PREVALENCE OF ANAPLASMA AND BABESIA INFECTIONS AND THEIR ASSOCIATED RISK FACTORS AMONG CALVES IN NAROK COUNTY, KENYA

A RESEARCH PROJECT THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF VETERINARY MEDICINE (MVetMed) DEGREE OF THE UNIVERSITY OF NAIROBI.

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J56/41561/2021 (BVM)

DEPARTMENT OF CLINICAL STUDIES

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

NOVEMBER, 2023

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DEDICATION

I dedicate my research project to my family and the livestock keepers in Narok County.

ACKNOWLEDGEMENTS

My Master's degree journey has only been made possible through God. I am grateful to Him for His mercy, faithfulness and grace.

I am greatly indebted to Prof. George Gitau, the Lead Supervisor, for linking me and supporting my research project through the Animal Health Innovation Laboratory-Feed the Future Programme, his countless hours of supervision while I was writing the thesis as well as his guidance throughout the entire process. I am filled with gratitude to Dr. Shepelo Getrude, for her prompt feedback and valuable time which made this research work a success. I am grateful to Dr. Daniel Muasya, for his willingness and committed support during data processing and analysis. I have received continuous support from all the members of Department of Clinical Studies and Faculty of Veterinary Medicine and for this, I am forever grateful.

Great appreciation goes to Animal Health Innovation Laboratory-Feed the Future for providing the fieldwork research support that enabled me to successfully complete my Master's program. Finally, I would like to thank the research team, Dr. Ng'etich Wycliff, Jeremiah Leyamyam, John Parkasio and Alex Otuni for their great support during the field work part of the research.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ELISA - Enzyme-Linked Immunosorbent Assay

GDP – Gross Domestic Product

TBDs – Tick Borne Diseases

ECF – East Coast Fever

SDG – Sustainable Development Goals

iNOS - Inducible Nitric Oxide Synthase

IFN – Interferon

USD – United States Dollar

FAO – Food and Agricultural Organization

GoK – Government of Kenya

RVF – Rift Valley Fever

LSD – Lumpy Skin Disease

AHS – Animal Health Specialist

BCS – Body Condition Score

ABSTRACT

Babesiosis and Anaplasmosis represent major constraints to livestock production in many developing countries. Both *Babesia* and *Anaplasma* species are transmitted by ticks. However, *Anaplasma* species can be transmitted by hematophagous arthropods or through fomites contaminated by *Anaplasma* species infected blood. Despite their economic impact, knowledge of their epidemiology is limited. Therefore, this study was aimed at estimating the seroprevalence of *Babesia bigemina (B. bigemina)* and *Anaplasma marginale (A. marginale)* infections and their associated risk factors among calves aged between 3 months and 12 months in Narok County, Kenya.

A cross-sectional study was undertaken in Narok County, Kenya, between February and May 2023. A total of 402 calves from 76 farms were randomly selected from 8 villages in two Sub-Counties of Naroosura Majimoto and Ololulunga. Data on individual calf and farm factors was collected via close-ended questionnaires administered to the owner or someone who was in charge of taking care of the calves. Serum and whole blood were collected from the calves for microscopy and serology, respectively. Microscopy was conducted on the blood smears stained with Giemsa to screen for the hemoparasites. A commercial enzyme-linked immunosorbent assay (ELISA) was used to estimate antibodies against *B. bigemina* and *A. marginale* infections using monoclonal antibodies based on an indirect competitive inhibition principle. Descriptive analysis was performed for both categorical and continuous variables. Mixed effect logistic regression analysis was used to determine the relationship between seropositivity and calf and farm level factors, with the random effect being the farm. The final model was assessed for fitness using Pearson chi square, deviance chi square, Hosmer-Lemeshow test and predictive ability of the model.

The seropositivity of *B. bigemina*, *A. marginale* and coinfections of *B. bigemina* and *A. marginale* was 60% (241/402), 60% (241/402) and 38.1% (153/402), respectively. The positive samples on microscopy for *Babesia bigemina, Anaplasma marginale* and coinfections of *B. bigemina* and *A. marginale* were 22.9% (92/402), 32.6% (131/402) and 11.4% (46/402), respectively. Factors significantly associated ($p \leq 0.05$) with the seropositivity included: increase in age (for *B. bigemina* ($p = 0.007$)*, A. marginale* ($p < 0.000$) and *B. bigemina* and *A. marginale* coinfections ($p = 0.019$), calves that receive acaricide treatment (for *A. marginale* ($p = 0.001$) and *B. bigemina* and *A. marginale* coinfections ($p = 0.007$)), purchasing of feed (for *B. bigemina* ($p = 0.009$) and *B. bigemina* and *A. marginale* coinfections ($p = 0.001$)), formal education for farm head (for *B. bigemina* ($p = 0.012$)), infection history on the farm (for *B. bigemina* and *A. marginale* coinfections ($p = 0.001$)), fever (for *B. bigemina* and *A. marginale* coinfections ($p = 0.028$)) and vaccinated calves (for *A. marginale* ($p = 0.034$)).

There is a relatively high seroprevalence of *Babesia bigemina* and *Anaplasma marginale* infections in the study population and particularly in apparently healthy calves. Strategic acaricide application should be explored further with establishing and maintaining endemic stability in mind to reduce the risk of clinical diseases from both infections.

Key words: *Babesia bigemina, Anaplasma marginale*, seroprevalence, calves, risk factors

CHAPTER ONE: INTRODUCTION

1.1 Background information

Kenya is a middle-income country with an economy where agriculture contributes about 37% of Kenya's GDP and employs three-quarters of the labor force (FAO, 2020). The vibrant livestock sector accounts for about 12% of Kenya's GDP (Behnke and Muthami, 2011). The livestock industry, therefore, indisputably contributes significantly to attaining food and nutritional security by supplying animal protein, economy, wealth, and livelihoods of the people in Kenya through traction power, hides, fuel, and fertilizer (Perry, 2016; Mukolwe, 2018; Waha *et al*., 2018).

Several factors constrain the health and productivity of livestock worldwide (Leta and Mesele, 2014; Dabasa *et al*., 2017), including poor management practices, a lack of appropriate extension services, scarce land, inadequate allocation resources , inadequate disease control policies, climate change that leads to low feed and water sources, poor market access and unreliable feed availability (Mukolwe, 2018). Diseases in cattle markedly constrain beef and dairy production worldwide (Emongor *et al*., 2000). Amongst these diseases, tick infestation and vector-borne infections, including theileriosis, babesiosis, and anaplasmosis (Emongor *et al*., 2000), have significant epidemiological, economic, and social impacts (Bock *et al*., 2004), especially in the tropics and subtropics, affecting approximately 80% of the world's cattle population.

Tick attachment and bites in heavily infested animals result in a negative economic effect on production and livelihood (Rodríguez-Vivas *et al*., 2017). Mastitis can be caused when tick bites on teat(s) and become secondarily infected with bacteria (Abbas *et al*., 2014; Vudriko *et al*., 2016). Other direct impacts of tick infestation include irritation and chronic stress, which alter the animals' behavior and lead to immunosuppression, loss of energy (de Castro, 1997; Abbas *et al*.,

2014), anemia due to excessive blood loss, and tick paralysis. Tick infestation causes indirect losses that emanate from the cost of tick control and treatment for clinical cases, lost potential due to maintaining less productive tick-susceptible breeds, tick-transmitted pathogen impact (Alim *et al*., 2012; de Castro, 1997), trade restrictions on livestock products and acaricide-contaminated animal products (Kariuki *et al*., 1995; Kivaria, 2006).

In East Africa, East Coast fever (ECF), babesiosis, and anaplasmosis have been reported as two of the most profit-hindering, important cattle diseases (Ocaido *et al*., 2009; Chenyambuga *et al*., 2010; Onono et al., 2013). According to Wesonga *et al*. (2017), the leading bovine vector-borne infections in Kenya, based on the economic losses exerted on farmers, include diseases caused by *Anaplasma marginale* (*A. centrale* having fewer reports) (Shepelo, 2020), *Babesia bigemina* and *Babesia bovis* (Githaka *et al*., 2022). In Kenya, previous reports have indicated extensive losses of up to 30 billion Kenyan shillings per year (Gitau *et al*., 1999; Maloo *et al*., 2001; Muraguri *et al*., 2005; Wesonga *et al*., 2010; Kiara *et al*., 2014; Kanduma, 2018).

