ingestion of unpasteurized milk from infected animals and secondarily from exposure to uterine discharges and infected tissues.

In many countries, the infections may be caused by *B.melitensis* especially where cattle are kept together with sheep or goats (Ocholi *et al.*, 2004). Despite the disease having a worldwide geographical distribution, it remains endemic in livestock and a major concern in terms of public health in Africa, Asia and Latin America. In developed countries, brucellosis disease has been or about to be eradicated, but has remain problematic in states with poor animal and human health control programs (Donev *et al.*, 2010). The disease has been documented as an occupational hazard to veterinarians/animal health technicians, animal farmers, workers in dairy processing, slaughter houses workers and laboratory. All these categories are considered high risk occupational groups. Anyone working with diagnostic sampling or testing must take careful precautions and use proper biosecurity and biosafety measures. All potentially contaminated materials including infected tissues and culture should be handled at Biocontainment Level 3 Laboratory. Ideally when brucellosis is diagnosed in animals, human health authorities should be notified immediately. This is because animals and humans normally act as sentinel herd for each other hence, whenever infections are confirmed in animals, the human involvement should be presumed and addressed with and vice versa. It's prudent for close cooperation between animal and medical health personnel. Brucellosis disease is an excellent example which can be dealt with the new One Health concept of collaboration between Veterinary and medical health professionals (Peninah *et al.*, 2019).

According to McDermont *et al.*, 2002, the pattern and distribution of brucellosis in both livestock and human beings are poorly understood. The author further reported on Sub Saharan Africa, where the average sero-prevalence in cattle populations varies widely from 1% - 50%. Faye *et al.*, (2005), reported sero prevalence of 15.8 % and 10.3 % in the South western and Western part of Uganda respectively. Domingo (2000) reported a 41% sero prevalence of cattle brucellosis in Togo, another sub Saharan tropical country with rudimentary animal disease control systems. In Kenya a survey done in Kajiado (Nakeel *et al.*, 2016), showed seroprevalence estimates of brucellosis were 21.92%, 8.6% and 7.3% for cattle, sheep and goats respectively. Also a study which was done in Baringo indicated a seroprevalence of 6.80% in cattle, 6.65% in goats and 4.90 % in sheep (Kosgei *et al.*, 2016). In spite, the prevalence being high and highly variable in several countries, surveillance for the brucellosis is generally poor (Donev *et al.*, 2010). Bale *et al.*, (1982) reported the factors normally assumed to be responsible for these variations in seroprevalence of brucellosis in animals include introduction of an infected animal for replacement or upgrading, communal dipping, common grazing and water sources, animal demographic factors, climate, livestock movement, management systems, herding of different species together and sharing of bulls, bucks and rams.

In Kenya the livestock industry contributes approximately 11.0% of the National gross domestic Product (GDP) therefore, deliberate attempt on disease eradication and control are paramount for development of the sub sector. The contribution of brucellosis in limiting livestock productivity and its impact on the industry is unknown since information on prevalence of this disease in domestic ruminant is inadequate. In addition, information on farmer's perception and their perceived impacts of brucellosis in animals and human is also scarce.

Nandi County is one of the high potential areas, with dairy farming as a principal activity of the residents. The livestock sub- sector is a vibrant and remains as major source of livelihood and is one of the key drivers of the county economy. It is dominated by smallholder dairy farmers who contribute to over 90% of the total milk production annually (CIDP., 2013). The county is considered to be among the highest producers of milk in the country and a source of milk to many neighboring counties. The records at the office of the Director of veterinary services in Nandi County (CDVS.,2015), showed an average of 25 cases of abortions are reported and suspected as brucellosis every month in cattle. Consequently, seroprevalence of brucellosis in cattle, sheep and goat was perceived to be significantly high and potential threat to source of infection in the country. However, according to a previously done study by Jerono *et al.*, (2012) on financial impact of misdiagnosis of human brucellosis in Nandi County, it was noted that residents of Nandi were at minimum risk of contracting brucella infection following high number of false positives.

1.2 Background information on Animal coxiellosis

Coxiellosis is regarded as neglected zoonotic disease. It is caused by the obligate intracellular bacterium called *Coxiella burnetii*. The disease has emerged as a worldwide significant human and veterinary problem (Sprong *et al*, 2012). The disease infects a range of animals including a large range of mammals, domestic and wild birds, reptiles and arthropoda. The infection causes mild disease in ruminants, and outstanding signs are abortion and stillbirth in cattle, sheep and goats. Coxiellosis is considered a worldwide endemic disease, except only New Zealand (OIE., 2018). Q fever mostly presents as asymptomatic and acute disease in man, it is mainly limited flu like sickness, pneumonia and hepatitis, whereas the chronic disease manifests with long term

fatigue and endocarditis. Symptoms of Coxiellosis include abortion, still birth or pre-mature births in pregnant women (Maurin., 1999).

In animals, particularly ruminants, Coxiellosis is asymptomatic in many cases but has been linked to reproductive disorders such as; infertility, late abortion, still births, weak offsprings and metritis. Infected animal normally shed the bacteria through placenta and birth fluid. These contaminants infect the environment resulting to air borne disseminations and infections of humans in close association with livestock (Cutler., 2007). Therefore, Q- fever is often regarded among occupational diseases affecting livestock producers, abattoir workers and veterinarians (Maurin., 1999). Domestic ruminants are implicated as the primary source of transmissions for human beings. Man is mainly infected by inhalation of polluted aerosols or through ingestion of contaminated milk or fresh dairy products. Domesticated pets, such as dogs, cats, geese (*Anser anser*) and rock doves (*Columba livia*) are known to be the other source of infection of coxiellosis (Marrie., 2011). Other investigations have documented occurrences of *C. burnetii* in migratory birds, rodents and ticks in South Cyprus (Ioannou., 2009).

In many Countries including the United States of America, Coxiellosis is among the listed reportable diseases. However, despite the infection having been reported in most African countries, it is not listed among the priority diseases under routine surveillance by the concern Authority (Knobel *et al.*, 2013). This could imply that Coxiella could be missed out in differential diagnosis when considering cases of abortions and infertility cases in livestock and in flu like and febrile symptoms in man.

In Kenya, *C. burnetii* organism is an important pathogen that is under reported and assumed to contribute the numerous undiagnosed reproductive cases in livestock and febrile related illness in

the farming communities (Nakeel *et al.*, 2016). The sero-prevalence rates from surveyed regions in Kenya, varied widely from 7.4- 51.1 % in cattle, 6.7- 20.0 % in sheep, 20.0- 46.0 % in goats and 20.0-46.0% in camels (Njeru *et al.*, 2016). According to a study done by Koka *et al.*, (2018), seroprevalence of Coxiellosis was reported at 12.1% in both human and animal population in five of the seven former provincial regions of Kenya surveyed and the risk factors was reportedly higher in grazed animals. In Baringo County, reported prevalence of coxiellosis was 26% and 12.2% in goat and sheep respectively (Kosgei *et al.*, 2016). In Kajiado the seroprevalence estimates in cattle, goats and sheep were 89.7%, 83.1% and 57.5% for Coxiellosis respectively (Nakeel *et al.*, 2016). Despite Nandi County being one of the high potential regions in Northern Rift Valley, where dairy is the major source of livelihood for the residents, data on Coxiella were not available.

1.3 PROBLEM STATEMENT

Coxiellosis and Brucellosis are zoonotic diseases that severely hinder increased livestock production and human health worldwide. The disease burden on low income Countries has led the World Health Organization (WHO) to rank the two infections as some of the world's leading Tropical Neglected Zoonoses (Franc *et al.*, 2018). In many countries, brucellosis in cattle and goats has emerged as a major hindrance in the expansion of the dairy sector development where both exotic and high yielding local cattle and goat breeds are raised (Mwangi., 2015).

In Kenya, brucellosis and coxiellosis just like other chronic animal diseases are ignored during resource allocation for disease control activities by the policy makers. They are also expensive and take time to manage owing to the complexity in their dynamics (Njeru *et al.*,2016). Further, brucellosis was not recognized as a notifiable disease until its gazettelement in the year 2011 despite being on the World Organization of Animal Health list. Therefore, prior to this, cases of

the disease were rarely reported to relevant authorities and therefore it was difficult to determine the status of the disease nationwide. To date, coxiellosis is not on the list of priority diseases and appears little known even to both veterinarians and human medical professionals. The free-range production system practiced in most parts of the country including Nandi County helps to maintain both diseases in animal and human populations. Further, cultural practices such as living in close association with domestic livestock, coupled with consumption of raw livestock products such as whole blood, meat or raw milk is still rampant in several parts of the county (Jerono *et al.*, 2012). Therefore, consumption of raw livestock products coupled with unsanitary conditions favour transmission to man. This scenario is attributed to lack of adequate information on the status of these two diseases and the impacts on livestock productivity in the county. However, the trend can be reversed if adequate data on brucellosis and coxiellosis are made available through research to inform the policy makers on the importance of the diseases in regard to public health and general economy. Importance of these diseases in dairy sub sector is a major concern because milk and milk products are the main sources of contamination and spread (Mangen *et al.*, 2002).

According to the records obtained from the Nandi County Veterinary Office, all cases reported as abortions, brucellosis remain as one of the differential diagnoses because there is no veterinary laboratory to confirm these suspected cases in the county. The situation of coxiella is even worse because it is not even mentioned among reported cases, which implied that the animal health experts are not aware or even keen to suspect its exposure in animals. The nearest Veterinary Investigation Laboratory (VIL) to Nandi County is in Eldoret town of Uasin Gishu County which is over 50 kilometres from the headquarters of Nandi County. Available data from VIL showed that few animal health practitioners utilized these services probably due to the

distance and cost implication. In this lab only Rose bengal plate test (RBPT) was carried out for diagnosis of brucellosis but, no confirmatory test is done, neither do they carry out any coxiella test. Currently in the County, there is no comprehensive control strategy tailored for Brucellosis and Coxiellosis owing to lack of adequate data and information on the diseases.

Therefore, the main goal of this investigation was to address these gaps in order to provide information on the current status of these two closely related zoonotic diseases.

1.4. JUSTIFICATION

The livestock industry is the major source of livelihood for the over 885,711 residents of Nandi County. The livestock population is estimated at 309,038 cattle, 121,461 sheep and 46,669 goats. (KNBS, 2019). The main livestock husbandry practised in the county is extensive and semi-intensive with a few in intensive system. It is also a common practice to find those farmers who keep cattle to also keep sheep and goats and this provided an opportunity for the spread of brucella and coxiella infections from cattle to sheep and goats and *vice versa*.

Currently, the County is one of the key sources of milk and milk products as well as livestock for both slaughter and breeding for many parts of the country, particularly the former western and Nyanza Provinces (Jerono *et al.*, 2012). The movement pattern of livestock as well as their products poses a potential risk of spreading brucellosis and coxiellosis to these regions. In addition, some animals are destined for neighbouring countries of Uganda, Tanzania, and Rwanda among others. In all these countries, brucellosis tops the lists of diseases that animals must be free from before they are issued with an import permit (DVS., 2011).

Brucellosis and coxiellosis have devastating impacts on socio-economic and public health. Due to scarcity of information of these two diseases, there is need to assess their level in livestock

population from time to time to reduce income losses due to abortions and infertility at the same time minimize the risk of humans contracting the diseases (Njeru *et al.*,2016)

This study was therefore designed to investigate the seroprevalence of Brucellosis and Coxiellosis in cattle, sheep and goats in Nandi County. Study further sought to identify the related risk factors and farmer's perception for these diseases among residents of Nandi County. The findings from this study will identify knowledge gaps in science. The study will also provide information on possible areas of intervention in order to minimize impact of the diseases on domestic ruminants and health of humans as well as socio-economic consequences and at same time allow Nandi County to continue enjoying the existing robust local and regional livestock trade.

1. 5: OBJECTIVES

1. 5. 1. General objective

To estimate the extent of infections of brucellosis and coxiellosis in cattle, sheep and goats, determine associated risk factors and assess the perception of the diseases by the livestock farmers in Nandi County, Kenya.

1. 5. 2. Specific Objectives

The specific objectives were to;

1. Estimate seroprevalence of brucellosis and coxiellosis in cattle, sheep and goats in Nandi County.
2. Determine the risk factors associated with the occurrence of brucellosis and coxiellosis in livestock in Nandi County.

3. Assess farmers' Knowledge, Attitude and Practices towards brucellosis and coxiellosis in Nandi County.

1.5.3 Null hypothesis

1. Brucellosis and coxiellosis diseases are not a problem in domestic ruminants in Nandi County.
2. Risk factors of brucellosis and coxiellosis in cattle, sheep and goats are not associated with occurrence of the infections in Nandi County.
3. Farmers in Nandi County have low level of perception on knowledge, attitudes and practices on the occurrence of brucellosis and coxiellosis animals.

CHAPTER TWO

2. 0. LITERATURE REVIEW

2.1 Brucellosis

2.1.1. Definition and brief history of brucellosis

Brucellosis is an infectious, and contagious bacterial disease of many animal species as well as humans (Blood *et al.*, 1989). The disease is caused by various species of Brucella organisms. The bacteria belong to gram negative, non motile, nonspore forming, rod shaped organism named after physician David Bruce (1855-1931). The different species of Brucella organism are genetically similar though each has different host specificity (Du Preez al 2018). The infection is known to be an economically important cause of abortion in cattle, goats and sheep. *B.abortus* also affects other wildlife species including the wild buffalo, bison and elk and some of these species serves as reservoir hosts for this organism (Alton, 2007). The author further noted that infections in wild animals can hinder eradication efforts in livestock. Brucella species is also a human pathogen which causes serious, debilitating and sometimes chronic disease that can infect a variety of organs.

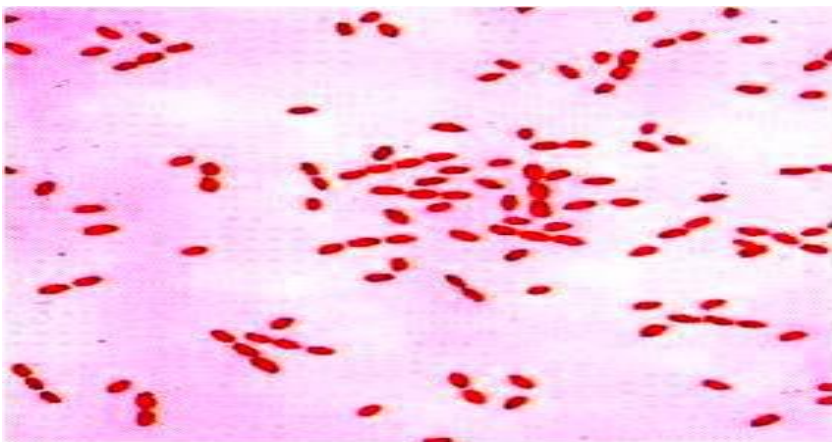


Figure 2.1 A micrograph showing brucella organisms (Alton., 2007)

2.1.2 Epidemiology

2.1.2.1 Etiology

There are 10 species defined in the genus *Brucella*. Each genus may infect different animal host species but, each *spp* has a preference for its host species (Xavier *et al.*, 2009):

No.	<i>Brucella</i> species	Host species
1.	<i>B.abortus</i>	Cattle, camel and buffaloes
2.	<i>B.melitensis</i>	Goats and sheep
3.	<i>B .suis</i>	Pigs
4.	<i>B. ovis</i>	Sheep and goats
5.	<i>B canis</i>	Dogs
6.	<i>B. microti</i>	Rodents (<i>Microtus arvalis</i>)
7.	<i>B. neotomae</i>	Rodents (<i>Neotoma lepidae</i>)
8.	<i>B. pinnipedalis</i>	Pinnipeds (marine animals)
9.	<i>B. ceti</i>	Cetacea (marine animals)
10.	<i>B. inopinata</i>	First isolated in human but the preferential host unknown

Table 2.1 *Brucella* organism species and host preference (Source: Scholz *et al.*, 2013).

The first three *brucella* species can be sub- divided in bio types (Ocampo-Sosa *et al.*, 2005). Eight bio types (1-7, 9) in *B. abortus*, three biotypes (1-3) have been identified in *B. melitensis* and five bio types (1-5) in *B. suis* (Whatmore, 2009). All the mentioned *Brucella* species are considered serious potential pathogenic for human beings, with the exceptions of *B.ovis*, *B. neotomae*, *B. microti* and *B. ovis* (Xavier *et al*, 2009a). *B. ceti* and *B .pinnipedialis* are the species isolated from marine animals (OIE, 2008), while the rest are terrestrial (Glynn, 2008).

2.1.2.2 Distribution and occurrence

The distribution of brucellosis is worldwide particularly in regions where cattle are raised except in Canada, Japan, Australia, New Zealand and some European Countries, where it has been eradicated and free from disease. In the US, eradication process of the disease for the domesticated herds are nearly completed (Robinson, 2003). However, the disease has remained a major public health constraint in Middle East, Mediterranean, Africa, Latin America, and parts of Asia (Pappas *et al.*, 2005).

2.1.2.3 Transmission

Brucellosis is principally a herd disease, where spread between different animal herds usually occurs by the introduction of an asymptomatic clinically infected animals (Nicoletti., 1992., Perry *et al.*, 2002). Normally initial infection in carrier species is often preceded by abortions, delayed or permanent infertilities. In animals, it present normally as a chronic and treatment is rarely undertaken (Mangen *et al.*,2002) making brucellosis prevalent in many countries that do not have good standardized prevention strategies (Gul *et al.*, 2007). Infected animals usually shed the organisms in uterine fluids following abortions or successful parturition as well as in colostrum/ milk (Mangen *et al.*,2002). Herd spread primarily followed ingestion of contaminated materials, though other studies have also mentioned venereal transmission (McDermott and Arimi., 2002; Mangen *et al.*, 2002). Congenital infection and peri- natal infections can also occur with development of latent infections. There is normally high level of bacteria in birth fluids (Akoko., 2010). According to Alton (2007), he reported that the organisms are usually transmitted by the infected animal via contact with aborted fetuses, placenta, fluids and vaginal discharges. It may also be isolated in milk, semen, urine, feaces and hygroma fluids. The shedding of organism in milk can occur for long period of time or may be shed intermittently

(Asakura *et al.*, 2018). Majority of infected animals become chronic carriers of the disease. It can also be spread through fomites including vehicles such as water and feeds. In regions with high humidity and lower temperatures or less sunlight, these brucella organisms may remain viable for many months in either water source, aborted fetuses, manure waste, wools, harvested hay, equipment and in cloths (Alton, 2007). *Brucella species* may withstand drying, especially when organic material is present. It can also survive for a long time in dusts and soils. The survival may be even longer when the temperature is lower, especially during freezing point.