Interactions that involve the environment, etiological agents, vertebrate hosts, and tick vectors reflect vector-borne disease occurrence and their importance (Norval *et al*.,1992). Vector-borne diseases dynamics are dependent on vectors' population density which in turn depends on the susceptibility of a host (Kocan *et al*., 2010), vector transmission capability, production systems and management practices, suitability patterns of temporal-spatial habitat (Gachohi *et al*., 2010), grazing management practices, availability and efficiency of veterinary infrastructure and resources, climate change, soaring rise of human population and land use patterns (Perry and Young, 1995; Gachohi *et al*., 2010; Keesing and Ostfeld, 2018). Therefore, this study aimed to estimate the prevalence of *Anaplasma* and *Babesia* infections and determine their associated risk factors among calves in Narok County, Kenya.

1.2 Problem statement

Anaplasmosis and babesiosis threaten the development and exploitation of livestock resources in great measure in southern, central, and eastern Africa (Perry, 2016) as exotic and cross-bred cattle are more susceptible to these vector-borne diseases (Gachohi *et al*., 2012) in what is generally referred to as "lost potential." These vector-borne diseases cause significant economic and production losses associated with reduced milk and meat production, morbidity, mortality, and loss of draft power (Mureithi and Mukiria, 2015; Kanduma, 2018), eventually leading to hunger and poverty (Okuthe and Buyu, 2006). These diseases also cause indirect losses through costly control measures that include treatment and acaricide use, loss of cash income, and reduced market access (Minjauw and McLeod, 2003; Homewood *et al*., 2006; Kivaria, 2006; Gachohi *et al*., 2012). In Uganda, according to Magona (2004), the overall loss of the calf crop due to these vector-borne diseases is projected to be 11% with anaplasmosis and 4.4% with babesiosis.

In developing countries, livestock-dependent small-scale households are the most affected by the socio-economic effects of vector-borne infections (Minjauw and McLeod, 2003). These vector-borne diseases such as, *Babesia divergens* and *Anaplasma platys* have zoonotic potential (Beattie *et al*., 2002). The increasing vaccination against *Theileria parva* infections (Gachohi *et al*., 2012) may lead to farmers' relaxation in tick control, which translates to an increase in babesiosis and anaplasmosis cases.

The current vector-borne disease control methods need to be improved as they have many essential limitations, including increased acaricide resistance (Magona *et al*., 2008; Rosario-Cruz *et al*., 2009) and the current disadvantages of live vaccines (De Vos and Bock, 2000; De Waal and Combrink, 2006; Kocan et al., 2010). In Kenya, as an example, the tick control advisory and

monitoring role was left to the livestock owners instead of the Directorate of Veterinary Services (Mutavi *et al*., 2018).

1.3 Justification

There needs to be more precise and appropriate information on the epidemiological profile of these vector-borne infections, including precise information on their socioeconomic impact (Pegram *et al*., 1989; Mukhebi *et al*., 1992). In most cases, there has been a disconnect between control efforts and the immensity of vector-borne infections (Norval *et al*., 1992; Perry, 1994). This is because earlier studies to determine the presence and magnitude of vector-borne diseases lacked production system or location specificity (Amir and Knipscheer, 1989; Miyama, 2020).

The need for more and updated details also widely constrains the development of improved control tools, particularly in Narok County, Kenya. Narok County is known to have large populations of cattle that freely move from one point to another particularly during long periods of drought. This movement also leads to interaction with wildlife due to sharing of forage and water source, creating a possibility of contact with tick vector and tick-borne diseases. Narok County is also a transboundary county famous for animal trade and market which is a risk factor for transmission of ticks and tick-borne diseases. The climate of Narok County is also suitable for the survival and reproduction of ticks vectors for anaplasmosis and babesiosis.

In recent years, a further shift in the epidemiology of vector-borne diseases may have occurred due to human activities such as transboundary animal trade, nomadic pastoralism, deforestation and agricultural intensification, and recent climatic changes (Githaka *et al*., 2021). To be able to develop sustainable mitigation efforts for vector-borne infections, a greater comprehension of the

patterns of vector-borne disease in a changing climate is a requisite (Baylis and Githeko, 2006; Van den Bossche and Coetzer, 2008; Thornton *et al*., 2009).

Therefore, studying the epidemiology patterns of *Anaplasma* and *Babesia* infections in calves in Narok County - Kenya will be crucial in elucidating the disease burden in livestock production and communities. The information on prevalence and associated risk factors may provide biological evidence for control strategies such as no intervention or dipping, innate resistance exploitation, or immunization (Norval *et al*., 1992; Perry and Young, 1995; Jonsson *et al.,* 2012). The information gathered from this study may be used generate hypothesis that often pave the way for other investigations conceiving longitudinal studies. This will increase livestock productivity from healthy cattle, thereby potentiating livestock's contribution to national GDP (Peter *et al*., 2020). Control strategies recommended from the findings of this study could positively impact on animal health, production and livelihoods value chains as envisaged in the Sustainable Development Goals (SDG).

1.4 Objectives

1.4.1 General objective

The general objective of the study was to estimate the prevalence of *Anaplasma* and *Babesia* infections and determine associated risk factors among calves in Narok County, Kenya.

1.4.2 Specific objectives

1. To estimate the prevalence of *Anaplasma* and *Babesia* infections among calves aged 3 – 12 months in Narok County, Kenya.
2. To determine the risk factors associated with *Anaplasma* and *Babesia* infections among calves aged 3 – 12 months in Narok County, Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Aetiology of bovine anaplasmosis and babesiosis

2.1.1 Aetiology, pathogenesis, clinical signs and pathology of babesiosis

Apicomplexan, intraerythrocytic protozoan, *Babesia bovis* and *Babesia bigemina* (*B. bigemina*) (Bock *et al.,* 2004; Wesonga *et al*., 2010), cause babesiosis, a severe and often fatal cattle disease, occurring throughout Southern Europe, Asia, Africa, South and Central America and Australia, (Bock *et al*., 2004). *Babesia divergens* has zoonotic potential and occurs mostly in Europe due to its tick vector, *Ixodes ricinus*, having limited distribution (Beugnet and Moreau, 2015; Rożej-Bielicka *et al*., 2015). *Babesia bigemina* and *B. bovis* are vectored by *Rhiphicephalus (Boophilus) decolaratus* and *Rhiphicephalus (Boophilus) microplus*, respectively hence these ticks determine the *Babesia* species geographical distribution (Chauvin *et al*., 2009).

Severe and sudden anaemia, jaundice and death result from rapid, sometimes massive, intravascular hemolysis causing more consistently and earlier haemoglobinuria than in *B. bovis* infection (Callow, 1984; Callow *et al*., 1986; Suarez and Noh, 2011; Tayebwa *et al*., 2018). The clinical symptoms seen in acute *B. bovis* infections such as hypotension, cytoadherence, cerebral involvement and coagulation disorders are not seen with infections of *B. bigemina* (Wright *et al*., 1988; Böse *et al*., 1995). *B. bigemina* acute cases are less severe than those of *B. bovis* infections. Complete and rapid recovery occurs in non-fatal cases. For 4 to 7 weeks, recovered animals can still infect ticks (Mahoney and Goodger, 1969; Johnston *et al*., 1978). For a few months, the recovered animals remain as carriers (Mahoney and Goodger, 1969; Johnston *et al*., 1978).

2.1.2 Aetiology, pathogenesis, clinical signs and pathology of anaplasmosis

Anaplasma marginale (*A. marginale*) is the major etiological agent of babesiosis (De Vos *et al*., 2004; Ngeranwa *et al*., 2008) in cattle, obligate intraerythrocytic rickettsiae, Order Rickettsiales, phylum Proteobacteria (Jabbar *et al*., 2015). Bovine anaplasmosis occurs simultaneously with their vectors, which finds optimal conditions for survival in regions with temperate winters particularly in tropical and subtropical areas (McCosker, 1981). The acute phase of the infection sees a rise in the levels of parasitemia (Kieser *et al*., 1990). Anaplasmosis results in an extravascular hemolytic disease in cattle as reticuloendothelial cells extensively phagocytize infected erythrocytes initiated by anti-erythrocytic antibodies and parasite-induced damage of red blood cells (DeVos *et al*., 2006) resulting in anemic and icteric conditions (Kocan *et al*., 2003) hence some of the clinical signs observed including: fever, dyspnea, anemia, jaundice, weakness, drop in production of milk, depression, constipation, loss of appetite, rapid body condition loss, dehydration, and often death (Richey, 1991; De-Whittier *et al*., 2007). Fetal death and abortion result from oxygen deprivation (De-Whittier *et al*., 2007).

Haemoglobinuria and haemoglobinaemia are not features accompanying extravascular hemolytic anemia (Rymaszewska and Grenda, 2008) due to the occurrence of extravascular erythrophagocytosis. The presence of bile pigments often makes urine dark brown in severely affected animals. Anemia creates hypoxic conditions that lead to degenerative changes in different organs. Phagocytic cells in the spleen excessively destroy infected erythrocytes leading to splenomegaly. The packed cell volume, hemoglobin values and erythrocyte count reduce markedly resulting in death due to severe anemia (De-Whittier *et al*., 2007).

2.2 General epidemiologic patterns of bovine anaplasmosis and babesiosis

Anaplasmosis and babesiosis have been potentially associated with the following factors: acaricide use frequency (Wesonga *et al*., 2017; Miyama, 2020), livestock production system (Gachohi *et al*., 2012), agro-ecological zone, age, breed, tick infestation (Gachohi *et al*., 2010; Byaruhanga *et al*., 2016; Kerario *et al*., 2017; Wesonga *et al*., 2017; Chiuya *et al.,* 2021), cattle inherent resistance to ticks and TBD (Shyma et al., 2013; Jonsson *et al*., 2014; Laisser *et al*., 2016; Robbertse *et al*., 2017), and vector tick distribution and infection rate in ticks (Norval *et al*., 1992).

2.2.1 Climate and seasonality

Particular tick species such as *Rhipicephalus* species, survival and development is better in lowlands than highlands, hence the role of climate suitability for the vector in agroecological zones (Rubaire-Akiiki *et al*., 2004; Gachohi *et al*., 2012). A blend of grass and tree cover provides a warm and humid area suitable for better tick reproduction and survival (Gachohi *et al*., 2012). However, other studies have reported that it is during the rainy seasons that tick populations are high (Kabi *et al*., 2014; Chenyambuga *et al*., 2010). Notably, Otim (2000) and Pegram (1986) reported ticks in particular *Rhipicephalus evertsi* lack seasonal peaks hence present throughout the year. *Amblyomma variegatum*, *R. decolaratus* and *Rhipicephalus appendiculatus* are widespread throughout the continent and lack seasonal variation in abundance (Kaiser *et al*., 1982).

Abundance of tick population in vegetation layers is regulated by microclimate (Childs & Paddock, 2003). Activity of ticks during different periods of the year is regulated by weather (Childs & Paddock, 2003). For the questing or molting stages, the long periods of high temperatures and high air desiccating power in the summer in temperate areas, lead to high mortality rates (Childs and Paddock, 2003). Therefore, climatic and environmental conditions greatly determine tick borne diseases prevalence (Duguma *et al*., 2012).

Movement of animals during dry seasons leads to exposure to heavy tick infestations due to communal watering and feeding points (Mugisha *et al*., 2008; Byaruhanga *et al*., 2018). In addition to that, ticks absorb atmospheric moisture, which helps maintain their water balance that allows them to survive long periods of starvation (Jongejan and Uilenberg, 1994). An abundance of hematophagous arthropods such as mosquitoes, *Culicoides, Stomoxys calcitrans,* and *Tabanus* is seen in wet seasons, which leads to higher occurrence of bovine anaplasmosis as compared to babesiosis (Ssenyonga *et al*., 1992; Byaruhanga *et al*., 2018).

2.2.2 Production systems and grazing systems

Cattle exposure to ticks is determined by livestock production and grazing systems (Gachohi *et al*., 2012). In extensive grazing system, practiced mainly by pastoralists, would have cattle constantly exposed to ticks with little or no acaricide application and an increased likelihood of developing endemic stability to vector-borne diseases (Homewood *et al*., 2006; Kipronoh *et al*., 2011; Byaruhanga *et al*., 2015). Such a production system sees a high population of ticks on cattle due to favorable climatic conditions and communal grazing, a greater number of vector-borne infections occur in such production systems (Rubaire-Akiiki *et al*., 2004). The animal movement in this production system favors ticks and vector-borne infections, spreading them to confined animals and pasture (Billiouw and Berkvens, 1999; Maloo *et al*., 2001; Muhanguzi *et al*., 2010).

2.2.3 Breed resistance

Studying the mechanisms of resistance to ticks among different breeds of cattle may contribute to the development of alternative control methods (Gasparin et al., 2007). Exotic cattle are more susceptible to ticks and the microorganisms they carry compared to indigenous cattle (Kabi *et al*., 2008), although the genetically determined factors involved have not been determined. This phenomenon is thought to be a result of the evolutionary relationship between *Bos indicus* cattle,

Boophilus spp. and *Babesia* (Dalgliesh, 1993). The phenomena of host resistance to ticks and enzootic stability to tick borne diseases are well documented (Latif & Pegram, 1992; Perry et al., 1985). Bonsma, 1980, through his research suggested that Zebu cattle were resistant to or repel ticks due to their phenotypic characteristics that include skin thickness, hair density, coat type and skin secretions (Muhammad *et al.,* 2008).

2.2.4 Age- related immunity

Anaplasma and *Babesia* infections are more severe in animals aged two years and older demonstrating an inverse age immunity (Riek, 1963; Jonsson *et al*., 2012). During the first 2 months of life, calves (upto 12 months) depend on passively acquired resistance from colostrum (Mahoney and Ross, 1972; Mahoney *et al*., 1973). For the next 3 to 9 months, it shifts to innate immunity (Mahoney and Ross, 1972; Mahoney et al., 1973). Strong, long-lasting immunity develops if calves have *Babesia* infection within the first 6 - 9 months; calves hardly come down with clinical disease (Trueman and Blight, 1978; Dalgliesh, 1993; Goff *et al*., 2001; Zintl *et al*., 2005) compared to adults. As a result, the high level of immunity protects calves in their adult life, from developing clinical disease and for the few infections, a low number of deaths (Perry and Young, 1995; Jonsson *et al*., 2012; Gachohi *et al*., 2013).

Goff *et al*. (2001), suggested that natural immunity involves inducible nitric oxide synthase (iNOS) mRNA expression and early induction of interferon (IFN)-c and interleukin (IL)-12 and not purely passive acquired immunity. In adult cattle, iNOS is not induced and there is late induction of IFN-c mRNA and IL-12 (Goff *et al*., 2001).

2.2.5 Tick infestation on cattle

Anaplasmosis and babesiosis epidemiology is determined by their vectors, ticks and their seasonal occurrences (Norval *et al*., 1992). It was observed that *Rhipicephalus appendiculatus* and

Amblyomma variegatum had no seasonal pattern of incidence, but the density of *Boophilus decoloratus* vary regularly with a frequency of approximately three months (Kaiser *et al*., 1982). The vegetation cover, for example, will increase *R. appendiculatus* density (Smith, 1969b, 1969a). Information on resistance estimations for different hosts, disease transmission dynamics and tick population dynamics can be derived from tick population on cattle (Norval *et al*., 1992). Interactions of numerous factors such as climatic conditions, management practices, and host diversity and resistance (Dipeolu, 1989; Dipeolu & Amoo, 1984; Punyua & Hassan, 1992; Rechav, 1982) cause variations in tick abundances within ecological zones and habitats and within seasons and years (Norval and Lightfoot, 1982).