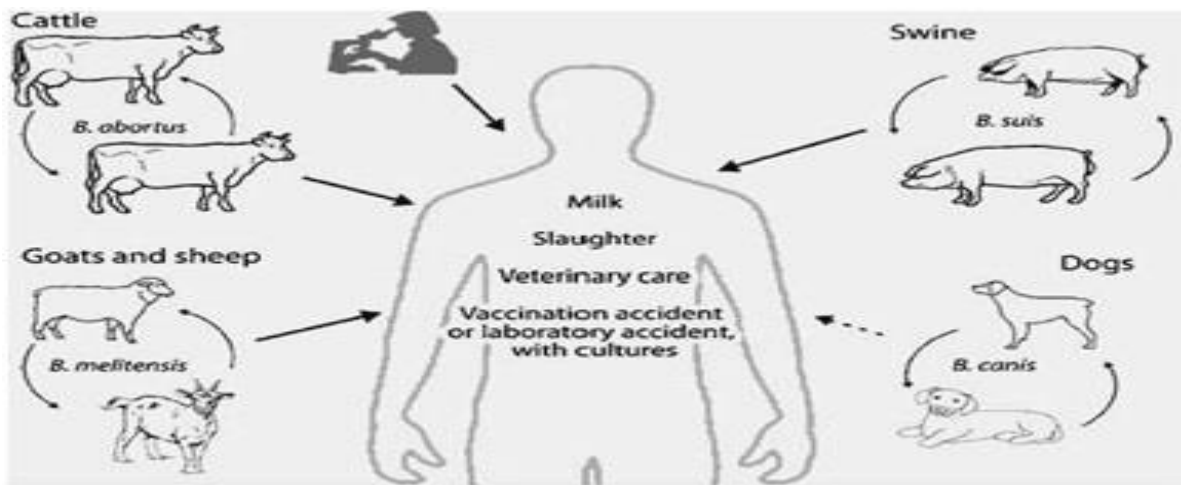


Figure 2.2 Pathways involved in the transmission of brucella (Source: Minja *et al.*, 2002)

2.1.3 Clinical manifestation

Brucellosis in animals causes abortions and stillbirths; abortions usually occur during the last trimester of the gestation (McDermont and Arimi., 2002). Some calves may be born alive, but weak and may die soon after birth. Usually, the placenta may be retained and secondary metritis can occur. Infection may cause lactation to be decreased. Following first abortion, subsequent pregnancies are generally normal however, animals may shed the organisms in milk and uterine discharges (CDC., 2005). Some of the symptoms in bulls includes inflammation of epididymis, seminal vasculitis, orchitis or abscesses in the testicles may be seen. Reports of Infertility

sometimes occurs in both sexes, due to inflammation of the uterus of scrotum/epididymis. Likewise, there inflammation of leg joints or hygroma which may lead to arthritis. In uncomplicated infections, systemic signs or death rarely occur, except in the fetus or newborn. Also, the infections in unpregnant females may not show any symptom (Seleem *et al.*,2010).

In human being, brucellosis is multi-systemic infections that may vary considerably and may last between three days to six months or occasionally for longer than a year (Hugh., 2000). The disease manifest as undulant fever with nonspecific signs such as general malaise, fever, fatigue, anorexia, sweats, muscles and joint pains (Wafa *et al*, 2009). Infection may be severe or chronic and may be followed by intermittent relapses (Stella *et al*, 2020).

2.1.4 Diagnosis

Brucella screening mostly utilize serological tests which are based on the detection of the antibodies produced against brucella antigens (Ewalt *et al.*, 2002). There has been indiscriminate use of most available tests, showing the possible absence of an ideal test for the disease (Ramon and Ignacio, 1989). The serological methods have been classified into to two; those that use whole smooth cells antigens and those that use soluble antigens. These tests include Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Complement Fixation Test (CFT), Coombs Test and Immuno-flourescent Test, while Enzyme Linked Immunosorbent Assay (ELISA), Radio-Immuno Assay (RIA) and Gel Precipitation Tests uses soluble antigens (Nielsen *et al.*, 2002).

For brucella diagnosis in animals, the gold standard remains isolation of *Brucella* bacterium through culture. However, the process to isolate the bacteria is normally time consuming and also resource intensive. It requires a Level 3 Bio-containment facility and highly skilled technical

person to handle samples and live bacterium for eventual identification and bio-typing. Risk of laboratory infection do occur when handling brucella samples and hence, strict biosafety rules must be observed. In order to minimize these infections, other methods have been newly developed such as, ELISA and Polymerase chain reactions (PCR). These methods have proven to be very beneficial and substantial progress has been made towards improving their sensitivity and specificity leading to lower cost and less technical. For bio typing of *Brucella spp*, new techniques such as the multiplex AMOS-PCR are in handy to isolate *B. Abortus*, *B. melitensis*, *B. ovis*, *B. suis* species is often used. This PCR protocol allow differentiation between *Brucella* organisms species and vaccine and wild-type strains (Bricker., 2002).

2.1.4 Treatment and Prevention

It's difficult to treat brucellosis, antibiotic use is long and relapses is common. The eradication process for brucellosis from herds is by normally testing and removal protocol. This can be done through frequent screening for brucellosis and herd health campaign in order to identify infected animals, so that they can be slaughtered to eradicate the disease and enhance practice food and occupational hygiene to prevent transmission of brucellosis to humans (McDermont, 2010). Farmers can also be trained on methods of controlling brucellosis to reduce its prevalence such as vaccination, promotion of use of artificial insemination to reduce venereal transmissions of brucellosis during breeding.

2.1.6 Risk factors associated with brucellosis

The disease incidence in animals may directly relates to that in man, is highly dependent on livestock husbandry practices, the interaction between humans. Animals and environment, hygienic practices, food customs and population density for both animals and humans (Bale *et*

al., 1982). Other factors include demography, wildlife interactions, livestock movement and sharing of bulls, dipping, grazing and water sources (Figure 2.3).

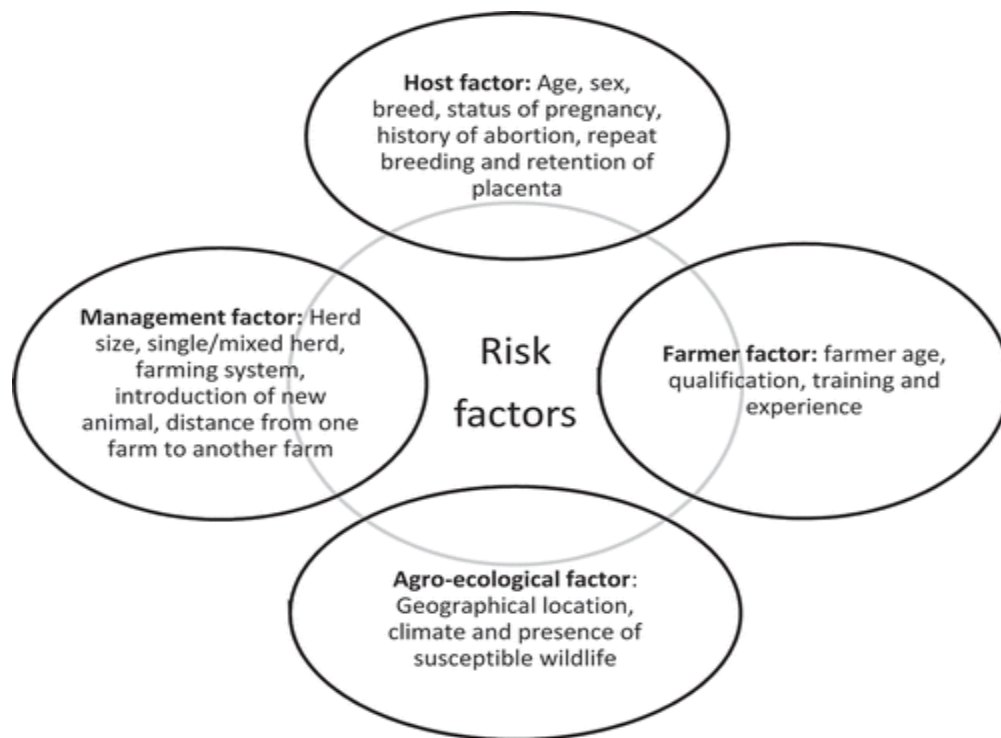


Figure 2.3. The risk factors for *Brucella* infection in animals classified into four groups (Ram *et al.*, 2019).

2.1.7 Farmer's perception on brucellosis

There are few studies done on the livestock farmer's knowledge, attitudes and practices on brucellosis in livestock especially in high potential areas. However, previous study done by Mwangi *et al.*, (2015), in Muranga County, Kenya showed farmer's knowledge was statistically significant to age and education level of the livestock farmers. According to this study the young and educated livestock farmers were more knowledgeable on brucellosis than those who were old and uneducated. This cannot however be generalized. Majority of these old and uneducated respondents had no idea on what causes brucellosis in cattle. This assumption too could probably not be true in all cases.

2.1.7 Impacts of brucellosis

Brucellosis is one of the most widespread bacterial zoonoses in the world, particularly in developing countries. The disease has been demonstrated to have immense important economic, veterinary and public health consequences (Pappas *et al.*, 2005, Franco *et al.*, 2007).

McDermont and Arimi (2002) reported that prevalence estimates of animal and human brucellosis are not readily available for many Countries of the world. There are no reliable data on the actual impact of this disease and its control in animal and human populations in sub-Saharan Africa including Kenya. However, a number of studies have attempted to seek experts' views on the potential bearings of animal diseases in the developing economies, including Kenya (Ogola *at el.*, 2014).

Many developing Countries especially with scarce resources are facing other serious priority diseases. They have not yet launched programs featuring any aspects geared towards brucellosis intervention and control (Henk *et al.*, 2004). The distribution of brucellosis in livestock and humans' population as well as its cost effective prevention strategies are not well understood. There are only limited information concerning brucellosis in sub-Saharan countries (McDermott and Arimi, 2002). Therefore, this disease may remain prevalent in domestic and wild animals and continue to cause economic loses and health problems in African countries WHO, (200). The disease may continue to be a barrier to trade on live livestock and its products, cause human illness therefore, causing impediment to smooth free movement of animals. This scenario could seriously impaired livelihood and health for livestock keepers, which are among the vulnerable group in rural society (Benkirane, 2006).

It is predicted that health and socio-economic impact of brucellosis in both animals and humans is greater than it is documented because of under reporting. This is associated to unavailable or ineffective data recording system (Franc *et al.*, 2018). A good example is Argentina where the economic cost was estimated at US\$60 million for 5% disease prevalence per year or equivalent to US \$1.20 per head of cattle. Similarly for Nigeria, the estimated economic losses were around US \$3.60 per head with 7 % to 1 2% seroprevalence. Economic impact of disease was substantial due to both direct and indirect effect on animals and humans. Recent research by Franc *et al.* (2018) tried to explained the impacts of both direct and indirect effects of the disease on animals (Figure 2.4) and humans (Figure 2.3). However, exact impression of this disease in the context of rural smallholder communities may be more complex due to integrated farming systems.

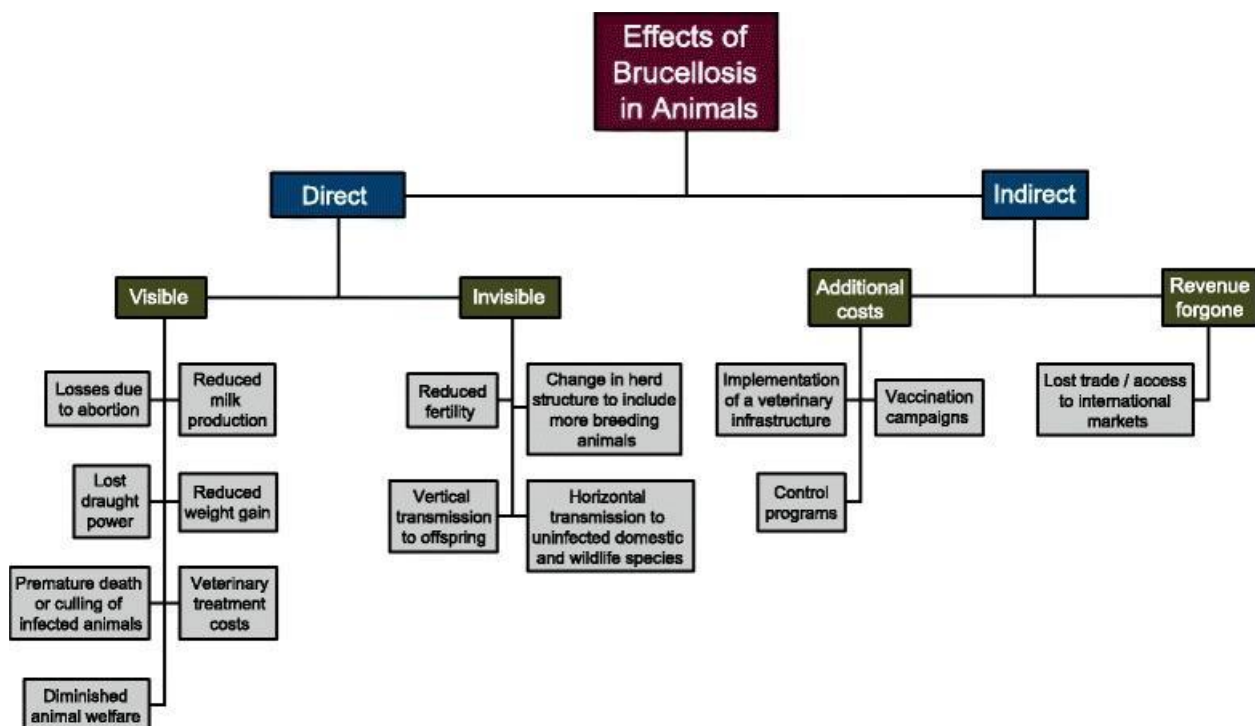


Figure 2.4. brucellosis impact on animals for an endemic region (Franc *et al.*,2018).

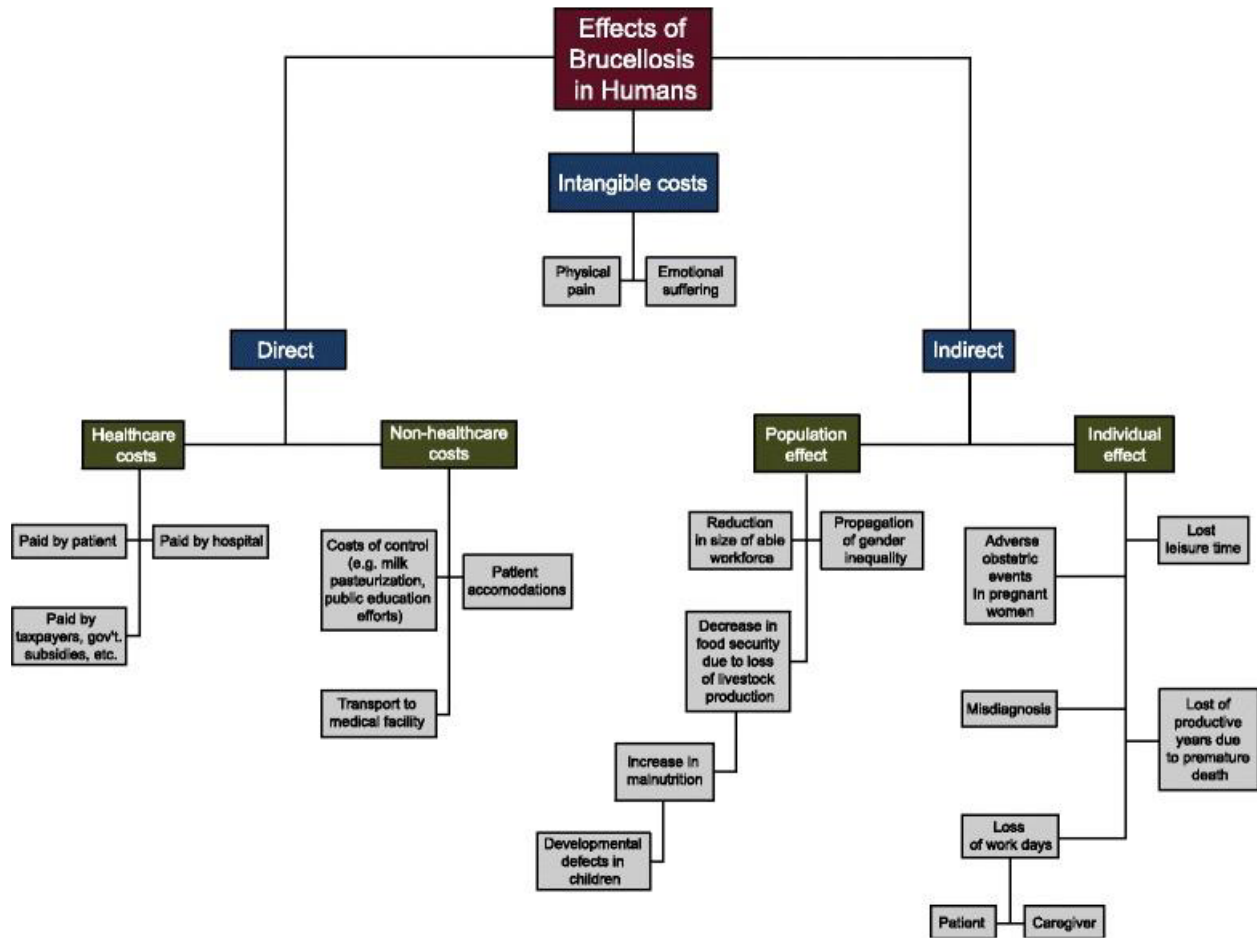


Figure 2.5. Brucellosis impact on human beings for an endemic region (Franc *et al.*,2018).

2.2 COXIELLOSIS

2.2.1 Definition and brief history of Coxiellosis

Coxiellosis (Q-fever) is also referred as Query fever. It is a worldwide zoonotic bacterial infection with important aspect of public health caused by *Coxiella burnetii*. The letter Q stands for the word query because the reason for the fever during 1935 outbreak in Australia among abattoir workers was not immediately known. However, in 1937 the causal agent was discovered by Edward Derrick. The disease can affect a wide variety of domestic and wild ruminants and these may act as reservoirs of *Coxiella burnetii* to other animals such as man, arthropods and even birds (Khalili et al, 2010).

2.2.2 Epidemiology

Coxiellosis in Kenya is neglected both by the veterinary and medical authorities, that's why the disease has remained silent (Nakeel *et al.*, 2016). To address the gap, a review study was carried out on the disease where literature was reviewed from previous investigation done between 1950 and 2015 in order to recognize the distribution pattern and any existing gaps of the coxiella infection. (Njeru *et al.*, 2016).

2.2.2.1 Etiology

Coxiella burnetii is the incriminating causal agent of Q fever (De Lange *et al.*, 2014). It is a gram negative bacteria of the genus *Coxiella*. *C. burnetii* is highly resistant to adverse physical conditions and chemical agents. It can survive for many months or even years in the environment. Its preferred target cells are macrophages located in body tissues and monocytes

circulating in blood stream (Maurin *et al.*, 1999). *C. burnetii* present in two distinct antigenic forms, phase I and phase II bacterial variants which can be discriminated by surface lipopolysaccharide (LPS) composition. The antigenic variation is important for both serological diagnosis and pathogenesis. Phase I variant are highly infectious form of the disease found in naturally infected hosts whereas phase II variants are less infectious and obtained after serial passages in cell culture systems or in embryonated eggs (Mege *et al.*, 1997)

2.2.2.2 Distribution and occurrence

Worldwide Coxiellosis has a vast distribution except New Zealand. It affects domestic and wild animals, arthropods and even birds. The disease is endemic in areas where domestic ruminants are kept (Plummer., 2015). Coxiellosis are one the World organization of Animal Health (WOAH) listed diseases on Terrestrial Animal Health Code. The member countries and territories are obligated to account all cases of the disease to the WOA.

2.2.2.3 Transmission

Circulation of *C burnetii* organisms occurs between wildlife and external parasites such as ticks and also between domesticated animals. Hard ticks have been document to act as carriers of this organisms. It is transmitted through vaginal discharges, feaces and urine. Contamination of environment occurs especially during parturition and during waste management (Larson *et al.*, 2019). The highest risk of coxiella transmission arises during parturition, aerosols, direct contact with birth fluids or placenta or ingestion(Sky *et al.*, 2014). High temperature pasteurization of milk effectively kills these microorganisms.