2.2.6 Endemic stability

An endemic stability state denotes an ecological balance between host (e.g., zebus cattle can develop immunity against the hemoparasites rapidly and effectively), tick (regular exposure and transmission to the host population), parasite, and environment (suitable ecological factors for the vectors); such that there is rare or no clinical disease (Perry, 1996; Bock *et al*., 2004). Cattle raised in areas with "sufficient" tick challenge and becoming infected early develop pre-immunity which is important for babesiosis endemic stability. Calves should have the appropriate level of challenge from a "sufficient" number of infected ticks (thus regularly boosting immunity). This will ensure the calves are exposed to the pathogen before the weaning off of innate and colostral immunity; subsequently, clinical disease incidence in adults, is low (Norval *et al*., 1992; Jonsson *et al*., 2012). In an endemic instability state, animals, depending on the breed, can develop life-threatening clinical diseases because of the failure of infection for a considerable period after birth (Callow, 1984).

Management practices including inconsistent tick control programs and climate variations may affect animals exposure to infection, limiting the establishment of endemic stability (Jonsson *et al*., 2012). Moreover, immunity against immunologically diverse heterologous strains may be lacking in seropositive animals (Jonsson *et al*., 2012). Hosts' genetic and immunological heterogeneity influences their susceptibility to disease (Jonsson *et al*., 2012).

Both cellular immunity and antibody-mediated immunity are involved in the response to *Anaplasma* infections (Jonsson *et al*., 2012). Pre-immunity does not appear in this immune response (Jonsson *et al*., 2012). *A. marginale* infection's endemic stability is favored by the additional transmission dynamics (Jonsson *et al*., 2012).

2.3 Transmission of bovine anaplasmosis and babesiosis

2.3.1 Ticks involved in transmission

B. bigemina is mainly vectored by *R. decoloratus* (Okon *et al*., 2011). *Rhipicephalus evertsi* and *R. decoloratus* vectors in Africa lead to *B. bigemina* infections to be more common compared to *B. bovis* infections (Friedhoff, 2018). *Babesia* species are transmitted transovarially (Riek, 1966; Mehlhorn and Schein, 1985; Hunfeld *et al*., 2008; Chauvin *et al*., 2009). Vector ticks for anaplasmosis include: *Hyalomma (H. excavatum)*, *Rhipicephalus (Boophilus) species (R. annulatus, R. decolaratus, R. microplus, R. bursa, R. simus), Ixodes ricinus, Ornithodoros lahorensis* and *Dermacentor (D. occidentalis, D. variabilis, D. andersoni, D. albipictus* and *D. hunteri*) (Aubry and Geale, 2011). Fomites contaminated with infected blood including dehorning saw, ear-tagging devices, tattooing instruments, needles, nose tongs, vaccination and treatment needles and castration instruments, have been implicated in mechanical transmission (Aubry and Geale, 2011). Tick vectors in South and Central America and Africa are absent; hence, transmission is primarily mechanical (Ewing, 1981) via hematophagous arthropods, *Stomoxys*, *Tabanus,* and

mosquitoes (Potgieter et al., 1981; Dreher *et al*., 2005; Kocan *et al*., 2010). Life-long carriers represent cattle that survived an acute disease and become reservoirs of infection (Aubry and Geale, 2011). Transplacental transmission during gestation from cow to calf can occur in *A. marginale* (Zaugg and Kuttler, 1984; Costa *et al*., 2016).

2.3.2 Tick biology as principal vector for tick-borne diseases

Several tick characteristics have been identified that enable them to adapted to their parasitism including highly sclerotized bodies, high reproductive potential, multi-year life cycles, blood sucking habit, engorgement habit, longevity, long starvation tolerance and relative freedom from natural enemies (Estrada-Peña *et al*., 2013).

Ticks use their chelicerae to cut through the skin of the host during feeding, then to ensure anchoring insert their barbed hypostome (Mandal, 2006). To reinforce this attachment, they then secrete saliva that has proteinaceous cement-like substance that has both host and tick derived biomolecules (Pacheco *et al*., 2021; Villar *et al*., 2020). This activity enables the ticks to remain attached to the host and feed for long durations (Mandal, 2006). Biologically potent substances in the saliva of the tick regulate process in the immune response in the host including vascular permeability, coagulation, vasodilation and cytolytic activity (Mandal, 2006). This acute inflammation that results from tick attachment provides continuous blood flowing into the feeding lesion hence feeding for ticks *(*Anderson *et al*., 2017; Villar *et al*., 2020). Despite their relatively small body, ticks are able to take in large volumes of blood by removing the excess water from blood (Desta, 2016). Pathogens vectored by ticks escape digestion in the gut of ticks due to the slow intracellular blood digestion (Mullen & Durden, 2009).

2.4 Diagnosis of bovine anaplasmosis and babesiosis

2.4.1 Thin blood smears microscopy

It is routinely the easiest way to detect parasites in the blood stream. Thin Giemsa-stained blood smear examined under a microscope can be used to demonstrate intraerythrocytic parasites such as *Babesia* as piroplasms and *Anaplasma* spp. as inclusion-bodies particularly during the acute phase of the diseases. In carrier animals, convalescent stages or the pre-clinical stage, the parasitaemia is low making the sensitivity of microscopy low (Figueroa *et al*., 1993). With microscopy, closely related species or identification upto species level is difficult; take *B. bigemina* and *B. bovis* as examples (Callow, 1984; de Vos *et al*., 1994). *B. bigemina* parasites appear as pear shaped forms in acute angle or irregularly elongated shape of up to 5 µm in length or relatively large round merozoite of 2.3 µm in size (Laha *et al*., 2015). On the other hand, *B. bovis* parasites appear as vacuolated ring forms or centrally-placed paired forms of 1.5–2 µm in size and often in obtuse angle (Laha *et al*., 2015).

2.4.2 Serological tests

Serological tests are used for export certification, epidemiological studies or research purposes. In cattle reared in babesiosis endemic areas parasites are present in the blood stream at very low numbers, below the threshold of direct detection techniques (Alvarez *et al*., 2019). These tests have limitations when differentiating between previous exposures in carrier animals and current infections as antibodies usually persist by variable periods of time even in *B. bovis*, *B. bigemina* cleared animals (Alvarez et al., 2019). These tests have been reported to have cross-reactivity of antibodies because antigenic similarity can lower the specificity hence limiting pathogen identification (Kocan *et al*., 2000; García-Sanmartín *et al*., 2006; Molad *et al*., 2006). Findings of high antibody titers are translated with caution as such does not prove protective immunity or parasite infection (Holman et al., 2005). Moreover, protected animals by sterile immunity are false

negatives, which is a phenomenon seen also in animals that have circulating parasites (Holman *et al*., 2005).

The tests previously described for detecting *Anaplasma* infections include: competitive ELISA (cELISA) (de Echaide *et al*., 2001; Strik *et al*., 2007); card agglutination test (Molloy et al., 1999); Compliment Fixation Test (Bradway et al., 2001; Coetzee et al., 2007); indirect ELISA (iELISA) (Uzal *et al*., 1996; Strik *et al*., 2007); dot ELISA (Montenegro-James *et al*., 1988); indirect immunofluorescence antibody technique (IFAT) (Goff *et al*., 1985; (OIE, 2008a) and Lateral Flow assay (Nielsen *et al*., 2007).

Serological tests previously described for Babesia infections include: Immunochromatography Test (ICT) (Guswanto *et al*., 2017), Indirect fluorescent-antibody test (Marana *et al*., 2009; Romero-Salas *et al*., 2016), Hemagglutination, Latex agglutination, Indirect immunofluorescence assay (de Souza *et al*., 2001; Marana et al., 2009), Rapid coagulation test, Test card, ELISA (Terkawi *et al*., 2011; Guswanto *et al*., 2017), Competitive ELISA (Marana *et al*., 2006), Indirect ELISA, Complement Fixation Test (Marana *et al*., 2006) and Fluoresent Antibody Test (Ross and Löhr, 1968).

2.4.3 Molecular tests

These methods have various advantages including: ability to differentiate between morphologically similar parasites, the ability to detect infection in the disease's latent phase and not affected by the immunity status of the animal (Kocan *et al*., 2010; Mans *et al*., 2015).

Several molecular tests have been used to detect *Babesia* species detection in ticks and blood including: reverse line blot hybridization assay (Gubbels *et al*., 1999; Brıgido *et al*., 2004; Oura *et al*., 2004), Loop-Mediated Isothermal Amplification Assay (Iseki *et al*., 2007) and nested PCR (Figueroa *et al*., 1993; Almería *et al*., 2001; Oliveira-Sequeira *et al*., 2005).

Molecular tests previously described for *Anaplasma* include: conventional PCR (Lew *et al*., 2002), nested PCR (Jilintai *et al*., 2009; Singh *et al*., 2012; Jaswal *et al*., 2014), real time PCR (Löhr *et al*., 2002; Futse *et al*., 2003; Courtney *et al*., 2004; Carelli *et al*., 2007; Reinbold *et al*., 2010) and Reverse Line Blot (Gubbels *et al*., 1999; Georges *et al*., 2001).

2.4.4 Animal inoculation

A splenectomized calf is inoculated with the suspect animal's blood. *A. marginale* parasites will be observed within 4-8 weeks, in blood smears from calves that have been splenectomized if the donor is infected (Coetzee *et al*., 2006). This is not a feasible method as the calves that have been splenectomized become infected after the sub inoculation of blood infected with *A. marginale* parasites hence, raising welfare issues. It is also a costly method as it can involve euthanasia (OIE, 2008).

2.5 Treatment of bovine anaplasmosis and babesiosis

Bovine babesiosis chemotherapy includes diminazene aceturate, given intramuscularly at a dosage rate of 3.5mg/kg, has rapid action and is well tolerated with protection of 2 and 4 weeks for *Babesia bovis* and *Babesia bigemina* respectively (Vial and Gorenflot, 2006), and imidocarb dipropionate, given subcutaneously at 3 mg/Kg and 1.2 mg/Kg for treatment of *B. bigemina* for 2 months and *B. bovis* for protection for 4 weeks (Taylor, 1979).

Babesia bigemina and *B. bovis* infection in carrier animals can be eliminated using imidocarb at a high dose; this dose, following live vaccination, will interfere with immunity development (De Vos *et al*., 1986). The use of long-acting oxytetracyclines does not interfere with immunity development but is still able to reduce parasitaemia and erythrocytes destruction (Jorgensen *et al*., 1993).

Anaplasmosis treatment includes the parenteral administration of imidocarb dipropionate and tetracyclines (Aubry and Geale, 2011). *A. marginale* from carrier animals can be eliminated by imidocarb (Otim, 2000). Supportive treatment, such as blood transfusion; administration of vitamins (B complex), anti-inflammatory drugs and fluid replacements, which aid erythropoiesis, may be necessary in severely affected animals in both cases of the diseases (Mosqueda *et al*., 2012).

2.5 Prevention and control of bovine anaplasmosis and babesiosis

2.5.1 Integrated control

Currently in Kenya, integrated control approaches are used and they include the strategic use of acaricides and/or tick vaccines, as well as safe antiprotozoal and antibacterial drugs for treatment and/or prophylaxis of bovine anaplasmosis and babesiosis, enzootic stability exploitation, the production of tick- and Babesia resistant cattle breeds, and the application of vaccines in cases of enzootic instability (Muhammad *et al*., 2008).

2.5.2 Vector control

Pasture management, manual plucking, antitick vaccines and the most widely used acaricide application either by dipping or spraying have been used to control ticks (de Castro, 1997; Mugisha *et al*., 2008; Chenyambuga *et al*., 2010; Byaruhanga *et al*., 2015; Vudriko *et al*., 2016).

Use of acaricides has several limitations including: acaricide environmental pollution (Parizi *et al*., 2012), milk/meat residues leading to public health concerns (Samish *et al*., 2004), relatively expensive to establish and maintain infrastructure of dip tanks (Vudriko *et al*., 2018), risk of tick populations that are resistant to ticks and consequently ineffective acaricides (Abbas *et al*., 2014; Vudriko *et al*., 2018).

Lack or limited national animal movement control and acaricide policies, as well as farmer-related factors (acaricide overuse and misuse), are key drivers of acaricide resistance (Muhanguzi *et al*., 2020). Super- and multi-acaricide resistant Rhipicephalus spp. ticks in Uganda have been reported by Vudriko *et al*., (2016).

2.5.3 Animal genetics

Tick and the vectored microorganisms resistance has been indicated in local breeds (Chenyambuga *et al*., 2010). Wikel and Whelen (1986) suggested that the resistance could be attributed to an inheritable immunity against ticks. The strategy has been used in Eastern Uganda by over 60% of farmers (Muhanguzi *et al*., 2014; Tayebwa *et al*., 2018).

2.5.4 Pasture management

Tick populations are scaled back by alternating between crop fields and pasture with livestock and combining with acaricide treatment before they enter a new paddock. Some ticks and their free-living larval stage do not survive in pasture kept free of cattle for a season (Young *et al*., 1988).

2.5.5 Exploitation of natural endemic stability

A herd that is constantly exposed to ticks and the associated infections, can develop endemic stability that is protective. However, in endemic areas, management strategies, climate and the host genetic make-up unavoidably affect transmission rate and subsequently, the likelihood of the development of endemic stability. Tick challenge is a prerequisite for the establishment and maintenance of endemic stability. Acaricide use frequency should be limited to when the herd is particularly susceptible or there is a seasonal upsurge in of tick populations. The "sufficient" number of ticks that justifies acaricide use for strategic control and the tick infestations' seasonal variations should be determined. Disease naïve tick-free herds experience high case fatality rates

in cases where tick control is unsettled; hence, it is more sustainable to maintain an endemically stable state (Jonsson *et al*., 2012).

2.5.6 Use of live vaccines

Live vaccines in Australia have been used to control outbreaks in naive herd (Domingos *et al*., 2013). Acaricide is applied on the targeted herd to prevent further exposure (Domingos *et al*., 2013). The *B. bovis* vaccine strain use in Argentina and the G vaccine strain of the *B. bigemina* are not infective for ticks (Bock *et al*., 2004).

Experimentally, G strain of *B. bigemina* and K strain of *B. bovis* from Australia, has shown to offer protection in South Africa (de Vos *et al*., 1982); Sri Lanka (Weilgama, 1986); Bolivia (Callow *et al*., 1976) and Malawi (Lawrence *et al*., 1993) and in many parts of the world, including Islands of the Caribbean, Ecuador and Venezuela in South America, Zimbabwe and Swaziland in Africa and the Philippines and Malaysia in Southeast Asia.

Drawbacks associated with live vaccines include: cold chain system is required, severe reactions, its shelf life is short, a potential spread of the species following vaccination (Bock *et al*., 2004), loss of viability, loss of protection and the potential for reversion to virulence and potential for transmission of a concurrent pathogens (Bock *et al*., 2004).

2.5.7 Anti-tick vaccines

Antitick vaccines against *B*. *microplus* were based on a recombinant antigen, Bm86 (Willadsen *et al*., 1995; De La Fuente *et al*., 1999). García-García *et al*. (2000), in several experiments, were able to prove the efficacy of anti-B. *microplus* is mainly used with acaricides in an integrated approach. Anti-tick vaccines can be effective and are environmentally friendly interventions. Active tick proteins that are immunological are inoculated into a host to stimulate antibody production (Nchu *et al*., 2012). Ticks that feed on the immunized hosts interact with the antigen-

specific antibodies, which then affect the targeted antigen function, providing protection (Merino *et al*., 2011). De la Fuente and Contreras, 2015 have identified and tested various candidate tick-protective antigens in controlled pen trials. Other on-station practices include an anti-tick vaccine based on the identified recombinant Subolesin being conducted in Uganda in collaboration with a Spain-based organization (Kasaija *et al*., 2021). Modifying vaccine formulations and developing multi-epitope-based antigens are other tests conducted by these two groups (Kasaija *et al*., 2021). Strain diversity, antigenic variation, and identification of protective tick antigens are some factors that limit anti-tick vaccine development (Rajput *et al*., 2006). Animal age, adjutant, and instant delivery are some factors that determine protective immune response generation (Rajput *et al*., 2006).