Most human infection results from consumption of contaminated under or unprocessed dairy products such as cheese or yoghurt. (Porter *et al.*, 2011).

2.2.3 Clinical manifestations

In domestic and wild mammals, Coxiellosis is frequently asymptomatic (Wardrop *et al.*, (2016). Goats and sheep show the following signs; anorexia, stillbirths, late abortions, early delivery or weak offspring, whereas in cows and camels may develop inflammation of the uterus and udder or infertility (OIE., 2018). New evidences have also linked *C. burnetii* with sub-clinical mastitis in lactating cows (Njeru *et al.*, 2016). Experimental infection have shown the disease to cause cats exhibit dullness, intermittent temperature and loss of appetite (Barandika *et al.*, 2007)

In humans, clinical presentation are non-specific and highly inconsistent, it varies from asymptomatic illness but accompanied by febrile illness, headache, fatigue, general ache, muscle and joint pains to a typical pneumonia or hepatitis. Aseptic meningitis, endocarditis and osteomyelitis are some of the complications of coxiellosis. Small percentage (1-2%) of acute clinical cases may progress to chronic infection (Honarmand *et al.*, 2012).

Q-fever is considered an occupational hazard of people who live together with their animals or animal products e.g vets, farmers, slaughterhouse and laboratory personnel (De Rooij *et al.*, 2012). In developing countries, the infection has been incriminated as the cause of non-specific malaria and febrile illness among what is referred in humans as community-acquired pneumonia (Knobel *et al.*, 2013). In Kenya diagnosis of Coxiellosis is rarely done. This scenario may be due to lack or scarcity of data and this has led to presumed low perception of the relevance of the disease compared to other endemic febrile illnesses. Therefore, this ‘silent’ disease may not often

be reported or missed out completely, hence its burden may be under estimated (Makungu *et al.*, 2014).

2.2.4 Diagnosis

The diagnosis of coxiellosis in domestic ruminants is a challenge, especially when distinguishing it with other causes of animal infertility and abortions. Traditionally, Coxiellosis has been diagnosed based on microscopic evaluation of clinical samples, in addition to positive serological outcomes (Burns *et al.*, 2018). Currently, there is no test for coxiellosis which can be referred as the gold standard, however other test such as PCR and ELISA which can detect and quantify the organisms may be the preferred methods of choice for disease clinical diagnosis (Vaidya *et al.*, 2010; Niemczuk *et al.*, 2014). Suggestions have been made for the harmonization of reporting system as well as monitoring for coxiella, to enable evaluation between different Countries (Vaidya *et al.*, 2010). The standardized analytic tests are needed for epidemiologic surveys for risky and suspected herds or flock in some regions. Thus, concerted efforts should be encouraged for development of these validation methods.

Recent infection can be detected by immuno-fluorescent test on paired serum taken two weeks apart. Other methods include culturing, immune-histochemical and Polymerase Chain Reaction tests may be utilized to classify *Coxiella burnetii* antibodies in animal tissues. According to OIE., 2018, these organisms are found occurring concurrently with other microorganisms in animal diagnostic labs particularly in cases of abortions suggesting coxiella do occur in mixed infections (OIE., 2018). Shedding of organisms has been documented to be at the peak during peri-parturient periods but drops to undetectable levels even in cases of persistent infections.

2.2.5 Treatment of Coxiellosis

Sample collection and laboratory testing is the only way to detect coxiella infection in a farm. There is no drug of choice for the infection. Attempts have been made with antibiotics which involves long term antibiotic therapy, but some evidence shown not to response to these treatments (Porter *et al*, 2011).

2.2.6: Zoonotic risk and bio safety requirements of Coxiellosis.

European Commission on disease control (2010) have raised alarms on risks associated with coxiellosis in European countries. They sought joint actions to address this challenge of disease outbreak by both human and veterinary health systems at both local and national levels. Impact of the infection on human health was limited and hence urgent need for enhanced awareness creation and surveillance system. In human epidemics, the key control strategy is to test and cull all pregnant animals and control of livestock movement. The disease is linked to incapacitating illness in expansive human population. Resistance in the environment due to its spore forming nature and aerosol spread, has led to Center for disease control (CDC) to classify *C. burnetii* as a group B biological agent due to its potential agent of bio-terrorism (Kersh *et al.*, 2010).

C.burnetii is regarded hazardous to human beings occupational zoonosis, hence bio-safety and bio-security is critical for its prevention and control (Khalili *et al.*, 2010, (De Lange *et al.*, 2014). Handling of live cultures/potentially infected/contaminated materials in the laboratory must be undertaken with great caution in bio-safety cabinet determined by bio risk analysis (Makungu *et al.*, 2014).

2.2.7 Prevention/control

One of the recommended way for prevention of coxiellosis is through vaccination of animals in areas where the infection is endemic. Other documented control measures include general hygienic practices such as removal of placenta and afterbirth fluids as well as proper cleaning and disinfection of animal bans (Njeru *et al.*, 2016). In the laboratory, strict biosafety and biosecurity controls are required to minimize infections. Handling of organisms is recommended to be done under bio- safety level 3 standard laboratory.

In order to control coxiellosi in both man and animals, domestic animals should be immunized using the only available inactivated *C.burnetii* vaccines (Hussein *et al.*, 2012, Muema *et al.*, 2018). The purpose of this strategy is to minimize the risk of abortion for the animals and reduce the shedding of these organisms. In order to produce appropriate immunological response with minimal safety hazards, vaccines produce should be efficient and targeted on those with phase I antigens (Zhang *et al.*, 2013)

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: Description of the Study area.

The study was conducted in Nandi County, which is located in western part of Kenya in the Northern Rift, occupying an area of 2,884.4 square kilometres. According to the Kenya National Bureau of Statistics (KNBS) of 2019 census, the County recorded a population of 885,711 persons made up of a number of Kenyan communities, the majority of whom belong to the native Nandi community. Nandi County borders Kakamega County to the West and South-west, Vihiga County to the South and South-west Kisumu County to the South, Uasin Gishu County to the North and North-east, Kericho County to the South-east (Figure 3.1).

Geographically, the County has a jug-shaped structure bound by the Equator to the South and extends Northwards. It lies within latitude 0° and $0^{\circ} 34''$ North and longitudes $34^{\circ} 45''$ and $35^{\circ} 25''$ East. Nandi County lies at an altitude ranging between 1,300 to 2,500 metres above sea level. It has a cool and moderately wet climate and receives an average rainfall of between 1,200mm to 2,000mm per annum. Most parts of the county experience mean temperatures of between $18-22^{\circ}\text{C}$ year round.

In terms of administration, Nandi County is made up of six Sub-Counties, namely, Chesumei, Mosop, Emgwen, Nandi hills, Tindiret and Aldai which comprises of thirty wards. Agriculturally, the County is situated in the highlands with dairy and crop production as the main activities. Main commercial crops grown in the county are maize and tea. In terms of Brucellosis and Coxiellosis, Nandi County was perceived as a high risk zone due to the presence of the large

livestock population and its sociocultural practices of living in close contact with the livestock (Jerono *et al.*, 2012).

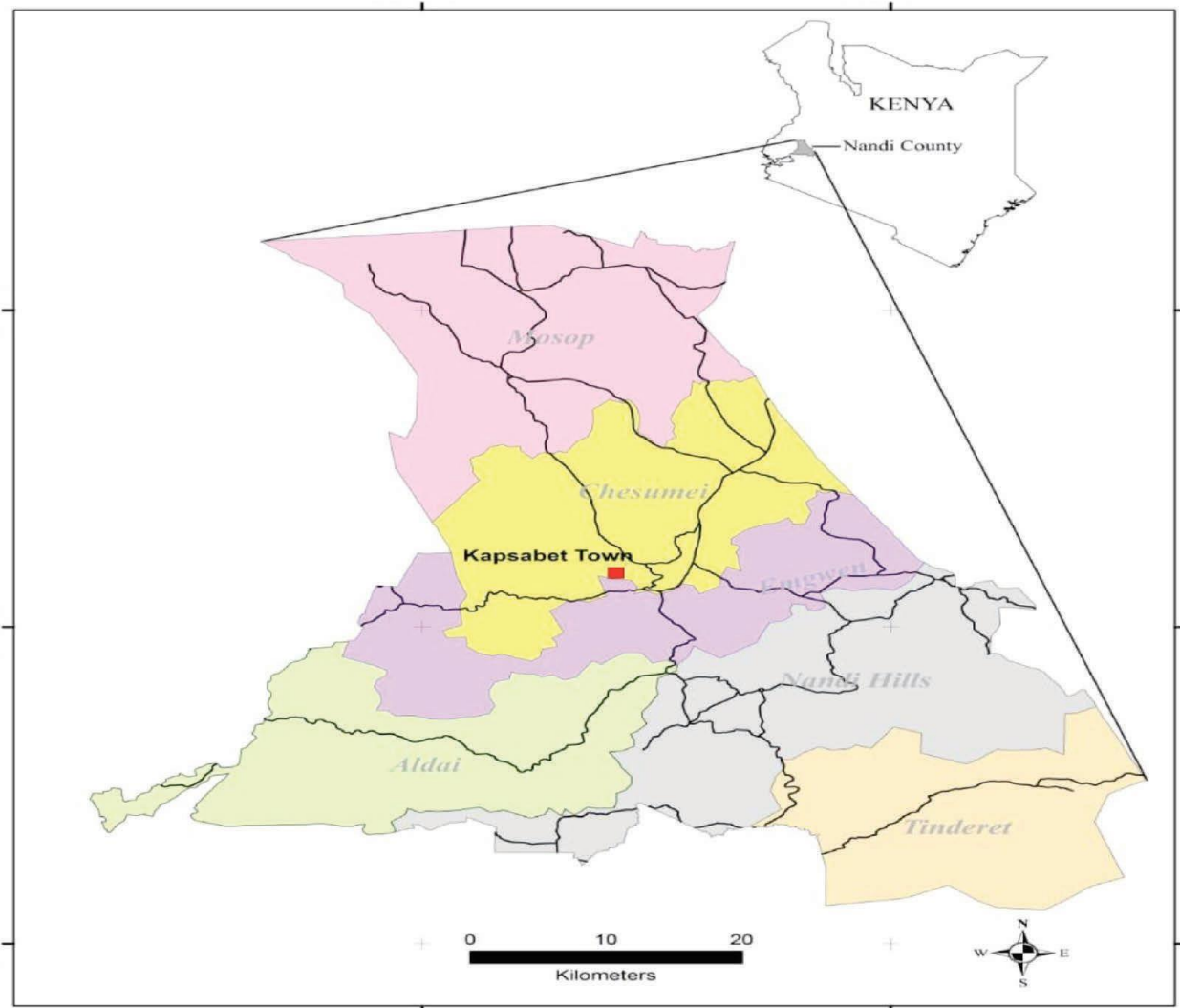


Figure 3.1: The Map of Kenya showing Nandi County location with the 6 sub-counties (2019).

3.2: Target population and study population.

The targeted population for the study was domestic ruminants in Nandi County, comprising of 309,038 cattle, 121,461 sheep and 46,669 goats (KNBS, 2019). The study population consists of cattle, goats and sheep above one- year of age because those below one year are at a lesser risk

(Ibrahim., 2021). The targeted group in cattle was breeding and mature bulls, first calf heifers and all mature cows (Alton., 2007).

3.3. Study Design

A cross-sectional study was used to identify the study households and the livestock where blood was collected for serology for both brucellosis and coxiellosis. For brucella, screening was done using Rose bengal plate test (RBPT) at Veterinary investigation laboratory (VIL) Eldoret. Confirmation of brucellosis was done by use of indirect Enzyme-linked immunosorbent assay (iELISA) at International Livestock Research Institute (ILRI) Nairobi. Coxiella was analyzed using the iELISA at the same facility.

Similarly, a cross sectional survey was undertaken to identify risk factors that were associated with incidence of brucellosis and Q fever infection using semi-structured and pre-tested questionnaire on the households. This was also used to capture farmer's knowledge, attitudes and practices of these two diseases on the residents of the county.

3.4: Sampling Technique

A multistage sampling technique was adopted, whereby primary units were the six Sub-Counties, namely:, Mosop, Emgwen, Chesumei, Nandi hills, Tindiret and Aldai. Secondary units were wards; one ward per Sub-county was selected using simple random sampling method. Sampled wards were Kilibwoni ward in Emgwen, Kabisaga ward in Mosop, Lelmokwo/ Ngechek in Chesumei, Lessos ward in Nandi Hills, Tindiret ward in Tindiret and Kaboi/Kaptumo ward in Aldai. Tertiary units were herds in the sampled ward. Household were visited and a verbal/written consent from the head of the household were obtained before any sampling was done. Herds were then sampled using systematic random sampling method until

the required sample size was achieved. Herds with 1-10, 11-20 and over 20 were categorized as “small, “medium” and “large” respectively.

3.5: Sample size determination

Determination of sample size was done according to the formula in Dahoo *et al.*,2010).

$$N = Z_{\alpha}^2 pq / L^2$$

Where,

n= required sample size.

Z= Standard normal deviate that provides 95% confidence intervals (1.96)

p= prevalence estimate (Kosgei *et al* 2016) where 6.8% for cattle, 6.6% for goats and 4.9% for sheep.

$$q = (1-p)$$

α =apriori estimate of the population variance

L^2 = Allowable error (0.02 for cattle and 0.05 for sheep and goats).

Therefore, the calculated sample size for blood collected for prevalence of brucellosis and coxiellosis was:

$$\text{Cattle (n)} = (1.96)^2 \times 0.068 \times 0.932 / 0.02^2 = 609.$$

Utilizing the same formula for sheep and goats, the target sample sizes were 94 and 263 respectively. Households/farmers were interviewed for demographic information, identification

of the risk factors and assessment for knowledge, attitude and practices ((KAPs) for both brucella and Coxiella infection.

3.6 Data collection

Questionnaire was developed (Appendix 1) for demographic and animal characteristics, to identify the risk factors associated with the occurrence of Brucellosis and Coxiellosis and assess participants' knowledge, attitude and practices (KAPs). The questionnaire was pre-tested in Ngechek/Lelmokwo ward using 10% of the calculated sample size (n=38) to assess for clarity of the questions and the time required to complete it. The survey tool was then updated based on the feedback received from the respondents.

Data were collected by administration of questionnaires via personal interview to the head of the household or a representative. Data collected included household and animal characteristics, risk factors such as herd size, animal production system, resources shared, history of brucellosis/coxiellosis, handling and disposal of livestock and their products, contact with wildlife or introduction of new animal into the farm among others. The risks factors were measured using logistic regression to analyze for association between exposed risk factors and the disease.

3.6.1 Assessment of knowledge, attitudes and practices on Brucellosis and Coxiellosis

The respondents were asked questions regarding knowledge on brucella and coxiella infections and scores rated based on a dichotomous scale where 1 was for a correct response and 0 for an incorrect or unknown response. The sum of scores obtained by the respondent was converted into percentage scores by dividing the sum scores obtained by the respondent with the possible

maximum scores then multiplied by 100. The sum of scores was then assessed based on Bloom's cut-off point. A person was considered knowledgeable about brucellosis or coxiellosis if he or she obtained 60% or more on the sum of scores. Regarding attitude, Likert scale was used. Items related to attitude were measured based on a Likert scale range from 1 to 5 (1= strongly disagree, 2= disagree, 3= Don't know; 4= agree, 5 = strongly agree) with higher scores indicating the most desired attitude. A sum of scores was then obtained and transformed into percentages. A person was considered to have a positive attitude if he or she scored 60% and above on the sum of scores in this section. Participants were also asked questions concerning routine precautionary measures taken against brucellosis and coxiellosis in the farms. All items in the practice subscale had dichotomous response with a score of 1 given for a correct practice and 0 for a wrong or unknown practice. Sum of scores were then obtained and transformed into percentages. A participant was classified to have good practice if he or she obtained a score of 50% and above on the sum of scores.

3.7: Blood collection

Approximately 10ml of blood sample was collected aseptically from the jugular vein from an individual animal with disposable needle and a vacutainer tube. The tubes were well labeled, placed in a rack and put in a cool box with enough ice. They were then transported to Regional Veterinary Investigation laboratory (RVIL) Eldoret within 12 hours of collection for analysis, where they were kept in the refrigerator (2-8°C) overnight.

3.8: Laboratory analysis

Blood samples were kept in the refrigerator (2-8°C) overnight and the following day sera were separated by centrifuging. Serum was then divided into two aliquots and each was labeled in

order to identify the animal. One aliquot was used to screen brucellosis at the facility using the RBPT sourced at Veterinary laboratories; New Haw Addlestone survey KT15 3NB Ref. 0060 Lot 292, United Kingdom. The testing protocol was done according to manufacturer's instructions and protocol (Appendix 3).

The other aliquot was preserved at -20°C at the facility for further testing for confirmation of brucellosis and coxiellosis at International Livestock Research Institute (ILRI), Nairobi using Indirect enzyme-linked immunoabsorbent assay (iELISA). This aliquot was further divided into two cryovials for the analysis of brucella and coxiella.

The Elisa kit for brucella was obtained from IDVet innovative Diagnostics, Louis Pasteur-Grabels, France. The kit is an indirect ELISA for multispecies for the detection of antibodies against *Br. abortus*, *Br. melitensis* and *Br. suis* in serum and plasma samples (Appendix 4). The ELISA kit for Q-fever was sourced from IDEXX laboratories, Inc, USA. The Q-Fever antibodies test is an enzymes immunoassay for the detection of antibodies against *Coxiella burnetii* in serum, plasma and milk samples of ruminant animals. The testing protocol for both iEISA for brucella and coxiella were done according to manufacturer's instructions(Appendix 5).

3.8.1: Procedure for Rose Bengal Plate Test (RBPT)

The antigen, control serum and test serum samples were removed from the refrigerator one hour before the test was done to attain room temperature. Every test plate, positive and negative control tests were set (Alton et al., 1975). The positive and negative control was derived from stabilized diluted rabbit serum containing antibodies to brucella antigen while the negative controls were diluted rabbit serum samples non-reactive to brucella antigen (sourced with RBPT kit).

Test serum(30µl) was placed on a white marked tile. The antigen bottle was shaken well but gently and 30µl of the antigen placed near the serum spot using pipette with sterile tips.

Immediately serum sample were mixed thoroughly with the antigen using clean applicator stick, to produce a circular zone of approximately two centimeters(2cm) in diameter. Gently the mixtures were then agitated for four minutes at ambient temperature on a rocker.

Interpretation of the reading of the test: Agglutination on the test zone was taken as positive sample with respect to the positive and negative controls on that test plates. Where there was no agglutination, it was taken as negative sample.

3.8.2 Procedure for Indirect ELISA for brucella.

All reagents were removed from the refrigerator and allowed to come to room temperature (21°C ± 5°C) before use. All the reagents were homogenized by inversion/Vortex. First 190µl of Dilution Buffer 2 were added to all wells. Then 10µl of the negative control was added to the wells A1 and B1 and then 10µl of the positive control were added into wells C1 and D1. Finally, 10µl of each sample or pools of 10 sera was added to the remaining wells. It was then incubated overnight at 21°C(±5°C). The wells were then emptied and washed 3 times with approximately 300µl of the wash solution. Drying of the wells was avoided between washings. The conjugate was prepared by diluting the concentrated conjugate 20X to 1/20 (overnight incubation) in Dilution Buffer 3. A 100µl of the Conjugate 1X was added to each well and incubated for 30 minutes ±3 minutes at 21°C (± 5°C). Wells were emptied and then washed 3 times with approximately 300 µl of the Wash Solution. A total of 100 µl of the Substrate Solution was added to each well and incubated for 15 minutes ±2 minutes at 21°C (± 5°C) in the dark. Thereafter,

100µl of the Stop Solution was added to each well in order to stop the reaction and read using the ELISA reader and record the O.D. at 450 nm.