2.6 Economic impact of bovine anaplasmosis and babesiosis

'Tick worry' has cost the Australian cattle industry USD 16.9 million annually based on the tick cost model spreadsheet developed by McLeod and Kristjanson (1999). Control costs and losses associated with *Babesia* and *Anaplasma* infections estimated using the model in Kenya was USD 5.1 million, Zimbabwe was USD 5.4 million, Tanzania was USD 6.8 million, South Africa was USD 21.6 million, China was USD 19.4 million, India was USD 57.2 million, Indonesia was USD 3.1 million, and Philippines was USD 0.6 million annually. In Australia, the losses in matters of money equals about USD 26 million (Sackett *et al*., 2006), while in China and South Africa, the amount equals USD 57.2 million and USD 21.6 million, respectively (Bock *et al*., 2004; Sackett *et al*., 2006).

Bovine babesiosis and anaplasmosis are profit-limiting diseases causing serious production and draft power losses in susceptible cattle through severe frailty, morbidity, and mortality. These

diseases continue to pose a significant threat to the 1- 2 billion cattle worldwide, especially in the presence of vector ticks and hemoparasites (Bock *et al*., 2004).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was conducted on cattle farms in Narok County, Kenya, situated in Rift Valley, the southern part. Between longitude 35° 28′ and 36° 25′ East and latitudes 0° 50′ and 1° 50′ South is where Narok County lies. The average annual rainfall is 500mm to 1800mm and the temperature is between 12℃ and 28℃; lying between 1500-2000 meters above sea level. Food And Agricultural Organisation in 2019 classified Narok County to be in between agro-climatic zones IV and V; the lowlands which experience unreliable and little rainfall, are suitable for pastoralism and animal rearing and wildlife conservation; and the highlands, which experience reliable and sufficient rainfall, hence supporting rain-fed agriculture. There are about 1.5 million cattle; 300,000 heads of both beef and dairy cattle kept on a small scale, almost 1 million goats and a little more than 1 million sheep in the county. Other livestock kept include: bees, donkeys, fish, poultry and donkeys. Narok County engages in pastoralism, mixed farming, marginal mixed farming, ranching and agro-pastoralism (GoK, 2015).

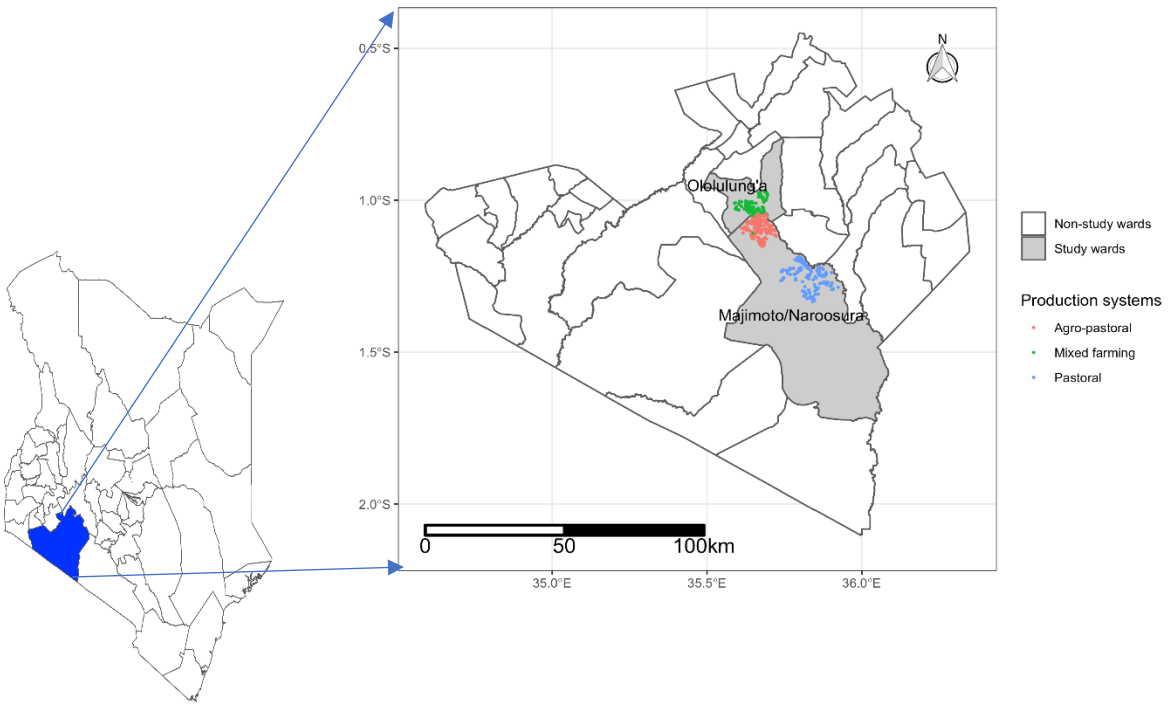

Figure 1: Map of Narok County highlighting the study area – Narok County. The green represents the villages in the mixed production system, the blue represents the villages in the pastoral production system and the red represents villages in agropastoral system. (Image source – Nge'tich *et al*., 2023. Accessed via https://www.eajsti.org/index.php/EAJSTI/article/view/765/234)

3.2 Study design

This was a cross-sectional study conducted between February and May, 2023. Two wards and three sub-locations were purposively selected based on livestock production systems: mixed farming, agropastoral and pastoral. Sampling of the villages, households and calves was done through a randomized process. Households from all livestock households listed in the chosen villages were randomly selected with the assistance of animal health officers, local chiefs, and research assistants in the respective sub-location. Since passively derived colostral antibodies may affect the results

of the study, the age of calves recruited for the study was capped between 3. Therefore calves between 3 to 12 months (Gitau *et al*., 1997; Gachohi *et al*., 2010) were recruited for the study. About 5-10 calves per household were sampled. The County veterinary personnel within the study area and the local chiefs were informed about the aim and protocol of the study. During each farm visit, the study objectives were explained to the farmers, who upon agreeing, signed and dated two consent forms and retained one copy.

3.3 Sample size estimation

The number of calves recruited for the study was computed through the method described by Dohoo *et al*., 2014 and Thrusfield, 2018; $n= [1.96^2 p (1- p)/L^2]$, where n is the estimated size of sample, p is the approximate prevalence of the infection and since the antibody prevalence of the *Anaplasma* and *Babesia species* infection is not known a priori, 50% prevalence is assumed; for a 95% confidence level, 1.96 is the Z value; L, which is the desired precision level, will be set at 5%. The number computed was 384 calves, which was increased to 400 calves in the study to increase the sample size power.

3.4 Data and Sample collection

3.4.1 Farm and animal data

The research team consisted of animal health attendants, laboratory technicians and researchers for the overall sample and data collection. The principal investigator administered a close-ended questionnaire designed in English to the animal owner or person normally in charge of livestock in Swahili or Maasai with the help of a local translator. The questionnaire was designed to collect general farm-level management factors and calf-level factors as well as social demographic information like respondents' gender, household main source of income, highest level of education and experience in cattle keeping. Information relating to the knowledge of ticks and vector-borne

infections was also collected. The farm-level factors include the following: grazing, source and type of fodder, housing practice, herd size, other animals present on the farm, source and introduction process of new animals, frequency of acaricide application, type of acaricide used, method of acaricide application, frequency of veterinary services, herd management related information and other disease control activities like vaccination and deworming. Some of the calf-level factors include breed, age, sex, source of calf (brought-in or homebred), body condition score (a scale of 1-5 visual scoring system using Nicholson and Butterworth, 1986), source of brought-in animals, tick species present and any clinical presentation of infections, at the time of sampling as well as disease history (clinical signs manifested, type of treatment).

3.4.2 Blood collection and serum harvesting

Using a disposable sterile venous blood collection needle, blood was collected using the jugular vein, from each selected and well-restrained calf into 4 ml plain vacutainer tubes (Becton Dickinson Vacutainer Systems, UK) which was used for ELISA tests as well as into 4 ml vacutainer tubes with EDTA, which was used to make blood smears, after disinfecting the collection site using 70% alcohol-soaked cotton wool.

The vacutainer tubes were labelled to indicate a unique individual calf sample number. Ice boxes containing ice packs were used to store the collected blood samples after collection for about 2-5 hours in the field until refrigeration was possible.

Corresponding records for each sampled calf including body condition score, sex, age, live body weight, tick burden including the number and tick type present and breed of the animal as well as location and ownership were recorded against the sample number. Other additional information

including vaccination status, disease history and source of animal (brought in or born in a herd) was recorded.

Later, to separate sera, blood in the plain vacutainer tubes underwent centrifugation for 15 minutes at 4000 times. A pasteur pipette was used to aspirate the sera and put it in 2 ml cryotubes (Greiner Bio One, Germany). The cryotubes were then stored at −20 °C in a freezer until transportation to International Livestock Research Institute (ILRI) where serological analysis was done.

The whole blood in the vacutainer tubes containing EDTA was used to make thin blood smears. Firstly, the blood smear slides were labelled with the corresponding calf identification at the serrated side. The corresponding whole blood was allowed to move up the capillary tube introduced into the vacutainer. A drop of blood was placed on one end of the slide. A second slide edge was lied on the smear slide, pulled until it contacted the drop of blood. The blood was allowed spread across the slide. Once blood had spread along the edge of the second slide, it was pushed away from the drop of blood firmly and swiftly to create a smear occupying three quarter of the slide, thin and with a feathered tail.

Using methanol, the blood smears were fixed for 2 minutes after being air dried and then Giemsa stained for 15 minutes as set out by Afridi *et al*., (2005). The stain that was excess on the slide, was rinsed off with running tap water and the stained smears were allowed to air dry and later put in and transported in slide boxes pending identification of Anaplasma and Babesia parasites in the Department of Clinical Studies laboratory, University of Nairobi. All the equipment used for blood sample collection was put on a disposal waste pin and transported to the Department of Clinical Studies for safe disposal according to the waste disposal policy.

3.5 Laboratory analysis

3.5.1 Microscopic examination of blood smears

On oil immersion under x1000 magnification on the light microscope, the already prepared blood smear slides were screened for *Babesia* species and *Anaplasma* species in red blood cells (Aktas and Özübek, 2017).

3.5.2 Serological testing of samples

Antibodies for *Anaplasma* and *Babesia* species were detected using an indirect enzyme-linked immunosorbent assay as described by Gitau *et al*., 1997 and Katende *et al*., 1998, based on a competitive inhibition principle. The ELISA test plate had 96-well plates for the test sera. Wells are coated with the appropriate antigen to specifically bind antibodies. Prior to binding the antibody from test samples, non-specific sites in the wells and on the antigen are blocked with casein to reduce the background signal in the assay and prevent false positives. Diluted test sera are then added to the wells. Antibodies in the test serum that bind to antigen are detected with a second antibody, an anti-bovine IgG monoclonal antibody conjugated to horseradish peroxidase (HRP). The HRP acts as a reporter molecule, which, in the presence of substrate and chromogen, generates a measurable coloured product. The coloured product intensity (which is proportional to the number of antibodies-antigens complexes) is measured with an ELISA plate reader.

The antigen used in the ELISA test was 200-kDa antigen and 19-kDa antigen for *Babesia bigemina* and *Anaplasma marginale* species respectively (Morzaria *et al*., 1999; Tebele *et al*., 2000). The tests sensitivity and specificity for *Babesia bigemina* and *Anaplasma marginale* are [97%, 98%] and [90%, 90%] respectively (Morzaria *et al*., 1999; Tebele *et al*., 2000). The ELISA is a five-step

method including: coating of the plate with antigen, blocking of non-specific sites, addition of test sera, enzyme conjugate step, substrate reaction and data analysis and interpretation.

Briefly, 15ml of lyophilized antigen was reconstituted with glycerol and diluted in buffered phosphate solution (DBPS). In each well, 150 µl of the diluted antigen were added and incubation was done for 2 hours at 37ºC. At the end of the incubation, the excess antigen was discarded into a sink and then drained by slapping the inverted plate onto a paper towel. The plate was then left in an inverted position on the towels for about 15 minutes to drain. Blocking of the wells was done with DBPS containing 0.2% of casein (DBPS-C) for 20 minutes at 37ºC. The excess blocking solution was then discarded and then the plates were washed 3 times. The residual fluid was removed by slapping the inverted plate onto paper towels.

Control (strong and weak positive and negative controls and conjugate controls) and test sera were diluted in dilution buffer (DBPS plus 2% of skim milk) and incubated at 37ºC for 45 min. For the *A. marginale* assay, the test sera and control sera are diluted to 1:40. Five µl of serum was put in duplicate wells and then 195 µl of serum diluent was added, to give a 1:40 dilution. 150µl of the sera was added to the coated plate. The plate was incubated at 37ºC in an incubator for 40 minutes. For the *B. bigemina* assays, the test sera and control sera are diluted at 1:100 first, then the sera diluted to 1:50. 5µl of serum was put in duplicate wells and 245 µl of serum diluent added, to give a 1:50 dilution. 75µl of the diluted sera was taken and transferred to the coated plate containing 75 µl of the serum diluent, resulting in a dilution of 1:100. The ELISA plate was incubated at 37ºC in an incubator for 40 minutes. At the end of the incubation, the excess antigen was discarded into a sink and then drained by slapping the inverted plate onto a paper towel. The plate was then left in an inverted position on the towels for about 15 minutes to drain. The wells of the plate were then

filled with 250 ml of washing buffer and incubated for 10 min at 37ºC in an incubator. The washing buffer was discarded by flicking out the contents into a sink then drained the plate on paper towels.

Anti-bovine IgG peroxidase conjugate (Sigma A-7414) diluted 1:40,000 in DBPS containing 0.2% casein and 0.1% Tween-20 (DBPS-C-T) was added in each well and incubated at 37ºC for 30 min. The excess diluted conjugate was then discarded and the plate was washed 5 times. The wells were then filled with 250µl of washing buffer, incubated at 37ºC in an incubator for 10 minutes. The wash buffer was then discarded and the plates were drained on paper towel.

The substrate H_2O_2 and chromogen 2,2'-azino-di [3-ethilbenzithianzoline-acid sulphonate(6)] solution was added. At the end of the incubation, the reaction was stopped by adding 25µl of stopping solution (3.2 M sodium hydroxide). The plate was then read at 405 nm with the ELISA reader.

Using the formula from Wright *et al*., (1993), the optical density (OD) values were expressed as PP (percent positivity), that is, (OD of test serum/OD of strong positive control) × 100. For a reading to be considered positive for antibodies to the *Anaplasma species* and *Babesia species*, it had to be 15 PP or above (Katende *et al*., 1998).

3.6 Data handling and analysis

Microsoft Excel version 2016 (Redmond, WA, USA) was used to manage data obtained from the questionnaire, blood smear results and ELISA results which was then put in STATA 18.0 (StataCorp LLC, College station, Texas, USA) for the data analysis. An accuracy check and coding of the data were done. Descriptive analysis was performed on continuous data to get median, mean and range and categorical variables to get frequency tables and charts.

The relationship between calf- and farm-level factors and seropositivity of *Babesia* and *Anaplasma* species in two independent models as separate outcomes was assessed using univariable mixed-effect logistic analysis. After univariable analysis, factors with a p-value of ≤ 0.1 were analyzed further. Variance inflation factor uncentered and variance covariance estimators corr were used to test for multicollinearity of factors that had a p-value ≤ 0.1 at the univariable analysis. The variable offered for subsequent multivariable mixed effects logistic analysis was one that was highly related, had biological plausibility and had stronger statistical significance.