Samples with results of a S/P % less than or equal to 110% were considered negative while, greater than 110% and less than 120 were considered doubtful and greater than or equal to 120% were considered positive.

3.8.3 Procedure for indirect ELISA for Coxiellosis

Antibodies to *C. burnetii* were detected by the commercial indirect Enzyme-Linked Immunosorbent Assay (ELISA) test. A 96-well polystyrene micro titer plates were pre-coated with *C. burnetii* antigen phase I and II strains (ID Screen® Q Fever Indirect Multi-Species, IDEXX laboratories, Inc, USA). Serially diluted sera in phosphate buffered saline containing 0.1% Tween 20 were added and then incubated. After incubation, the plates were washed to remove any unbound materials. Antibodies were detected with alkaline phosphatase conjugated Rabbit anti-human IgG, IGM and IgA at optimal dilution. Both anti phase I and II antibodies were detected. Positive and negative control sera were included in each plate.

Color developed in the presence of bound enzyme, and the optical density was read with an ELISA plate reader. As recommended by the manufacturer an animal was considered to be ELISA positive when the optical density (OD) was over 80% (strong positive). An OD between 50% and 80% was considered positive. A doubtful ELISA result was noted if the OD was between 40%- 50%, while an $OD \leq 40\%$ was considered a negative animal. The sensitivity and specificity of the ELISA test kit as provided by the manufacturer was 99% and 98% respectively.

3.9: Data handling and Data analysis

All data obtained from both the serological tests and questionnaires survey were entered, cleaned and stored in Microsoft Excel spreadsheet programme (Microsoft Corp). Data was then imported to Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA, 2002).

Tables of descriptive statistics were then generated including frequency, mean, median, mode and range. Tests of association between the independent and the dependent variables were done using univariate logistic models and multivariate models. Significance for the univariate analysis was set at $p \leq 0.05$. The variables that were significant in the univariate analysis were used to build a multivariate model using the backward elimination procedure.

3.10 Inclusion criteria

- Livestock farmer in Nandi County
- Over 18 years.
- Mature cattle, sheep and goats.

3.11 Exclusion criteria

- Cattle, sheep and goats below one year.
- Farmers below 18 years
- Non-resident of the county
- Farmers who decline to participate.

3.12. Limitations of the study

- Financial constraints.
- The desired number of goats was not attained because the animals were not available or was too few in the wards.

3.13 Ethical considerations

Ethical approval was sought from University of Nairobi Faculty of Veterinary Medicine Biosafety, Animal Care and Use committee before the research commenced. Also permission was requested and granted by the County Government of Nandi County to undertake the research.

Consent was also sought from participants before starting the research where adequate explanation was given to the farmers whose animals were sampled and those interviewed on the purpose of the research. They were required to either freely give their informed written/verbal consent or decline. The blood samples collected were coded and only referred to as such without reference to any individual. However, positive samples for brucellosis were communicated to the owners and the local veterinarian so that they can be advised accordingly. Identities of the participants remained confidential.

CHAPTER FOUR

4.0 RESULTS

4.1 Seroprevalence estimates of brucellosis and Coxiellosis in cattle, sheep and goats in Nandi County.

Blood samples from cattle, goats and sheep were collected from six wards, each from the six Sub-Counties in Nandi County as shown in Figure 4.1. A total of 1140 blood samples were collected from 366 households across the selected sites as shown in Figure 4.1.

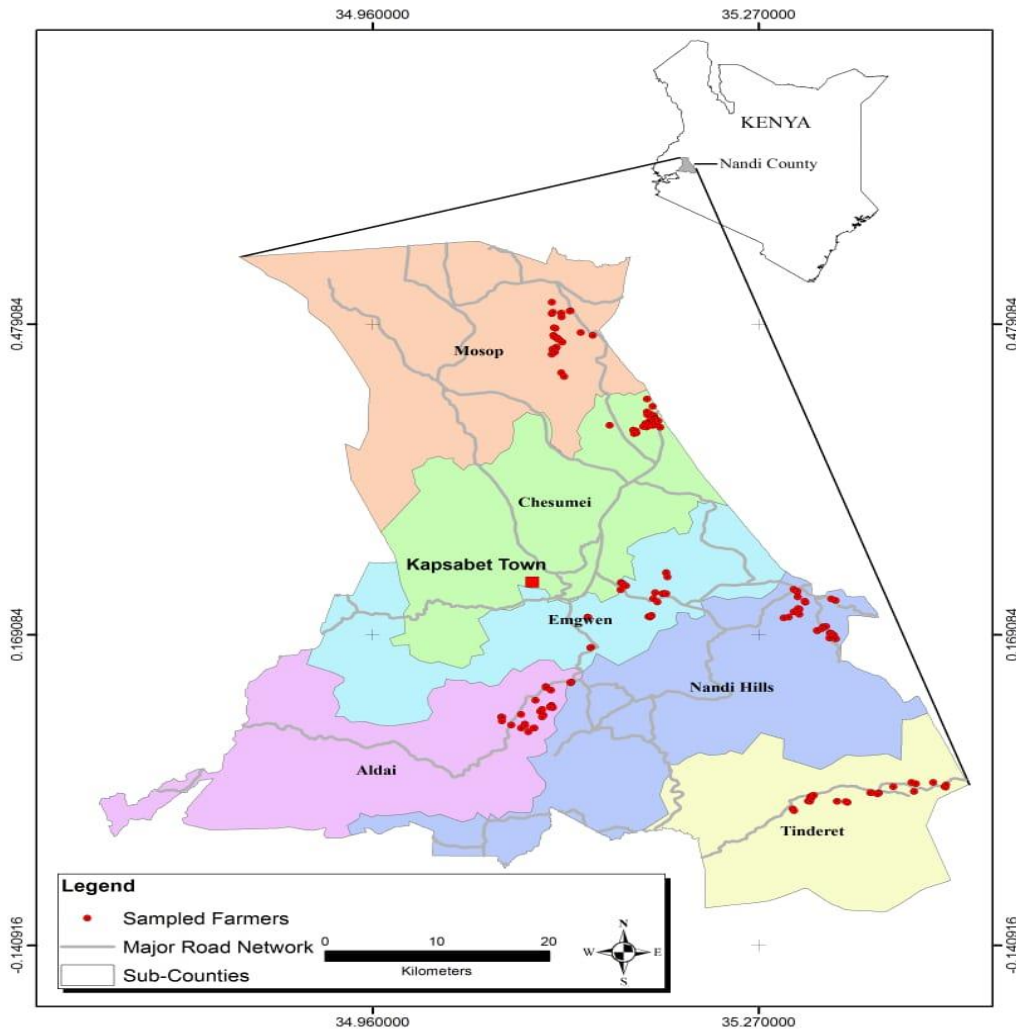


Figure 4.1: Map of Nandi showing the sampling sites (2019)

4.1.1: Sero-prevalence of brucellosis in cattle, sheep and goats in Nandi County

A total of 1140 (n= 1140) serum samples were sampled from 725 cattle (64%), 283 sheep (25%) and 132 goats (11%) from 366 households in six wards in Nandi County (Fig 4.2).

Seroprevalence was determined by dividing the number of positive cases by the total number of sampled population. Analysis of brucellosis was obtained from two tests, the first was on RBPT as a screening test and the second was on iElisa as a confirmatory test. Pie chart and tables of descriptive statistics were generated to illustrate these results

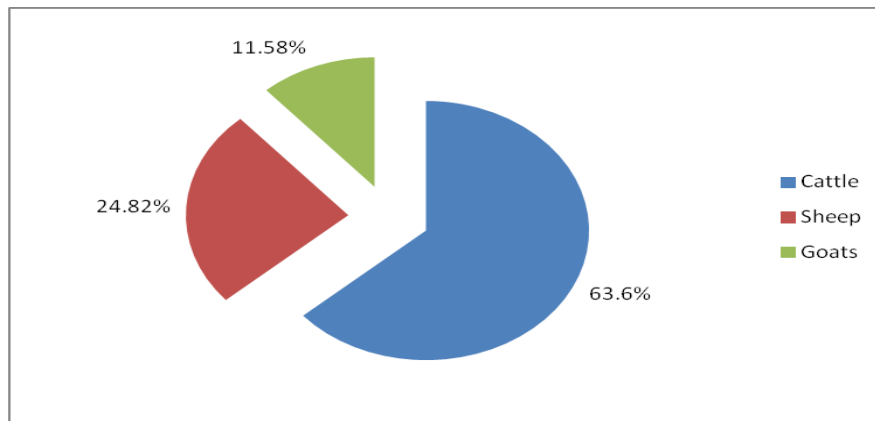


Figure 4.2 Pie-chart showing the blood samples collected from the three animal species

4.1.1.1 Seroprevalence of brucellosis on RBPT.

Overall animal seropositivity in Nandi County was 1.228% (14/1140) on RBPT. However, the prevalence with respect to species showed that it is more prevalent with sheep as out of 14 positive cases, 9/283 cases was reported on ovine; this was a percentage prevalence of 3.180%. Cattle and goats seropositivity were 0.414% and 1.515% respectively. The results further demonstrated brucellosis to be more prevalent in Chesumei Sub-County with positivity rate on RBPT at 3.627% (7/193) followed by Mosop Sub-County at 2.326% (4/172) and one case each

from Aldai, Nandi Hills and Tindiret Sub-Counties. There was no positive case from Emgwen Sub-County (Table.4.1).

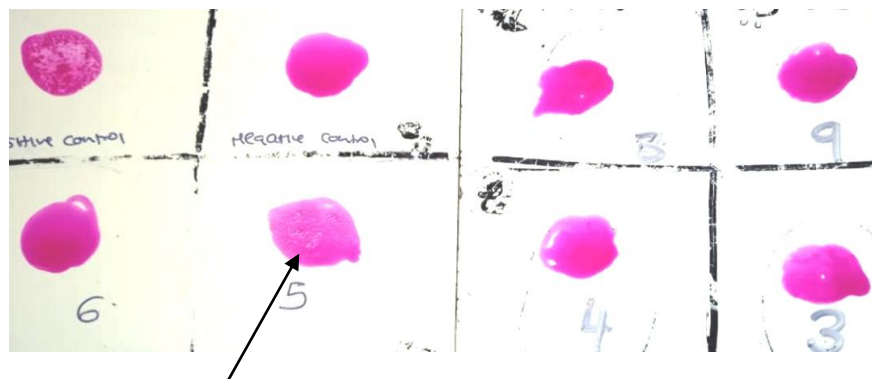


Figure 4.3 A mammogram showing a positive sample on RBPT for brucellosis.

Table 4.1 Species distribution of sera testing positive to brucella antibodies on RBPT as per Sub-County.

Sub-County	Livestock species								
	Cattle			Sheep			Goats		
	Number Tested	Number Positive	Sero prevalence (%)	Number Tested	Number Positive	Sero prevalence (%)	Number Tested	Number Positive	Sero prevalence (%)
Aldai	128	0	0	61	1	1.64	0	0	0
Chesumei	143	2	1.40	44	3	6.82	6	2	33.33
Emgwen	104	0	0	66	0	0	2	0	0
Mosop	128	0	0	44	4	9.091	0	0	0
Nandi Hills	130	0	0	64	1	1.56	3	0	0
Tindiret	92	1	1.09	4	0	0	121	0	0
Total	725	3	0.414	283	9	3.180	132	2	1.515

4.1.1.2 Seroprevalence of brucellosis on indirect ELISA

The seroprevalence of brucella on iElisa analysis showed that, out of 1140 blood samples collected and analyzed only one sample was confirmed positive (0.0877%). The positive case was from a Jersey cow from Chesumei Sub-County which also had tested positive on RBPT translating to 0.70% (1/143) prevalence rate. Therefore, overall prevalence rate of brucellosis in Nandi County was 0.138 % (1/725) in cattle and zero percent in sheep and goats since there was

no positive case on iELISA, despite recording 9 positive cases in sheep and 2 cases respectively on RBPT analysis (Table 4.2).

Sub-County	Livestock species								
	Cattle			Sheep			Goats		
	Number tested	Number Positive	Sero prevalence (%)	Number Tested	Number Positive	Sero prevalence (%)	Number Tested	Number Positive	Sero prevalence (%)
Aldai	128	0	0	61	0	0	0	0	0
Chesumei	143	1	0.70	44	0	0	6	0	0
Emgwen	104	0	0	66	0	0	2	0	0
Mosop	128	0	0	44	0	0	0	0	0
Nandi hills	130	0	0	64	0	0	3	0	0
Tindiret	92	0	0	4	0	0	121	0	0
Total	725	1	0.138	283	0	0	132	0	0

Test	Proportion on positive test (%)	Proportion on Negative test (%)	Total bovine tested N (%)
RBPT	0.414 (3)	99.586 (722)	725 (100)
iELISA	0.138 (1)	99.862 (724)	725 (100)

Figure 4.4 Pictures showing the Principal investigator and the assistant collecting blood samples in various sites in Nandi County.



Figure 4.5 The main supervisor Dr. Gathura P. in Kilibwoni ward, Nandi County supervising data collection, (2019).

4. 2 Seroprevalence of Coxiellosis on indirect ELISA in cattle, goats and sheep.

The results indicated that out of the total 1140 blood samples collected and analyzed in Nandi County using the indirect ELISA at ILRI, Nairobi, sixty four (64) sera samples tested positive for Coxiellosis. Overall, the animal sero-positivity in the County was 5.614%. However, seroprevalence in cattle was 8.138% (59/725), sheep 1.413% (4/283) and goats 0.758% (1/132). These results showed that Coxiellosis is more prevalent among cattle than sheep and goats in Nandi County. Emgwen Sub-County recorded the highest prevalence figure in cattle at 11.54% (12/104), followed closely by Tindiret at 10.87% (10/92), Aldai at 9.38% (12/128) and Chesumei at 8.39% (12/143). Nandi hills and Mosop Sub-Counties recorded the lowest seropositivity rate of 4.62% (6/130) and 5.43% (7/128) respectively (Table 4.4).

Sub-County	Livestock species								
	Cattle			Sheep			Goats		
	No. Tested	No. Positive	Prevalence (%)	No. Tested	No. Positive	Prevalence (%)	No. Tested	No. Positive	Prevalence (%)
Emgwen	104	12	11.54	66	0	0	2	0	0
Tindiret	92	10	10.87	4	0	0	121	1	0.83
Aldai	128	12	9.38	61	2	3.28	0	0	0
Chesumei	143	12	8.39	44	1	2.27	6	0	0
Mosop	128	7	5.47	44	0	0	0	0	0
Nandi Hills	130	6	4.62	64	1	1.56	3	0	0
Total	725	59	8.138	283	4	1.413	132	1	0.758

4.3: Risk factors associated with occurrence of brucellosis in cattle, sheep and goats.

Results showed brucella prevalence on RBPT with respect to species was 3.18% in sheep, since 9 out 14 positives were from ovine samples. These results demonstrated brucellosis was more prevalent in sheep compared to 0.414% and 1.515% for bovine and caprine respectively.

Multivariate analysis (Table 4.5) showed a statistical significance to support the hypothesis that all species are susceptible to brucellosis diagnosed on RBPT-; bovine (P-value 0.002), caprine

(P-value 0.002) and ovine (P-value 0.031). The result also indicated that, medium farm size recorded eight (8/370) positive cases of brucellosis representing a percentage prevalence of 2.162%. Large and small farm size recorded 3/511 and 3/259 positive cases each, representing a percentage prevalence of 0.587% and 1.158% respectively. Some cases were reported with medium size farms although not statistically significant (p value 0.107).

From the findings, females had more positives cases than male animals. However, ratio of female to male was 1027:110 since 3 animals were not specified in the data. Out of the 14 positive cases, bovine female cases were 0.446% (3/672), caprine female cases were 0.840% (1/119) and female ovine cases were 3.390% (8/236). In males, caprine male cases were 9.091% (1/11) and caprine male positive cases were 2.340% (1/47) with no case for male bovine. However, these results on sex showed that brucellosis was reported more in male caprine than female ovine and female bovine. It is statistically true to say that, there was association between female ovine and brucella disease on RPBT (p value 0.015). On breed seropositivity, dorper breed of the ovine species had a significant (p value 0.001) percentage prevalence of 64.29% (below the standard p-value of < 0.05). Brucella disease was more prevalent in dorper breed and had a positive association at a P-value of 0.001. Amongst the bovine species, Jersey breed showed a significant (P value 0.001) positive association with brucella on RBPT at P value =0.001 with a prevalence percentage of 8.69%.

Age wise, 8 yearlings tested positive representing a percentage prevalence of 21.62%, with ovine yearlings reporting more compared to other species although without statistical significance of association. Among the bovine, the data showed that seroprevalence of brucellosis in heifers on RBPT was higher compared to adults and yearlings, with a significant test of association (p-value 0.021 and p-value 0.00 respectively). Animals from semi-intensive production system

(6/582) tested positive, representing a percentage prevalence of 1.031%. However, the test of association showed no statistical significance ($p = > 0.05$). A total of 3 out of 357 and 5 out of 154 cases tested positive for extensive and tethering respectively with test of association showing no statistical significance ($p = > 0.005$). No positive case was recorded from zero grazing units. Further test was subjected to these samples to confirm the seropositivity using the iELISA.

Table 4.5. Multivariate analysis for prevalence of brucellosis using iELISA							
Dependent variable	Independent variable	Pairwise group	N	Proportion positive (%)	95%CI	Lower bound	Upper bound
Brucellosis	Species						
	Bovine	Caprine	725	1 (0.138)	.000	.000	.040
		Ovine			.001	.001	.018
	Caprine	Bovine	132	0 (0.00)	.000	.000	-.105
		Ovine			.097	.097	-.066
	Ovine	Bovine	283	0 (0.000)	.001	.001	-.066
		Caprine			.097	.097	-.006
	Breed						
	Arshyire	Friesian	243	0	0.733	.016	.022
		Guernsey			0.842	.134	.109
		Jersey			0.037	.108	.003
		Zebu			0.808	.112	.087
	Fresian	Ayrshire	448	0	0.735	.022	.016
		Guernsey			0.800	.136	.105
		Jersey			0.024	.111	.008
		Zebu			0.752	.114	.083
	Guernsey	Ayrshire	4	0	0.842	.109	.134
		Friesian			0.800	.105	.136
		Jersey			0.513	.174	.087
		Zebu			1.000	.155	.155
	Jersey	Ayrshire	23	1(4.35)	0.037	.003	.108
		Friesian			0.024	.008	.111
		Guernsey			0.513	.087	.174
		Zebu			0.439	.067	.154
	Zebu	Ayrshire	6	0	0.808	.087	.112
		Friesian			0.756	.083	.114
		Guernsey			1.000	.155	.155
		Jersey			0.439	.154	.067
	Age						
	Adult	Heifer	588	1	.042	.045	.001
		Yearling			0	.000	.755
	Heifer	Adult	135	0	.042	.001	.045
Yearling				0	.000	.777	1.238
Yearling	Adult	1	0	.000	1.214	.755	
	Heifer			0	.000	-1.238	.777

Results from the iELISA analysis confirmed only one sample as positive from the total sample size of 1140 collected and analyzed, reflecting an overall animal seroprevalence rate of 0.088%. The positive case recorded was from a female Jersey cow (1/725) representing a seroprevalence of 0.138% (P = 0.001). Therefore, from the finding significant potential predictors for seropositivity of brucellosis were animal species (p-value 0.00, CI95%), age (p-value 0.042, CI95%) and breed (p-value 0.037, CI95%). The data further, showed no significance association among the other variables e.g breed (Ayrshire p-value 0.3, Friesian p-value 0.22, Zebu p-value =0.067 and Guernsey p-value =0.08).