A backward stepwise elimination approach was used for the multivariable logistic regression model. Factors that had a p-value of ≤ 0.05 were left in the final multivariable model. Confounding effects were assessed by checking regression coefficient changes $\geq 20\%$ with and without possible confounding variables, but only where a confounder had a plausible relationship with both the outcome and the variable. Two-way and three-way interaction variables were explored among the significant variables in the final model. Model diagnosis was therefore done without controlling for cluster, as the intracluster coefficient was negligible using Pearson Chi square test and Hosmer and Lemeshow Chi square. Standardized residuals, overall predictive ability and area under the curve were examined on the final model to determine the goodness of fit of the model

CHAPTER FOUR: RESULTS

4.1 Farm and calf demographics

4.1.1 Distribution of household and farm characteristics

Seventy-six farms were involved in the study, 63.2% (48/76) from Naroosura Majimoto and 36.8% (28/76) Ololulunga Wards (Table 4.1). The majority of the farms were male-headed, 88.2% (67/76), while the rest were female-headed, 11.8% (9/76). The mean age of farm heads was 43 years ranging from 21 to 67 years, while the median was 41 years (Table 4.1). Most of the farm heads, 60.5% (46/76), lacked any formal education. In the majority of the farms, the female spouses (mothers) from the households, 71.1% (54/76), took care of the animals, although under the supervision of the male spouses. Other people in the household who also took care of the livestock were husbands, 63.2% (48/76), children 59.2% (45/76) and employees 44.7% (34/76) (Table 4.1).

The average number of years a livestock farmer had been practicing livestock farming was 15.3 years with a median of 15 while the average land size owned by the study farms was 53.3 acres and a median of 40 acres (Table 4.1). The mean herd size of the study farms was 19.7 with a median of 14 cattle. Besides owning cattle, the other livestock kept were sheep, 79.0% (60/76), goats, 77.6% (59/76) and chicken 76.3% (58/76) (Table 4.1).

Table 4.1: Distribution of socio-demographic factors among cattle farms in Narok County-Kenya between February and May 2023

Variable	Frequency (n=76)	Percentage (%)
Ward		
Naroosura Majimoto	48	63.2
Ololulunga	28	36.8
Livestock production systems		
Agropastoral	31	40.8
Mixed	28	36.8
Pastoral	17	22.4
Gender		
Male	67	88.2
Female	9	11.8
Education		
None	46	60.5
Preschool, Primary	18	23.7
Secondary	6	1.9
Vocational/ Tertiary	6	1.9
Marital Status		
Married	73	96.1
Widowed	2	2.6
Single	1	1.3
Religion		
Christian	73	96.1
Other	2	2.6
Traditionist	1	1.3
Occupation		
Mixed farmer	41	54.0
Pastoralist-Livestock	21	27.6
Business	8	10.5
Fulltime employment	5	6.58
Others	1	1.3
Animal care		
Wife	54	71.1
Owner	48	63.2
Children	45	59.2
Employee	34	44.7
Animals owned		
Sheep	60	79.0
Goats	59	77.6
Chicken	58	76.3
Donkeys	27	35.5

4.1.2 Knowledge on *Anaplasma* and *Babesia* infections by cattle owners

The cattle owners that were reported to be aware of the *Anaplasma* and *Babesia* infections were 86.8% (66/76) and 89.5% (68/76) respectively, while 10.5% (8/76) did not know about the two diseases (Table 4.2). Indigenous knowledge was reported as the major source of the information 63.2% (48/76), on the two diseases with the lowest source of information coming from the death of an animal, 2.6% (2/76) (Table 4.2). The most common clinical signs of anaplasmosis reported by farmers were anorexia 65.8% (50/76), lethargy 57.9% (44/76) and rough hair coat 39.5% (30/76) (Table 4.2). On the other hand, the common clinical signs of babesiosis reported by farmers were red urine 71.1% (54/76), lethargy 47.4% (36/76) and anorexia 44.7% (34/76) (Table 4.2). The main transmission routes of the two diseases reported by the farmers included animal movement 47.4% (36/76), communal grazing and watering points 36.8% (28/76) (Table 4.2.)

Table 4.2: Description of the clinical and epidemiological features on *Anaplasma* and *Babesia* infections among cattle owners in Narok County-Kenya between February and May 2023

Variable	Frequency (n=76)	Percentage (%)
Awareness		
Know babesiosis	68	89.5
Know anaplasmosis	66	86.8
Do not know	8	10.5
Information source		
Indigenous knowledge	48	63.2
Neighbours/Friends	14	18.4
Animal health specialist	10	13.2
Agrovet attendants	5	6.6
Extension services	5	6.6
Sick animal	4	5.3
Death of an animal	2	2.6
Cannot recall	2	2.6
Anaplasmosis clinical signs		
Anorexia	50	65.8
Lethargy	44	57.9
Rough hair coat	30	39.5
Constipation	18	23.7
Hard feces	17	22.4
Weight loss	10	13.2
Others	14	18.4
None	6	7.9
Babesiosis clinical signs		
Red urine	54	71.1
Lethargy	36	47.4
Anorexia	34	44.7
Movement reluctance	19	25.0
Others	16	21.05
None	5	6.6
Possible transmission route		
Animal movement	36	47.4
Communal watering and grazing points	28	36.8
Ticks	13	17.1
Do not know	11	14.5
Seasonality		
Anytime	6	7.9
During dry seasons	32	42.1
During rainy seasons	20	26.3
Do not know	18	23.7
Possible tick vector		
Blue tick	4	5.3
Red spotted tick	2	2.6
Do not know	7	2.6

4.1.3 Distribution of calf characteristics

Four hundred and two calves were involved in the study. Of these, 67.7% (272/402) of the calves were recruited from Naroosura Majimoto Ward while 32.3%, (130/402) were from Ololulunga Ward (Table 4.3). The number of calves recruited from Pastoral production system, Agropastoral production system and Mixed production system were 20.7% (83/402), 47% (189/402) and 32.3% (130/402) respectively (Table 4.3). All the calves recruited for the study were raised on the homestead.

Of the calves recruited, 51.2% (206/402) were female and 48.8% (196/402) were male (Table 4.3). The main calf breed was Boran crosses 96.5% (388/402), while the rest were Sahiwal breed 3.5% (14/402) (Table 4.3). The predominant age category ranged from 3-6 months 75.1% (302/402) while those between 10 and 12 months were at 8.0% (32/402) (Table 4.3). The majority of the recruited calves 49.8% (200/402) had a body condition of 2/5, with a few 2.7% (11/402) having a body condition of 4/5 (Table 4.3). During the visit, 2.2% (9/402) of the calves were clinically ill (Table 4.3).

Table 4.3: Distribution of various calf level factors among calves from Narok County-Kenya between February and May 2023

Variable	Frequency (n=402)	Percentage (%)
Ward		
Naroosura Majimoto	272	67.7
Ololulunga	130	32.3
Sublocation		
Agropastoral	189	47.0
Mixed	130	32.3
Pastoral	83	20.7
Sex		
Female	206	51.2
Male	196	48.8
Breed		
Boran crosses	388	96.5
Sahiwal	14	3.5
Age		
3-6 months	302	75.1
7-9 months	68	16.9
10-12 months	32	8.0
Suckling status		
Yes	341	84.8
No	61	15.2
Body condition score		
BCS 2	200	49.8
BCS 1	105	26.1
BCS 3	86	21.4
BCS 4	11	2.7
Tick present		
Yes	205	51.0
No	197	49.0
Tick burden		
0	198	49.3
1-10	153	38.1
11-50	41	10.2
>50	10	2.5
Other ectoparasite identified		
No	376	93.5
Yes	26	6.5
Other ectoparasite identified		
Lice	17	4.2
Fleas	7	1.7
Flies	2	0.5

Disease presence last 1 month		
Not sick	393	97.8
Sick	9	2.2
Disease known		
No	7	77.8
Yes	2	22.2
Treatment provided for sick		
Yes	6	66.7
No	3	33.3
Treatment provider		
Nothing	3	4.0
Self-medication	1	1.3
Self-medication and Para veterinarians	