4.4. Risk factors associated with Coxiellosis infection in cattle, sheep and goats in Nandi County.

The results showed prevalence of coxiellosis in cattle was 8.138 % (59/725), sheep was 1.413% (4/283) and goats were 0.758% (1/132). Risk factors for coxiellosis in animals considered in this study were animal species, breed, sex, age and production systems. From this finding, coxiella was more prevalent in cattle (OR 7.260) than in goats and sheep. Therefore, animal species (p-value 0.015, CI 95% OR 7.260) was the only potential predictor for the three considered species for the presence of *C. burnetii* antibodies. Breed, sex, age and production system had no statistically significant association for coxiella infection since p-value was > 0.05.

4.4.1. Sero-prevalence of coxiellosis based on Sex, Breed and Age categories.

Findings demonstrated coxiella seroprevalence was highest in male caprine at 9.09 % (1/11), followed by female bovines at 8.48 % (57/672) and male bovines at 3.85 % (2/52). Seroprevalence for female sheep was 1.70 % (4/236), but 0 % for both male sheep and female goats. In terms of breed, Ayrshire and Jersey recorded the highest figures of 15.23 % (37/243)

and 8.70 % (2/23) respectively. According to the age category, adult bovines recorded higher seroprevalence of 8.33 % (49/588) when compared to heifers 6.67 % (9/135) (Table 4.6).

Species	Sex			Breed					Age				Sero positive animals
	Female	Male	N / S	Arshyire	Fresian	Guernsey	Jersey	Zebu	Adults	Heifers	yearlings	N/S	
Bovine	57/672 (8.48 %)	2/52 (3.85 %)	1	37/243 (15.23 %)	20/448 (4.46 %)	0/4 (0%)	2/23 (8.7 %)	0/6 (0%)	49/588 (8.33%)	9/135 (6.67%)		2	59/725 (8.14%)
				Galla	Saanen	Toggenburg							
Caprine	0/119 (0%)	1/11 (9.09 %)	2	0/94 (0%)	0/3 (0%)	1/26 (3.85%)			1/123 (0.81%)		0/9 (0%)		1/132 (0.76%)
				Dorper	Merino								
Ovine	4/236 (1.7%)	0/47 (0%)	0	4/290 (1.38 %)	0/2 (0%)				4/246 (1.63%)		0/37 (0%)		4/283 (1.41%)
	Total samples	1140			1140					1140			

4.4.2: Sero-prevalence of coxiella based on Production system category.

In Nandi County, cattle, sheep and goats were raised under four production systems namely; extensive, semi-intensive, zero grazing, and tethering. Results pointed out the seroprevalence of coxiellosis was higher in zero grazing units at 12.12 % (4/33) compared to semi-intensive production system 8.24 % (48/582), extensive production system 2.8 % (10/357) and tethering at (1.29 %) 2/154. However, test of association was negative since the p value >0.05.

4.4.3. Association of Coxiellosis seroprevalence with Species, Breed, Sex, Age and Production Systems.

Multivariate logistic regression was adopted to measure the effects of the independent variables (species, sex, age, breed, and production system) against the dependent variable (coxiella).

Following the analysis, the only variable that was statistically associated with sero-positivity of coxiellosis was animal species. From the result, it can be concluded that cattle were more prone

to coxiella infection 8.138 % (59/725) compared to sheep 1.413% (4/283) and goats 0.758 % (1/132) (OR 7.260, p-value 0.015 CI 95%) (Table 4.7).

Dependent Variable	Independent Variable	N	Proportion positive (%)	Odds ratio (OR)	Seropositivity (%)	CI (95%)	P-value
Q fever	Bovine	725	59 (8.138)	7.260	8.14%	2.8-18.23%	0.015
	Caprine	132	1 (0.758)	0.110	0.76%	0.14-7.27%	0.317
	Ovine	283	4 (1.413)	0.028	1.41%	1.0-7.78%	0.076

The other variable analyzed was sex of the animal, where results demonstrated Q-fever was more common in female than male animals. From this observation, 61 out of 64 positive cases were females and 3 were male animals. Indeed, in cattle, females recorded a higher figure 8.482% (57/672) and were two times (OR 2.320) more likely to be positive than sheep and goats. These findings however, had no statistical significance to show a positive association between coxiella infection and sex variable (p-value 0.065). Also, the test of association amongst the breeds and age categories of the animal species under investigation did not yield any statistical significance with coxiella infection (p-value > 0.05), likewise to the production system (p value >0.05).

4.5 Socio-demographic characteristics of the study households for the assessment of KAPs on Brucellosis and coxiellosis.

Demographic data were obtained from a total of 366 households (n=366) where, the head of the household/representative of household was interviewed using a semi-structured questionnaire.

The data collected included participant's demographic information and animal characteristics, as

well as knowledge, attitudes and practices (KAPs) among the livestock farmers. Descriptive statistical analysis was used to identify respondent's demographic and animal characteristics for frequencies and percentages.

From the findings, 64/366 (17.49%) respondents were from Chesumei Sub-County, 62/366 (16.94%) from Aldai, 62/366 (16.94%) came from Nandi Hills, 62/366 (16.94%) from Mosop, 60/366 (16.39%) from Emgwen and 56/366 (15.30%) from Tindirret Sub-Counties (Figure 4.6).

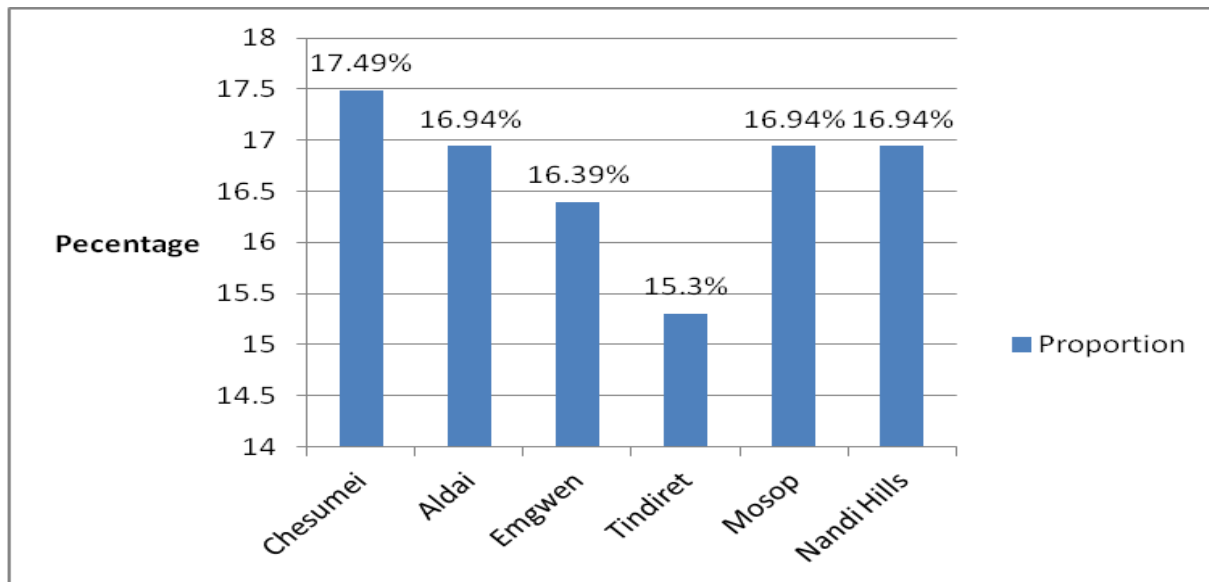


Figure 4.6 Household respondents in the Sub-Counties of Nandi County

On gender, majority of the respondents were male (271/366) who formed 74.04%, while the female respondents were 25.96% (95/366) as illustrated in figure 4.7.

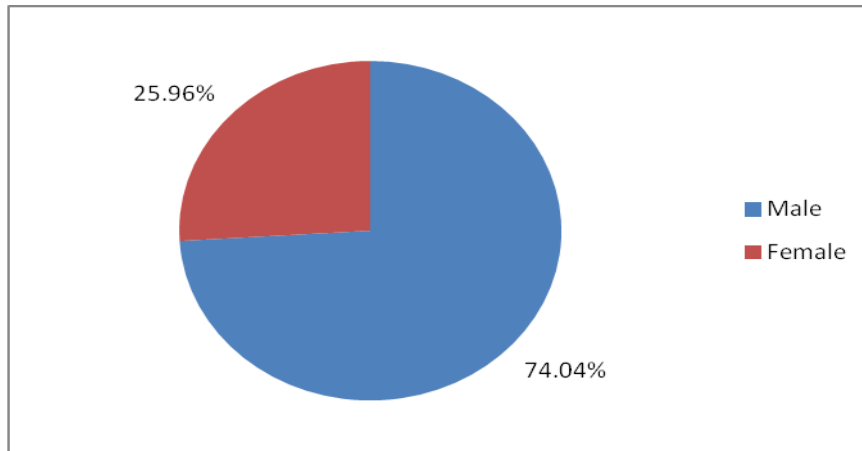


Figure 4.7 Pie chart showing gender response frequencies

Another variable in the study was age where the findings showed most of the respondents across the Sub-Counties were those between 18-35 years of age and they formed 37.16% (136/366), followed closely by those of age above 50 years at 35.52% (130/366), while the least of the respondents are of age 36-50 years at 27.32% (100/366) as shown in Figure 4.8.

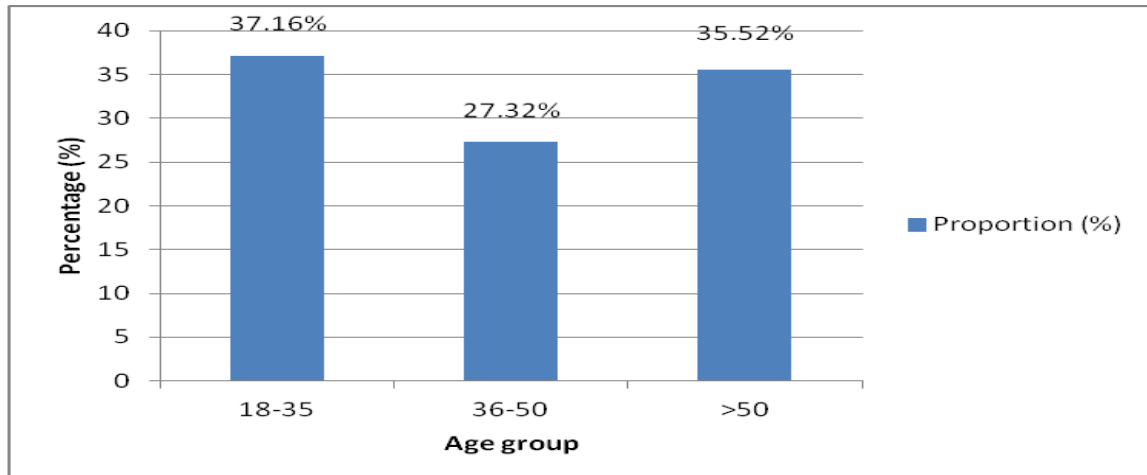


Figure 4.8 Participants response as per the age group.

The measure of central tendency of age was also considered as shown in Table 4.8. The result indicated, the mean age of the respondents was 1.98, median age was 2.0 (36-50) and mode was 1 (18-35). The age standard deviation as a measure of dispersion was 0.85; that was a smaller

value which significantly implied that the statistical values are close to the mean of the data set. Minimum age was 18 years and maximum age was above 50 years while the age data span or range was 2.

Table 4.8: Show mean, median, mode and std. deviation, range, minimum and maximum respondents' age

N	366
Mean	1.98
Median	2.00
Mode	1
Range	2
Minimum	1
Maximum	3
Std. Deviation	0.854

In terms of level of education, over half of the respondents had secondary education at 51.6% (189/366), followed by those with technical college education 20.2% (74/366), university education 15.8% (58/366), primary education 10.9% (40/366) and 1.4% (5/366) reported to have no formal education (Fig 4.9).

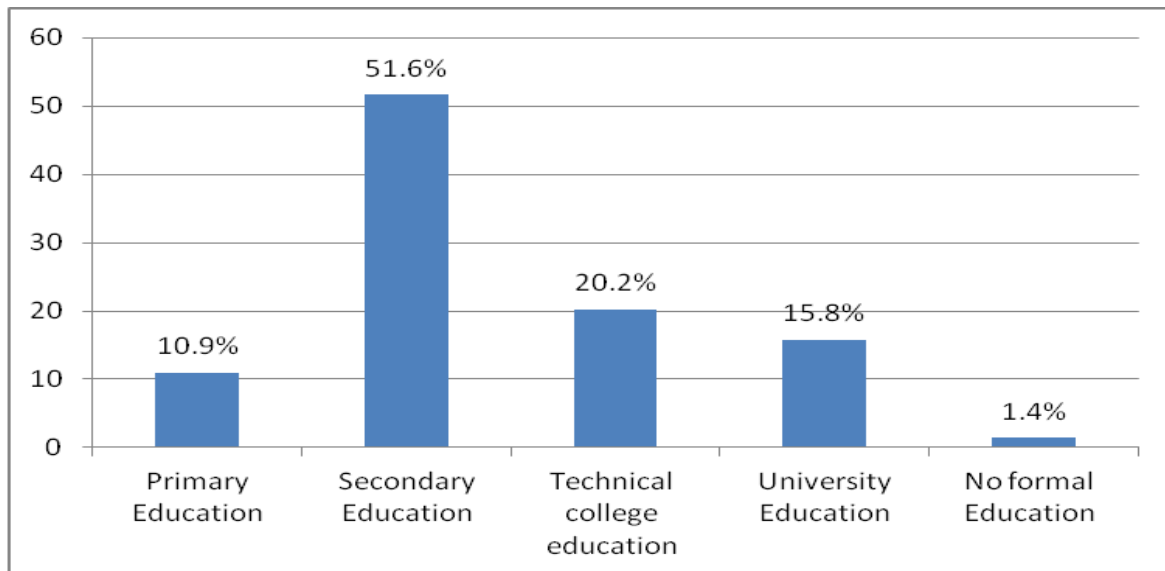


Figure 4.9 Response frequencies of farmers as per the level of education in Nandi County.

The study further collected data from the respondents on practices that may contribute to the occurrence of brucellosis and coxiellosis. These includes type of livestock species kept, farm management system, shared livestock resources, breeding methods, contacts with wild animals and introduction of new animals into the farm.

From these findings, most of the respondents reared only cows; however, others kept cows and sheep, cows and goats and cows and poultry. Some farmers reared sheep only, while some reared sheep and goats, goats only, cows and donkey and cows and pigs (Fig 4.10).

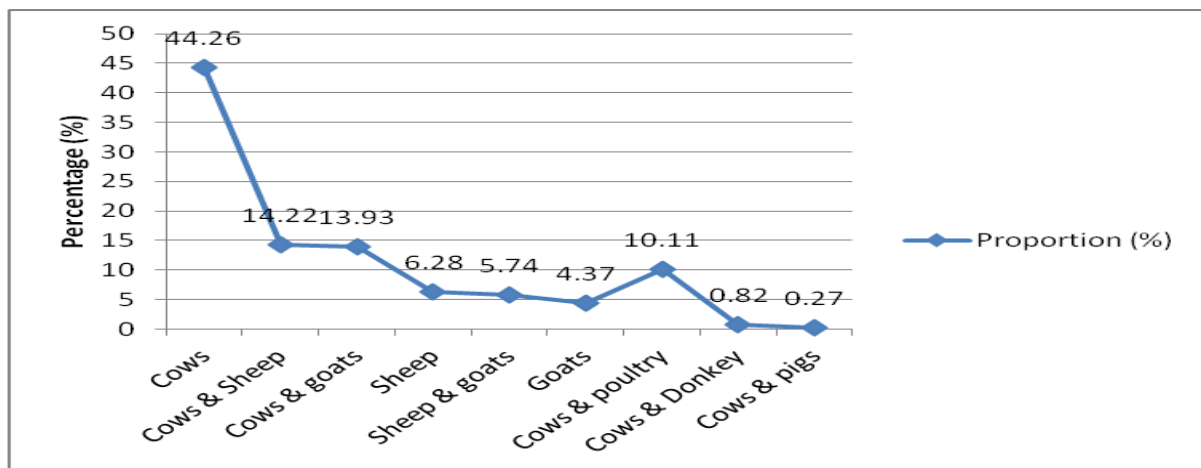


Figure 4.10 Farmer’s response on the type of livestock kept in the farm.

On farm management system, a bigger number of the respondents practiced semi-intensive farm management system who formed 66.94% (245/366), 24.05% (88/366) practiced intensive and 7.92% (29/366) farmers practiced zero-grazing. Only four farmers 1.09% (4/366) used tethering management system (Fig 4.11).

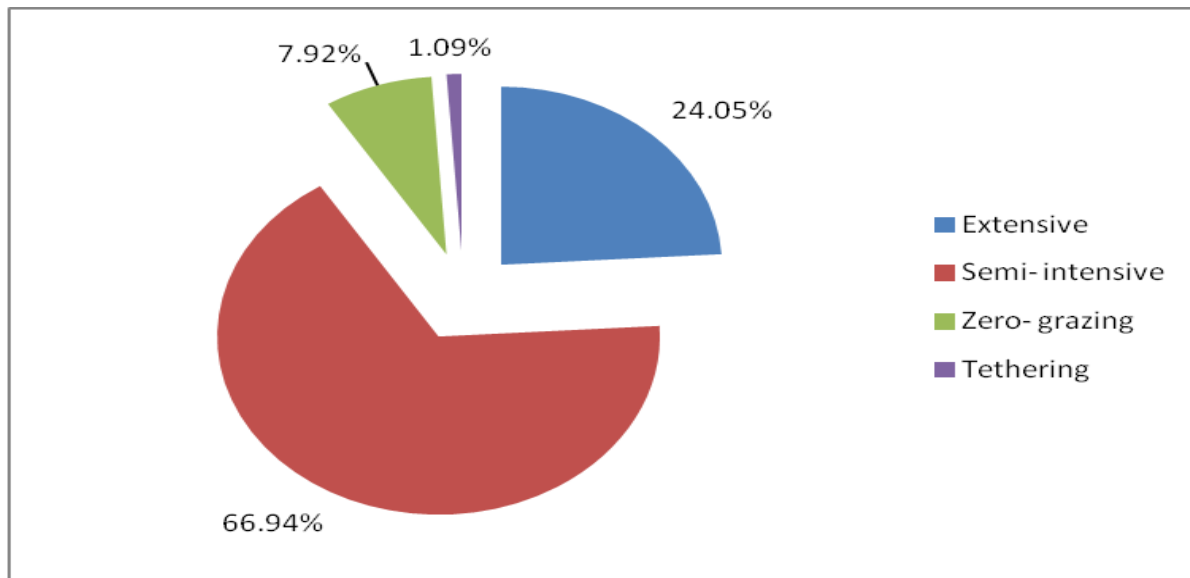


Figure 4.11 Pie chart showing the response on the type of production system in the farm

Below are some of the pictures on different productive systems in Nandi county.



Figure 4.12 Cattle in the semi- intensive production system in Tindiret Sub-County, Nandi County, 2019.



Figure 4.13 Cows in a zero grazing unit in Chesumei Sub-County, Nandi County, 2019.

A number of livestock resources normally shared in the County were considered in the investigation as shown in Figure 4.14. The findings showed the plunge dip as the most popular community resource with 306 out of the 366 farmers using communal dip reflecting 83.61%. Grazing and watering points followed marginally at 9.01% (33/366) and 1.64% (6/366). Sixteen out of 366 (4.37%) and five out 366 (1.37%) farmers shared dips/watering points and dip/grazing point respectively.

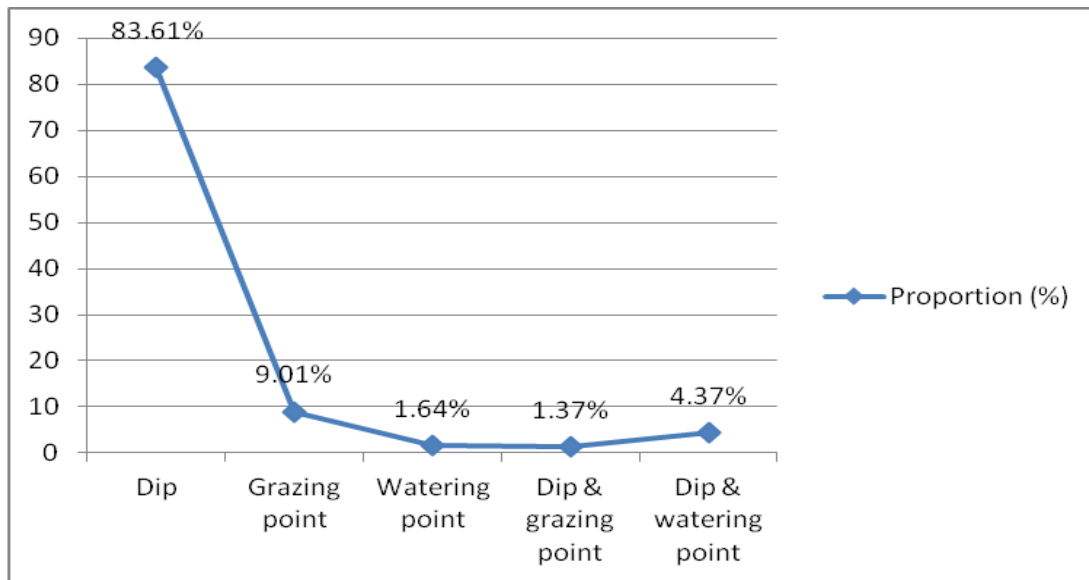


Figure 4.14 Participants response on the commonly shared resources in the County

Respondents were also asked the type of breeding method they used in their farms. It was noted that the use of own bull/ram/buck and artificial insemination were almost the same in the County. However, few farmers relied on neighbor’s bull/ram/buck for breeding their cows, sheep and goats (Figure 4.15).

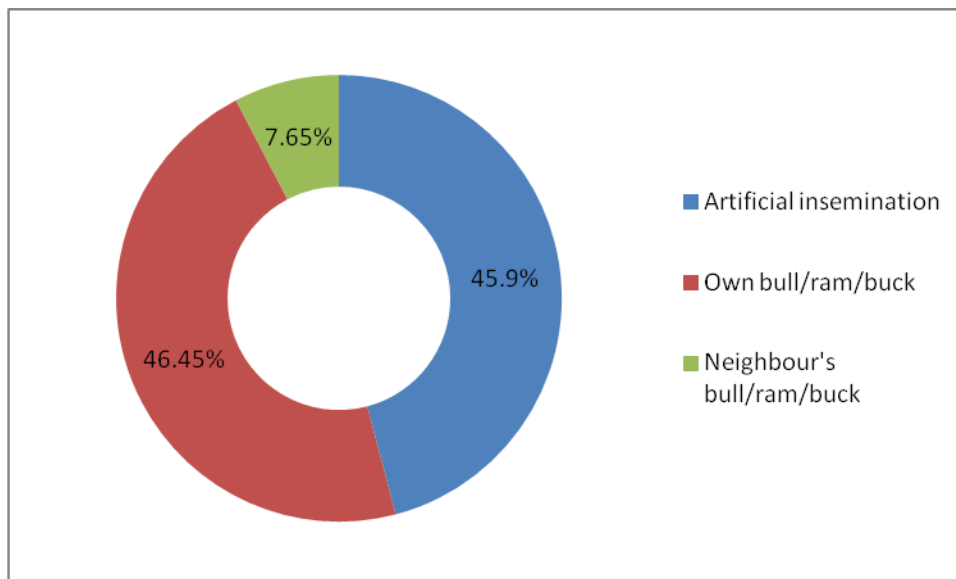


Figure 4.15 Respondent’s response on the breeding method used in the farm.

Information on whether their animals came in contact with wild animals was sought during the investigation. A total of 291 out of 366 respondents who formed 79.51% of the total respondent's population indicated that their animals usually did not come into contact with wild animals. The remaining 20.49% (75/366) of the respondents acknowledged that their animals usually interact with wild animals in the grazing field. The wild animals listed were hyenas, rabbits and monkeys (Figure 4.16).

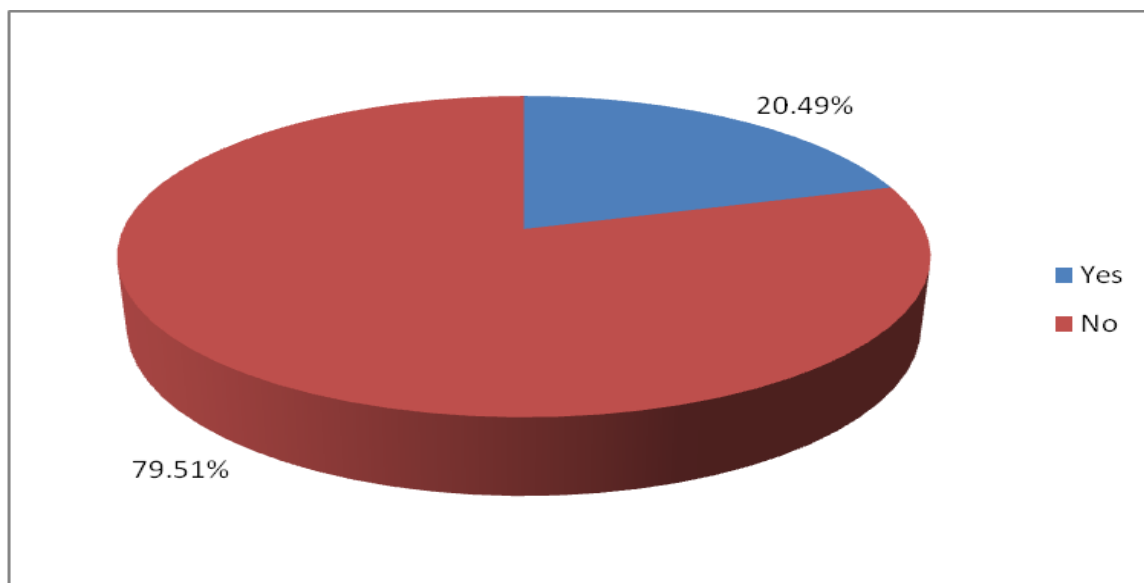


Figure 4.16 Response on whether their animals come in contact with wild animals.

Results on whether a cow, sheep or goat had been introduced into the farm in the last 12 months showed only 28.96% (106/366) of farmers reported to have introduced an animal into their farm while the rest 71.04% (260/366) were to the contrary.

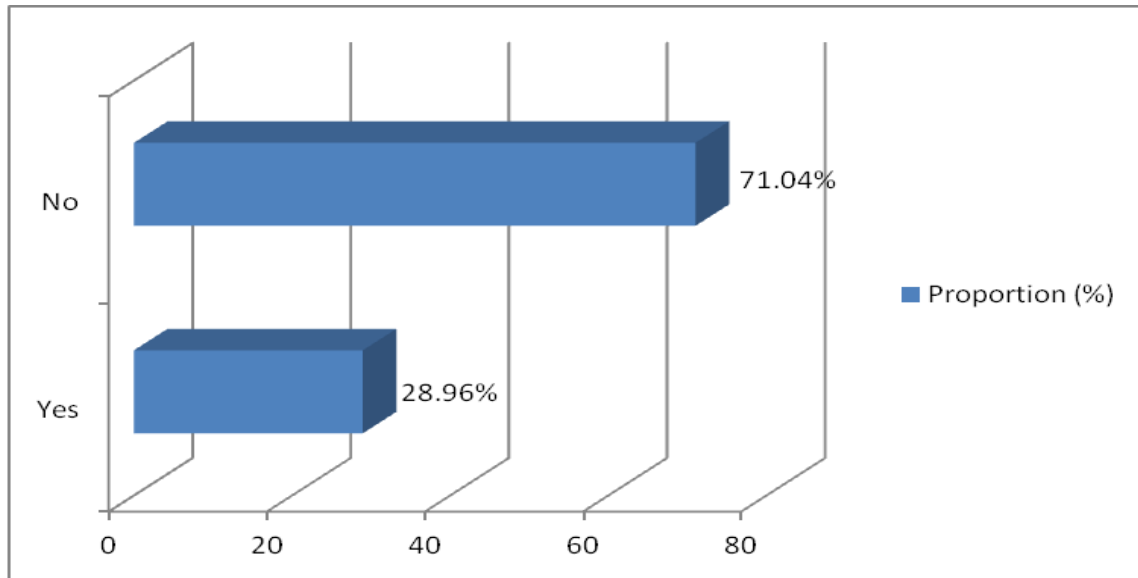


Figure 4.17 Participants’ response on whether they have introduced a new animal in the farm.

4.6 Assessment of farmers’ knowledge, attitudes and practices (KAPs) on Brucellosis and Coxiellosis in livestock in Nandi County.

A total of 366 households were assessed on KAPs on the two diseases by administration of semi-structured questionnaire to the head of the household or representative.

4.6.1 Farmers’ knowledge on brucellosis in livestock in Nandi County.

Livestock farmers were interviewed to assess their knowledge on brucellosis in cows, sheep and goats in Nandi County (Table 4.7).

Table 4.9 Farmer's responses on knowledge on brucellosis in livestock.

No.	Variable	Proportion (%) 95% CI			
		Option	Score	Frequency (n)	Valid Percent (%)
1.	Heard of brucellosis	Yes	1	325	88.8
		No	0	41	11.2
				366	100.0
2.	They believed brucellosis is a problem in your area	Yes	1	215	66.2
		No	0	22	6.8
		Do not know	0	88	27.1
3.	They knew what causes brucellosis	Yes	1	36	11.1
		No	0	268	82.5
		Do not know	0	21	6.5
4.	Knew some of the symptoms of brucellosis	Yes	1	253	77.8
		No	0	35	10.8
		Do not know	0	37	11.4
5.	Knew how brucellosis is transmitted in animals	Yes	1	194	59.7
		No	0	78	24.0
		Do not know	0	53	16.3
6.	They believed brucellosis can be cured in animals	Yes	1	231	71.1
		No	0	73	22.5
		Do not know	0	21	6.5
7.	Knew how brucellosis can be prevented in animals	Yes	1	251	77.2
		No	0	42	12.9
		Do not know	0	32	9.8
8.	They thought brucellosis can affect human beings?	Yes	1	178	54.8
		No	0	119	36.6
		Do not know	0	28	8.6

Results showed that, out of the total respondents 88.8% (325/366) said to have heard about brucellosis while 11.2% (41/366) were to the contrary. Among the 325 respondents who had heard about brucellosis, 138 (42.5%) heard it from farmer's training, 101(30.0%) from exhibition, 64 (19.7%) from animal health experts and 22 (6.8%) from school. The findings further showed that 66.2% believed brucellosis was a problem in their area, 6.8% thought it was not a problem, while 27.1% were not sure. The effects of the disease were sought from those who were in agreement that brucellosis was a serious problem in the area. Some of the highlighted effects were abortions by 59.5% (128/215) of the respondents, 25.1% (54/215) associated it with deaths in livestock and 15.3% (33/215) linked it to low milk production.

Of the 325 respondents who said to have heard of brucellosis, 77.8% knew some of the symptoms associated with brucellosis in livestock, 10.8% did not know and 11.4% were not sure. Among the symptoms listed by the respondents included; abortion in livestock, retained placenta, low milk production and loss of calf and adult animal. On disease transmission, 59.7% agreed to know how brucellosis can be transmitted, 24.5% did not know and 16.3% did not know whether it can or cannot be transmitted. Of those who agreed, they attributed the use of an infected bull as one of the ways brucellosis can be transmitted in livestock. On prevention of brucellosis in livestock, 77.2% (251/325) agreed to know how brucellosis can be prevented, 12.9% (42/325) did not know how it can be prevented and 9.8% (32/325) did not know whether it can be prevented or not. Vaccination, isolation of sick animals, cleaning of animal pen, use of AI and avoiding sharing of resources are some of the ways brucellosis can be prevented. Preventive measures identified were associated with artificial insemination (25.1%), animal pen cleaning (25.9%), resource sharing (23.9%) and vaccination (6.0%). In terms of curative measures, 71.1% (231/325) of the respondents were of the opinion that brucellosis can be cured, 22.5% (73/325) disagreed that brucellosis can be cured and 5.5% (21/325) did not know if it can be cured or not. Majority of participants 71.1% (231/325) believed treatment as a way of curing brucellosis in livestock. Information on disease transmission to humans were sought where some respondents 54.8% (187/325) believed that brucellosis could affect human beings, 36.6% disagreed and 8.6% did not know whether it could affect human beings or not. Some participants 29.3% (52/178) associated human transmission with lack of proper handling of animal products, 25.8% (46/178) associated with taking of raw milk and meat and 16.9% (30/178) associated with taking of raw meat.

Participants overall knowledge was calculated using the Bloom's cut-off point, where a score of between 60% and 100% and a score less than 60% was regarded as good and poor respectively.

This was done by finding the percentage average across different level; $\frac{k_1 + k_2 + k_3 + \dots + k_n}{n}$

(Alicia *et al* 2019). Therefore, average knowledge level of brucellosis amongst the respondents

was; $\frac{88.8\% + 66.2\% + 11.1\% + 77.8\% + 59.7\% + 77.2\% + 71.1\% + 54.8\%}{8} = 63.34\%$.

These results showed that residents of Nandi County had good knowledge about brucellosis disease in livestock.

4.6.2 Assessment of Farmers' Knowledge on Coxiellosis disease in cattle, sheep and goats.

Table 4.10 Farmers' responses on knowledge on coxiellosis in livestock

No.	Variable	Proportion (%) 95%CI			
		Option	Score	Frequency (n)	Valid Percent (%)
1.	Heard of Q-fever	Yes	1	21	5.7
		No	0	345	94.3
				366	100.0
2.	They believed Q-Fever is a problem in the area	Yes	1	8	38.1
		No	0	0	0
		Do not know	0	13	61.9
3.	Knew what causes Q- fever	Yes	1	10	47.6
		No	0	8	38.1
		Do not know	0	3	14.3
4.	Knew some of the symptoms of Q- fever	Yes	1	9	42.9
		No	0	12	57.1
		Do not know	1	0	0
5.	Knew how Q- fever is transmitted in animals	Yes	1	12	57.1
		No	0	9	42.9
		Do not know	0	0	0
6.	They believed Q- fever can be cured in animals	Yes	1	8	38.1
		No	0	5	23.8
		Do not know	0	8	38.1
7.	Knew how Q- fever can be prevented in animals	Yes	1	11	52.4
		No	0	6	28.6
		Do not know	0	4	19.0
8.	They thought Q- fever can affect human beings	Yes	1	10	47.6
		No	0	5	23.8
		Do not know	0	6	28.6

Findings showed that only 5.7% (21/366) have heard about Q-fever compared to 94.2% (345/366) who reported to have never heard. Among those who had heard about Q- fever 52.4% (11/21) said to have heard it from the veterinary staff, farmers training 33.3% (7/21) and those who learned from school were 14.3% (3/21). Over half of these group 61.9% (13/21) were not sure whether the disease was a problem in the area while 38.1% (8/21) agreed it was a problem. . Majority of the respondents (76.9%) associated the infection with animal deaths in the area, 15% said it caused infections in animals and 7.7% attributed to infections in human beings.

The findings further showed that 47.6% knew what causes Q- fever as bacteria, but 38.1% said they did not know, while 14.3% were not sure on what causes the infection. Regarding their knowledge on symptoms 42.9 % knew some of the symptoms associated with Q-Fever infection but 57.1% do not know. Some of the symptoms highlighted included; abortion (44.4%), loss of body weight (11.1%) and low milk production (44.4%).

On transmission of Q-fever, 57.1% of them believed the livestock disease can be transmitted but 42.9% said it cannot. Most of them (75%) associated it with infected bull, while 25% of the 21 respondents said it can be transmitted through production systems or practices.

On treatment, 38.1% believed that an animal infected with Q-fever can be treated and recover, 38.1% did not know whether it can be cured or not, while 23.8% said the infection in animals cannot be cured. Eight of the respondents (62.5%) agreed that Q-fever can be cured through treatment while 37.5% did not know how it be cured. On prevention, 52.4% of the 21 respondents who have heard about Q-Fever said the disease can be prevented, 28.6% said it cannot be prevented while 19% did not know whether it can be prevented. Some of the preventive practices mentioned were use of AI (27.3%) as an effective method of preventing livestock against Q-fever, 9.1% said vaccination can be used to prevent Q-fever while 63.6% said culling of infected animal is the only way to prevent the occurrence of the disease.

The participants were asked on whether Q-fever was zoonotic and 47.6% agreed that the infection can be transmitted to human beings, 28.6% did not know, while 23.8% said it cannot be transmitted to human beings. Among the 9 respondents who agreed that Q-fever can be transmitted, 55% said it can be transmitted to human beings through consumption of raw meat while 44.4% thought it can be transmitted through intake of raw milk.

Knowledge level amongst the respondents was measured using Bloom's cut off point by calculating the average percentages across different level; $\frac{K_1 + k_2 + k_3 + \dots + k_n}{n}$, therefore, average

knowledge of Q-fever amongst the respondents was;

$$\frac{5.7+38.1+38.1+42.9+57.1+52.4+38.1+47.6}{8} = 40\%.$$

Therefore, knowledge level of Q- fever amongst the respondents was at 40%.

4.6.3 Farmers' attitudes towards brucella infection in cattle, sheep and goats.

Likert's scale was used to determine the attitude of brucellosis among the respondents. Those who agreed and those who strongly agreed were considered to have the desirable attitude. The overall level of attitudes was categorized using original Bloom's cut-off point, where it was regarded as positive attitude if the score was between 60-100% and negative when the average score was less than 60%. Positive attitude towards the disease means having a perception that brucellosis disease is actually a public health problem and preventable, if appropriate strategies are devised.

Table 4.11 Assessment of farmers' response on the attitude on brucellosis diseases.

No.	Variable	Proportion (%) 95%CI			
		Option	Score	Frequency (n=366)	Valid Percent (%)
1.	Brucellosis is a serious disease in livestock	Strongly disagree	1	5	1.4
		Disagree	2	28	7.7
		Don't know	3	81	22.1
		Agree	4	194	53.0
		Strongly agree	5	58	15.8
2.	Animals can be affected with brucellosis.	Strongly disagree	1	3	0.8
		Disagree	2	38	10.4
		Don't know	3	147	40.2
		Agree	4	155	42.3
		Strongly agree	5	23	6.3
3.	You can be affected with brucellosis	Strongly disagree	1	5	1.3
		Disagree	2	25	6.9
		Don't know	3	132	36.2
		Agree	4	146	39.9
		Strongly agree	5	58	15.7
4.	Brucellosis is curable.	Strongly disagree	1	3	0.8
		Disagree	2	31	8.5
		Don't know	3	110	30.1
		Agree	4	122	33.2
		Strongly agree	5	100	27.4
5.	You are well informed about brucellosis disease.	Strongly disagree	1	2	0.5
		Disagree	2	92	25.3
		Don't know	3	135	37.0
		Agree	4	123	33.5
		Strongly agree	5	14	3.7
6.	Farmers need more information on brucellosis to increase awareness	Strongly disagree	1	5	1.4
		Disagree	2	27	7.4
		Don't know	3	93	25.4
		Agree	4	185	50.5
		Strongly agree	5	56	15.3

Findings from this study, results showed majority of the respondents 53.0% (194/366) agreed that brucellosis is a serious livestock disease, 15.8% (58/366) strongly agreed and 22.1% (81/366) did not know whether it is a serious livestock disease, 7.7% (28/366) disagreed and 1.4% (5/366) strongly disagreed. On whether they believed the disease could affect their animals,

42.3 % of the respondents agreed and 6.3 % strongly agreed. However, almost a similar number 40.2% did not know, 10.4% disagreed and 0.8% strongly disagreed.

On public health perspective, 39.9% and 15.7% agreed and strongly agreed that human being can contract brucellosis respectively, whereas 36.2% had no idea whether human beings can contract brucellosis. The remaining 6.9 % and 1.3 % of the respondents disagreed and strongly disagreed respectively. Over half of the respondents agreed with the perception that brucellosis can be cured; 27.4% strongly agreed and 33.2% agreed. However, 8.5% disagreed and 0.8% strongly disagreed but 30.1% did know if the disease can be cured.

Information about brucella infection appears scanty among the respondents in Nandi County, because 37% of the respondents did not know if they had information about brucellosis, 33.5% agreed to have information, 25.3% disagreed 3.7% strongly agreed while 0.5% strongly disagreed. Despite the grim scenario portrayed above, most of them 65.8% (15.3% strongly agreed and 50.5% agreed) wished they could access more information on the disease. Others disagreed 8.8% (7.4% disagreed and 1.4 strongly disagreed), whereas 25.4% were undecided.

Overall attitude was based on the percentage of those who strongly agreed and those who agreed across different levels and divided by the total number of questions (n=6).

53	42.3	39.9	33.2	33.5	50.5	
15.8	6.3	15.7	27.4	3.7	15.3	
68.8	48.6	55.6	60.6	37.2	65.8	
34.4	24.3	27.8	30.3	18.6	32.9	168.3
						28.05

Therefore, attitude level amongst the respondents was 28.05%. This indicated that there is a negative attitude towards brucellosis in livestock in Nandi County since the overall score was significantly below the desirable level of 60% and above.

4.6.4 Determination of Coxiellosis attitudes among the respondents.

The attitudes of the participants were assessed using the Likert scale to find out their perception

about coxiellosis disease in livestock and its public health implications in the County.

Table 4.12 Farmers’ responses on attitudes towards Coxiellosis disease in animals.

No.	Variable	Proportion (%) 95% CI			
		Option	Score	Frequency (n=366)	Valid Percent (%)
1.	Q- fever is a serious disease in livestock	Strongly disagree	1	24	6.6
		Disagree	2	29	7.9
		Don't know	3	281	76.8
		Agree	4	23	6.3
		Strongly agree	5	9	2.5
2.	Your animals can be affected with Q- fever.	Strongly disagree	1	34	9.3
		Disagree	2	44	12.0
		Don't know	3	242	66.1
		Agree	4	31	8.5
		Strongly agree	5	15	4.1
3.	You can be affected with Q- fever	Strongly disagree	1	37	10.1
		Disagree	2	29	7.9
		Don't know	3	192	52.5
		Agree	4	90	24.6
		Strongly agree	5	18	4.9
4.	Q- fever is treatable disease.	Strongly disagree	1	25	6.8
		Disagree	2	63	17.2
		Don't know	3	181	49.5
		Agree	4	44	12.0
		Strongly agree	5	53	14.5
5.	You are well informed about Q- fever disease.	Strongly disagree	1	248	67.8
		Disagree	2	21	5.7
		Don't know	3	68	18.6
		Agree	4	20	5.5
		Strongly agree	5	9	2.5
6.	Farmers need more information on Q- fever to increase awareness.	Strongly disagree	1	5	1.4
		Disagree	2	3	0.8
		Don't know	3	60	16.4
		Agree	4	281	76.8
		Strongly agree	5	17	4.6

The findings showered that 76.8% of the respondent did not know if Q-Fever is a serious livestock disease, 7.9% disagreed, 6.6% strongly disagreed, 6.3 % agreed while 2.5 % strongly agreed that Q-fever is a serious livestock disease. On further probing 66.1% of them did not know if their animals can be affected with Q-Fever, 12% disagreed, 9.3% strongly disagreed,

8.5% agreed while 4.1% strongly agreed. The participants were asked whether they think human beings can be affected by this disease. Response was 54.5% did not know if they can be affected by Q-fever, 24.6% agreed, 10.1% strongly agreed while 7.9% and 4.9% disagreed and strongly agreed respectively. On treatment, 49.5% did not know if an animal infected with Q-Fever disease can be cured, 17.2% disagreed, 14.5% strongly agreed 12% agreed and 6.8% strongly disagreed.

Respondents were asked whether they were well informed about coxiellosis disease and a small number were of the opinion that they were conversant (5.5% agreed and 2.5% strongly agreed), but majority strongly disagreed 67.8% and 5.7% disagreed and the rest 18.6% were not committal on if they agree or not. However, most respondents agreed that they need more information on the disease to increase awareness in the County for the sake of their livestock and themselves. This was shown by 76.8% of the respondents agreeing that they need to be well informed about coxiellosis infection with 4.6% strongly agreeing. On the other hand, 16.4% did not know whether to have the information or not whereas, 1.4% strongly disagreed and 0.8% disagreed.

The Likert scale was utilized to collect the above responses to these predictors of precautionary practices in the farm towards prevention and control of coxiella. This scale was re-stratified as 1 for strongly agreed and /agreed and two as strongly disagreed/disagreed/do not know.

6.3	8.5	24.6	12	5.5	76.8	
2.5	4.1	4.9	14.5	2.5	4.6	
8.8	12.6	29.5	26.5	8	81.4	
4.4	6.3	14.75	13.25	4	40.7	83.4
						13.9

The respondent attitude towards coxiellosis was therefore calculated and the result was significantly low at 13.9%.

4.6.5 Precautionary practices towards prevention of Brucellosis.

The respondents were assessed on some of the precautionary practices they undertake in the farm geared towards prevention/ control of brucellosis disease in animals and humans. It was done using the Bloom's cut- off point by calculating the average percentages across different level.

Table 4.13 Farmers' response on precautionary measures for Brucellosis in animals

No.	Variable	Proportion (%) 95% CI			
		Option	Score	Frequency (n)	Valid Percent (%)
1.	Do you protect yourself when handling sick animals?	Yes	1	318	86.9
		No	0	48	13.1
	If yes which protective gears, do you use?	Gumboot		295	92.8
		Mask		14	4.4
		Gloves		5	1.6
		Overall		4	1.3
2.	Has there been any abortion in your farm?	Yes	1	148	40.4
		No	0	218	59.6
	At what age was the pregnancy	1st trimester		87	58.8
		2nd trimester		22	14.8
		3rd trimester		39	26.4
3.	Do you seek expert assistance when your animal aborted?	Yes	1	219	59.8
		No	2	147	40.2
	Who did to call to treat your sick animal?	Vet		219	59.8
		Neighbor		1	0.3
		Treat myself		71	19.4
		No action		75	20.5
4.	Do you know any of the farm practices used to prevent brucellosis in livestock?	Yes	1	363	99.2
		No	0	3	0.8
	Name the most livestock farm practice use to prevent brucellosis?	Use of AI		191	52.2
		Use of own bull/ram/buck		104	28.4
		Vaccination		68	18.6
		Don't know		3	0.8

The findings indicated that 86.9% (318/366) of the respondents protected themselves when handling aborted animals while 13.1% (48/366) on the contrary do not take any precautionary measures. The protected gear included gumboots (92.8%), mask (4.4%), gloves (1.65%) and overalls (1.3%). Information on the incidence of abortion in the farm showed that 59.6% (218/366) had not had cases of abortion in their farms while 40.4% reported to have had cases of abortion in their farms. Among the latter group 58.8% (87/148) had their animals aborted in the

first trimester, 14.8% (22/148) in the second trimester and 26.4% (39/148) had their animals aborted at the third trimester of pregnancy.

Respondents were also asked whether they seek technical expertise when their animals are sick. Over half of them 59.8% (219/366) reported to always sought for services from a veterinarian or para-veterinarian. The other participants said they don't consult an expert. One farmer said he uses a neighbor, 19.4% treated their animals by themselves and 20.5% did not take any action. Incidentally almost every participant 99.2% (363/366) was familiar with at least one preventive measure for brucellosis except three 0.8% (3/366) who were not.

Overall precautionary practice for brucellosis was $89.9+40.4+59.8+99.2/4 = 71.58\%$ which indicated that their precautionary practice was positive towards brucella control.

4.6.6 Precautionary practices towards Coxiella prevention.

The respondents were assessed on some of the precautionary practices they undertake in the farm in an effort to prevent or control Q- fever disease in their animals.

Table 4.14. Farmers' responses on precautionary practice for coxiellosis disease in animals

No.	Variable	Proportion (%) 95% CI			
		Option	Score	Frequency (n)	Valid Percent (%)
1.	Do you clean animal pen/shed?	Yes	1	362	98.9
		No	0	4	1.1
	How often do you clean animal pen/shed?	Weekly		67	18.3
		Fortnightly		206	56.3
Monthly			89	24.3	
	Never		4	1.1	
2.	Do you use any form of protective gears when handling sick livestock or its products	Yes	1	364	99.5
		No	2	2	0.5

	If yes which protective gear, do you use when handling livestock?	Gumboots		341	93.7
		Mask		15	4.1
		Gloves		5	1.4
		Overall		3	0.8
3.	Do you control ticks in your animals?	Yes	1	366	100
		No	0	0	0
	If yes, mention method you use?	Dipping		254	69.4
		Hand spray		112	30.6
		Spray race		0	0

The results indicated that almost all respondents 98.9% (362/366) affirmed that they normally cleaned their animal pens/sheds. A few of them 1.1% (4/366) said they do not clean the animals' pen/shed. However, the frequency of cleaning differed among the respondents with 18.3% cleaning on a weekly basis, 56.3% fortnightly and 24.3% monthly. From the SPSS output, majority of the respondents (99.5%) acknowledged that they do use at least one protective gear while handling livestock while only 0.5 % don't use any protective gears while handling animal products and livestock. Although most of the respondents confirmed to use personal protective gears while handling livestock and livestock products, gumboot (93.7%) was the dominantly used protective gear followed dismally by use of mask at 4.1%, gloves at 1.4% and overall, at 0.8%.

Tick control in the County showed 100% compliance since all the respondents (366) practiced at least one of the tick control strategies available. Dipping (69.4%) appeared the most popular control method among the livestock farmers followed by hand spraying (30.6%). There was no farmer in the survey who used spray race. Overall precautionary measures were $98.9+99.5+100=99.5\%$.

CHAPTER FIVE

5.0 DISCUSSION

5.1 INTRODUCTION

According to the Nandi County Integrated Development plan (2013), livestock sub sector was regarded as the key driver for socio-economic activities and supports the livelihood of many rural poor households. Dairy cattle, sheep and goats are the main livestock species kept in the County. Dairy farming is the heartbeat of the community's livelihood since apart from getting food and income; they also have a special sentimental attachment to their livestock. Majority of the farmers practiced mixed farming with maize, tea and sugarcane as the major commercial crops. According to Muturi et al. (2021), zoonotic diseases are regarded as public health priority with Rift valley, Brucellosis and Coxiellosis as the three top priority diseases in the country.

Brucellosis and coxiellosis are considered endemic diseases across the African continent (Franc *et al.*, 2018). The two pathogens are contagious zoonotic diseases affecting almost all domesticated animals, wildlife and humans. They are multi-burden diseases which cause huge economic losses due to abortions, infertility and reduced milk in animals and disability in man (Adamu., 2009). In addition, the diseases are barriers to regional and international trade in livestock and livestock products (Franc *et al.*, 2018). One of the important key elements for effective prevention or control for brucellosis and coxiellosis is to improve community knowledge, attitudes and practices (KAPs) through sensitization and awareness creation on the diseases (Diez *et al.*, (2013).

The main aim of this study was to gather critical information regarding status of brucellosis and coxiellosis for cattle, goats and sheep in Nandi County with a view to raise awareness for their

economic and potential zoonotic significance among the farming community. This was the first ever study to be carried out involving a large sample of animals representing the whole of Nandi County on estimates of sero-prevalence of brucellosis and coxiellosis in cattle, sheep and goats. The result from the study indicated that *Brucella* and *Coxiella* organisms are circulating in ruminants in Nandi County with seroprevalence ranges that are quite variable within individual animal species and Sub-Counties. Risk factors for the two diseases showed association variability between the independent and dependent variables. Likewise, perception on the two diseases was also variable among the farming community in Nandi County. Residents were more familiar with brucellosis disease, since they even have a local name for it “koroitab chego” translated loosely as “the sickness of milk” or *ugonjwa ya maziwa* in swahili”. However, from the findings, Q- fever disease appeared unknown to the community, despite reporting significant seroprevalence level in ruminants.

5.2 BRUCELLOSIS

The current study established a low seroprevalence rate of brucellosis of 0.41% in cattle, 3.18% in sheep and 1.52% in goats on RBPT. Whereas on confirmation with iELISA, the figure was even lower with cattle recording only 0.14% and sheep and goats recorded 0% seropositivity. The results of brucellosis on these two tests were not consistent, despite RBPT being considered a very sensitive test as it misses very few infected animals (Ducrotoy *et al*, 2015). However according to Mugambi, (2001), he reported that RBPT was not an adequate test on its own, since it had high false positive reactions due to antibodies against other bacteria such as *Campylobacter* and *Yersinia* species. In this study, all samples were confirmed with iELISA, because it was considered to have better diagnostic performance, in addition to being simple and

rapid test with high sensitivity and specificity in detecting antibodies to *Brucella* organisms (Godfroid *et al*, 2004).

These findings indicated that brucellosis may indeed currently not be a major problem in livestock for the majority households sampled during the study in Nandi County. However, this does not mean that the disease is not a threat to livestock and human health because, the presence of confirmed circulating brucella isolates may serve as sources of outbreak. These results agreed with a study previously done by Osoro *et al*, (2015) where they reported low seroprevalence ranges of between 0.8% - 2.4 % in cattle, 2.4 % in sheep and 0-1.3% in goats in small holder herd farms of Kenya. However, according to Nanyende, (2010) in pastoralist herds, these results differed where higher figures on seroprevalence were recorded as 9.90 % -16.90 % for cattle, 11.90 % for sheep and 13.0% -16.10% for goats. From these findings, the variations were quite significant between the different livestock production systems and agro-ecological zones. In pastoral communities, common practices such as grazing together of large numbers of domestic animals, livestock movement in search of pastures and water, sharing of grazing zones with wild animals and converging of these animals at water points were linked to higher spread of brucellosis. Low seroprevalence in highlands such as in the current study area was lower, because livestock are usually well managed in individual confined farms, most farmers practice artificial insemination or use their own bull or ram or buck for breeding purposes which minimizes disease spread. Other possible reasons could include minimal movement of livestock, limited shared resources and domestic animals rarely come in contact with wildlife.

This study was further supported by a review survey done in 12 Sub-Saharan Africa countries, where results showed prevalence of 1.0%- 36.6% among cattle raised under various livestock production system (Njeru *et al.*, 2016). In East Africa, brucellosis prevalence in ruminants

ranged between 0.2% to 43.8% which was comparable, but higher than seroprevalence in Sub-Saharan Africa. Cattle brucellosis in Uganda, Tanzania, Kenya and Burundi reported seroprevalence range of between 1.8 % - 25.4% (McDermont *et al*, 2002) which was comparable to the current study. According to Mwangi (2015), outbreaks of brucellosis in highlands are commonly associated with introduction of an infected animal to the farm.

This study also assessed potential risk factors associated with brucellosis in cattle, sheep and goats. The predictors were mainly animal demographics such as species, sex, age, breed and management factors such as farm size and livestock production systems. The results showed that animal species (p-value<0.01), age (p-value=0.042) and breed (p-value=0.037) were the identified factors associated with occurrence of brucellosis in cattle in Nandi county. Cattle were more commonly associated with brucella infection than sheep and goats. This observation was supported by Nikeel *et al.*, 2016 and Kosgei *et al.*, 2016, where they found the seroprevalence in cattle was more than in sheep and goats but conversely, the figures were slightly higher than the current study. These results also agreed with previous study conducted in India by Ram *et al*, 2019 and CDC 2005 which gave similar risk factors. Brucellosis was also associated with adult cows as compared to heifers which were similar with the finding by Franc *et al.*, 2018. This observation could be attributed to the length of exposure to the organisms and breeding methods. The study was also in agreement with Bebe *et al* 2003, where breed association with seropositivity was linked to exotic breeds possibly due to genetics. Sex, farm size and production systems, however, did not show any significant association with brucellosis occurrence. Therefore, these findings provided significant data on seroprevalence estimates of brucella infection in goats, sheep and cattle as well as its associated risk factors in Nandi County.

The study further assessed the level on knowledge, attitudes and practices of livestock farmer on brucellosis in animals. These findings supported that brucellosis still remains a burden in many countries of the world, but despite this, the disease was rarely prioritized by both animal/human health systems in many developing countries and was considered a neglected zoonosis (Nicoletti P., 2010). The current study explores the gaps in KAPs towards brucellosis in goats, cattle and sheep. The findings revealed over 60% study population had good knowledge on brucellosis which could be attributed to the low sero-positivity in animals. This is true because according to Jerono *et al.*, 2012, there was minimal risk of brucellosis infections in human beings in Nandi County, since what was reported in the health facilities as positive on conventional SAT and treated as such were confirmed through CFT as false positives for brucellosis ((98%).

It was evident that majority of the respondents were familiar with the disease (86.4%) and this was attributed to their participation in farmers' training and agricultural exhibition. This was similar to what was documented in Uganda (Kansiime *et al.*, 2014) where it showed experts and community health workers were the major sources of information on brucellosis. Even in the current study only small number of respondents mentioned media or internet. This study supports the findings by Ekram *et al.*, 2016 on the role of the media on dissemination of only health informations in times of outbreaks, but unfortunately diseases of enzootic nature are continually neglected. This therefore, highlighted powerful role of animal health experts and the community animal health personnel plays key role in terms of relaying important animal/human health messages or information to livestock owners. Over 50% of respondents knew the impacts of the disease on their animals and were even conversant with public health significance, transmission, symptoms and preventive interventions. According to Diez *et al.*, (2013), knowledge on infections and preventive herd/flock management practices have previously been recognized as

one of the most important factors required for minimizing disease risks in animals. Current study differed with earlier studies done in Nigeria by Buhari *et al.*, (2015), where they reported brucellosis in animals poorly understood by their study subjects, where they reported more than two thirds of the respondents had not heard of the disease or even its possible cause of transmission. This study was however consistent with reports in Kenya and Egypt by Obonyo *et al.*,(2015) and Safaan *et al.*, (2016) respectively.

The attitude by the participants of Nandi County regarding brucellosis may negatively impact on individuals' control or preventive methods of the disease at the humans and animal interface because of misconception on its determinants. It was evident in this study where farmers' attitude was reported as negative at 28.05% and this could be a barrier to brucellosis control as well as enhanced susceptibility and risk behavior. However, Sjabera L., (2000) disputed this notion and stated that high awareness level does not necessarily go hand in hand with accurate behavior or practices, as the perception may be influenced by many other factors such as culture and life experience. Overall precautionary practice for brucellosis was good 71.58% which indicated that their precautionary measures were positive towards brucella control. Among the measures highlighted included use of personal protective gears and seeking technical expertise to attend to their animals

Therefore, the findings from this study calls for a more concerted joint effort by both veterinarians and medical doctors for awareness creation and sensitization on brucellosis through new one health approach concept. Actions should therefore be undertaken geared to incorporate all aspects of animal health education for the livestock owners including zoonoses.

5.3 COXIELLOSIS

This study was the first to be undertaken in the County to investigate the status of Coxiellosis in cattle, sheep, and goats and its associated risk factors. According to Njeru *et al.* (2016), infection impart immense socio-economic burden due to production and reproductive losses such as abortions, stillbirths and infertility. This study was carried out to establish the true status of coxiella infections in Nandi, given that the County is home to over 300,000 cattle of which, the majority are exotic and their crosses particularly Friesian, Ayrshire, Jersey, Guernsey, 120,000 sheep mainly Dorper and Merino, and 45,000 goats (Galla goats and Toggenburg). Results indicated a seroprevalence 8.138% (59/725) in cattle, 1.413 % (4/283) sheep, and 0.758 % (1/132) goats. Despite the status of the disease being unknown, these data were an eye opener because it proved the widespread of coxiella infection in ruminants, hence portraying real threat to human health particularly amongst people living in close proximity to their animals. The findings further demonstrated the disease was more prevalent in cows followed by sheep and goats respectively. These findings were in agreement with the previous study where seroprevalence of coxiella in cattle was estimated at 7.4-51.1 % and 8.77 % in Kenya (Njeru *et al.*, 2016) and Ethiopia (Feyissa *et al.*, 2020) respectively. However, in small ruminants these results differed because the prevalence was lower than the reported figure of 20-46 % in goats and 6.7-20 % in sheep. Furthermore, other surveys undertaken in neighbouring countries reported higher *C. burnetii* prevalence than the current study in domestic ruminants. Tanzania, reported a prevalence of 13.3 % in cattle, 13.6 % in goats, and 17.1 % in sheep (Hummel, 1976). Likewise, in Sudan, it reported seroprevalence of 24 % in goats, 40.4 % in cattle, 53 % in goats, and 62.5 % in sheep (Hussien *et al.*, 2012). In Ethiopia, the prevalence was 31.6% in cattle, 54.2 % in goats, and 90 % in camel Gumi *et al.*, 2013). Whereas in Kenya, the seropositivity of

Coxiellosis was higher (28.2- 57.1 %) in livestock from pastoralist communities (Nakeel *et al.*, 2016, Mwololo., 2016) than in highland regions. Though the precise reason for this diverse variation was not clear, agro-ecological zones, livestock production systems, livestock and human population density appeared to play a significant role. The pastoral communities context where , practices such as mixing of huge numbers of livestock, livestock movement in search of pasture/water, sharing of grazing zone with wildlife and convergence of animals in one water points are linked to the higher spread of coxiellosis among goats, cattle and sheep. Seropositivity in highlands tends to register lower prevalence rates because, they are well managed in individual confined farms. These farmers mostly use either artificial insemination or their own bull, ram and buck for breeding purposes which minimizes the transmission . Other possible reasons may include limited shared resources, minimal movement and limited contacts between domestic and wild animals. This could be true for the current study because, Nandi County is generally cold and wet and livestock farming systems are sedentary with minimum livestock movement or shared resources. Moreover, animals are kept in enclosed and well managed farms with no wildlife interaction.

In this study, the following factors in domestic ruminants were investigated: species, breed, sex, age, and animal production systems. However, results showed animal species was the only identified risk factor associated with coxiella infection. It exhibited *C. burnetii* sero-prevalence was higher in cattle (8.138 %) than both sheep (1.413 %) and goats (0.758 %). It further demonstrated that cattle were 7 times more prone to coxiella infection compared to the other species (OR= 7.260). Data on the burden and distribution of coxiellosis were not readily available particularly in terms of the actual prevalence and source of infection. According to Ioannou *et al.*, (2009); he documented that some types of hard ticks are linked to the transmission

of coxiellosis where they acted as reservoir/carrier of the pathogen. In Nandi County this could be correct where ticks might be playing a key role in coxiella transmission since 80% of diseases reported yearly are tickborne diseases mainly Anaplasmosis, East coast fever and Babesiosis among others (CDVS, 2015). It is recommended that further investigations on the role of ticks in the spread of coxiellosis in Nandi County may be essential to establish this assumption.

In terms of sex, overall seroprevalence of coxiellosis was 5.94 % (61/1027) in females and 0.29 % (3/1027) in males. Male goats were leading in the seropositivity at 9.091% (1/11) followed by female cows at 8.482 % (57/ 672) and female sheep at 3.482 % (4/236). There were no positives in female goats and male sheep. These results were in contrast with a study conducted in Ghana, where both males and females were infected in equal measure irrespective of the sample size (Sherry *et al.* 2019). Despite these results, the current study however confirmed sex variable was not a risk factor for Coxiella infection. Results outcomes further illustrated adult cattle had higher seropositivity rate (4.737 %) compared to heifers (0.877 %). These findings were in agreement with study done by Mwololo *et al.*, (2016) in Bura, Tana River County, where he reported low seroprevalence in young domestic animals. Possible explanation could be linked to more exposure of mature ruminants to *C. burnetii* antibodies. On breed characteristics, there was no statistically significant difference in the seropositivity of coxiella in the County. In this study, Jersey breed had a slightly higher seroprevalence of 8.69% when compared to Fresian and Ayrshire which recorded a prevalence of 8.231 % and 8.259 % respectively. In goats toggenburg breed had a seroprevalence of 3.846%, and the Dorper breed in sheep was 1.379 %. Therefore, this may be associated with exposure to the causative agent and individual animal genetics.

In terms of production systems, sero prevalence of coxiellosis showed zero grazing and semi-intensive production system had higher seroprevalence rate of 8.88% and 8.233% respectively,

compared to 2.801 % and 1.299 % for the extensive system and tethering. Findings corresponded to an earlier investigation done by Ibrahim et al. (2021) which reported extensive production systems to have a lower coxiellosis seropositivity compared to semi- intensive and zero-grazing. The possible explanation could be linked to contamination following hygienic challenges in intensive production systems particularly, during management of animal wastes and manure. In spite of these results, multivariate analysis model found no statistically significant association between coxiella infection and the investigated independent variables since p-value was >0.05 for sex, age, breed, farming production systems.

Responses on knowledge on coxiellosis in animals among the participants recorded an overall low level at 40.0 % with 94.2% of the respondents confirming to not having heard about the disease. This could be true because according to Bwatota *et al.*, 2021 who reported that information regarding coxiellosis in Kenya is limited. This resultss are consistent with study done in Tana River (Mwololo *et al*, 2016. The attitude level was even worse in the county at 13.9% which poses a great challenge in addressing this emerging zoonotic disease. However, a good number (92%) of the participants felt that they require more information regarding this ‘new’ disease. In spite of all the scenario, the precautionary practiced by the respondents was exceptionally high at a score of 99.55%. The possible explanation for this finding could be that fact that the same practices used for other known diseases such as brucellosis also applied to Coxiellosis prevention and control strategies. Another reason could be that all farmers in Nandi County dip or hand spray their animals against ticks since tick- borne diseases are endemic and in the process are perceived to be do the right precautionary measure against coxiella which could not be true.

Lastly, gaps observed in this study and needs to be addressed was that Coxiellosis was not among the priority list of diseases under surveillance by the veterinary authority in Nandi County, coupled with lack of diagnostic facility within the region that has the capacity to test for coxiella. This therefore, implied that Coxiellosis could be missed out in differential diagnosis during investigation cases of abortions, stillbirths and infertility in livestock.

CHAPTER SIX

6.0: STUDY CONCLUSIONS AND RECOMMENDATIONS.

6.1. Conclusions

Based on these findings, several conclusions were drawn on Brucellosis and Coxiellosis in cattle, sheep and goats in Nandi County, Kenya.

1. Detection of Brucellosis and Coxiellosis diseases in the study area, demonstrated that cattle, sheep and goats within Nandi County are widely exposed to *Brucella species* and *C. burnetii* antibodies and may pose a public health and socioeconomic challenge amongst the inhabitants of the County.
2. The risk of brucella infections in cattle, goats and sheep was minimal in Nandi County due to report of low seropositivity, but the fact that there was presence of circulating brucella organisms in cattle means that the infection remained a serious risk to the of animal and human health, since small pockets may serve as a source of outbreaks.
3. The sero-prevalence of Coxiellosis in cattle was significantly higher, compared to goats and sheep which confirmed the infection as an important zoonosis in the County. Despite the disease and its status being unknown, these results were an eye-opener that revealed coxiella was widely spread and could be one of the reasons of missed diagnosis or misdiagnosis for abortion and infertility in animals.
4. The identified risk factors that were associated with incidence of Brucellosis in ruminants were animal species, breed and age, whereas for Coxiellosis it was only animal species.
5. Residents of Nandi County were knowledgeable on brucellosis, but there was a huge knowledge gap on Coxiellosis. The perception towards the two diseases was negative and may influence their practices towards prevention and control of the two diseases.

However, their general precautionary practices were good and remarkable.

Recommendations

1. The department of Veterinary Services in Nandi County should enhance brucella surveillance, monitoring, awareness and risk factors to maintain the current favourable situation. Likewise, farmers and stakeholders should be enlightened on the causative agent, modes of transmission, risk factors and control measures against coxiellosis to enhance awareness and participation in surveillance and control programs.
2. Laboratory facility should be set up to strengthen the capacity for the diagnosis of brucellosis and coxiellosis as well as other animal diseases in the County. All positive cases of brucellosis on RBPT should be confirmed to minimize false positives and unnecessary treatment.
3. County zoonotic disease unit among veterinary and medical personnel should be established to enhance awareness on brucellosis and coxiellosis, in addition to other zoonoses under the one health concept.
4. Disseminate information from this study to stakeholders in the County regarding the status of these two diseases in regard to the prevalence, risk factors and the KAPs assessment in order to impart key knowledge and improve their attitude and precautionary practices which will assist in reduction on the occurrence of these diseases in animals.
5. Further studies should be conducted in Nandi County that involves a wider population of other livestock including wildlife to better understand their role in epidemiology, scope and impact of these two diseases in animals and humans. Also, a study should be done to investigate the status of the diseases in human population and its zoonotic potential.

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7. Which breeding method do you use in your farm?
- 1. Artificial insemination
 - 2. Own bull/ram/buck
 - 3. Neighbour's bull/ram/buck

8. Do your animals come in contact with any wild animals?
- 1. Yes
 - 2. No

If yes, name the wild animals.....

9. Have you introduce a new animal in the farm in the last 12 months?
- 1. Yes
 - 2. No

d). Knowledge on brucellosis infection in Livestock

10. Have you heard of brucellosis disease?
- 1. Yes
 - 2. No

If yes, where did you first learn/hear about brucellosis.....

11. Do you think brucellosis is a problem in this area?
- 1. Yes
 - 2. No
 - 3. Do not know

If yes, explain.....

12. Do you know what causes brucellosis?
- 1. Yes
 - 2. No

3. Do not know

If yes what do you think causes brucellosis?

- 1. Virus
- 2. Bacteria
- 3. Other (Specify).....

13. Do you know some of the symptoms of brucellosis in livestock?
- 1. Yes
 - 2. No
 - 3. Do not know

If yes can you list some of these symptoms of brucellosis you know.....

.....

...

14. Do you know how brucellosis in livestock can be transmitted?
- 1. Yes
 - 2. No
 - 3. Do not know

If yes, how is it transmitted?.....

15. Do you know how brucellosis can be prevented?
- 1. Yes
 - 2. No
 - 3. Do not know

If yes, how can it be prevented.....

.....
.
16. Can brucellosis in livestock be cured?

- 1. Yes []
- 2. No []
- 3. Do not know []

If yes, how can it be cured?.....

17. Do you think livestock brucellosis can affect human beings?

- 1. Yes []
- 2. No []
- 3. Do not know []

If yes, how can it be transmitted to humans.....

e). Attitudes towards brucellosis infection in livestock.

18. Brucellosis is a serious livestock disease.

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

19. Your animals can be affected with brucellosis.

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

20. You can be affected with brucellosis.

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

21. Brucellosis is curable

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

22. You are well informed about brucellosis disease.

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

23. Farmers need more information on brucellosis to increase awareness.

- Strongly agree []
- Agree []
- Don't know []
- Disagree []
- Strongly Disagree []

f). Practices on prevention of livestock brucellosis

24. Do you use personal protective gears when handling the sick animal or assisting livestock to give birth?

- 1. Yes []
- 2. No []

If yes, which personal protective gear do you use?

- 1. Gumboots []
- 2. Mask []
- 3. Gloves []
- 4. Overall/dustcoat []

25. Has there been any abortion in your farm?

- 1. Yes []
- 2. No []

If yes what age was the pregnancy?

- 1. 1st trimester []
- 2. 2nd trimester []
- 3. 3rd trimester []

26. Do you seek expert assistance when your animal is sick?

- 1. Yes []
- 2. No []

If yes, who did you call to treat your sick animal?

- 1. Vet []
- 2. Neighbor []
- 3. Treat it myself []
- 4. No action []

27. Which breeding method do you use in your farm?

- 1. Artificial insemination []
- 2. Own bull/ram/buck []
- 3. Neighbour's bull/ ram/buck []

28. Which are some of the other practices you think you can do to prevent livestock from getting infected with brucellosis?.....

d). Knowledge on Coxiellosis infection in Livestock.

29. Have you heard of coxiellosis disease?

- 1. Yes []
- 2. No []

If yes, where did you first learn/hear about coxiellosis

30. Do you think coxiellosis is a problem in this area?

- 1. Yes
- 2. No
- 3. Do not know

If yes, explain.....

31. What do think causes Coxiellosis?

- 1. Virus
- 2. Bacteria
- 3. Other (Specify).....

32. Do you know some of the symptoms of Coxiellosis in livestock?

- 1. Yes
- 2. No
- 3. Do not know

If yes can you list some of these symptoms of Coxiellosis you know.....

.....

...

33. Do you know how Coxiellosis in livestock can be transmitted?

- 1. Yes
- 2. No
- 3. Do not know

If yes, how is it transmitted?.....

34. Do you know how Coxiellosis can be prevented?

- 1. Yes
- 2. No
- 3. Do not know

If yes, how can it be prevented.....

.....

.

35. Can Coxiellosis in livestock be cured?

- 1. Yes
- 2. No
- 3. Do not know

If yes, how can it be cured?.....

36. Do you think livestock Coxiellosis can affect human beings?

- 1. Yes
- 2. No
- 3. Do not know

If yes, how can it be transmitted to humans.....

e). Attitudes towards Coxiella infection in livestock.

37. Coxiellosis Q-fever is a serious livestock disease.

- Strongly agree
- Agree
- Don't know
- Disagree
- Strongly Disagree

38. Your animals can be affected with Coxiellosis.

- Strongly agree
- Agree
- Don't know

Disagree []
 Strongly Disagree []
 39. You can be affected with Coxiellosis.
 Strongly agree []
 Agree []
 Don't know []
 Disagree []
 Strongly Disagree []

40. Coxiellosis is curable
 Strongly agree []
 Agree []
 Don't know []
 Disagree []
 Strongly Disagree []

41. You are well informed about Coxiellosis Q- fever disease.
 Strongly agree []
 Agree []
 Don't know []
 Disagree []
 Strongly Disagree []

42. Farmers need more information on Coxiellosis to increase awareness.

Strongly agree []
 Agree []
 Don't know []
 Disagree []
 Strongly Disagree []

f). Practices on prevention of Coxiellosis livestock.

43. Do you clean animal pen/shed?
 1. Yes []
 2. No []

If yes, how often do you clean animal pen/shed?
 1. Weekly []
 2. Every 2 weeks []
 3. Monthly []
 4. Never []

44. Do you use personal protective gears when handling sick animal or assisting livestock to give birth?
 1. Yes []
 2. No []

If yes, which personal protective gear do you use when handling sick livestock?
 1. Gumboots []
 2. Mask []

- 3. Gloves
- 4. Overall
- 45. Do you control ticks in your farm?
 - 1. Yes
 - 2. No
- If yes, which method do you use?
 - 1. Dipping
 - 2. Hand spray
 - 3. Spray race

APPENDIX 2

CONSENT FORM

Investigator: **Jerono Kiptanui**

Department of Public Health, Pharmacology & Toxicology
University of Nairobi.
P.O. Box 29053
Nairobi.

TITLE OF THE RESEARCH: SERO-PREVALENCE ESTIMATES OF BRUCELLOSIS AND COXIELLOSIS IN CATTLE, SHEEP AND GOATS; ASSOCIATED RISK FACTORS AND PERCEPTION IN LIVESTOCK FARMERS IN NANDI COUNTY, KENYA.

Purpose and background

The general purpose of this study is to investigate the seroprevalence of brucellosis and Coxiellosis in cattle, sheep and goats; associated risk factors and perception in livestock farmers in Nandi County, Kenya. This will be through collection of blood samples from cattle, sheep and goats and administration of questionnaires to the farmers. The findings will provide information on the burden and extent of brucellosis and coxiellosis diseases in the County that will guide future appropriate control strategies. This will also allow the farmers in the County to exploit its competitive advantage in dairy production for their socio- economic development.

Procedure- People aged 18 and above will be interviewed on issues that relates to Brucellosis and coxiellosis. Blood samples will be collected from animals over one year for testing in the laboratory.

Benefits and risks- There will be no direct benefit for those participating in the study. There will be no risk too to the participants since laboratory technicians involved in blood collection are qualified and will adhere to bio-security measures.

Confidentiality- All information given in the study will be considered confidential and will be used only for the purpose of this study.

Voluntary participation- The participation in the study is voluntary and participants are free to accept or refuse to take part in the study and are also free to withdraw at any time.

CONSENT

I have read/heard the nature of the study and voluntarily agree to participate in the study.

Signature of the respondent: _____

Date: _____

Signature of the researcher: _____

Date: : _____

APPENDIX 3

Approval of Proposal by Biosafety, Animal use and Ethics committee.



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Dr Jerono Kiptanui
c/o Dept of PHP&T.

REF:FVM BAUEC/2018/150

Dear Dr Kiptanui

12/02/2018

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Sero-Prevalence of Brucellosis in Livestock and species biotyping; risk factors and perception among livestock farmers in Nandi County, Kenya

By Jerono Kiptanui (J87/5188/2017)

We refer to the above proposal that you re-submitted to our committee for review and approval. We have now reviewed the proposal and are generally satisfied that you addressed the issues we had raised in our letter to you dated 05/02/2018. This concerned Occupational health, biosafety and creating appropriate community awareness. We hereby approve your work as per the proposal you have re-submitted.

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine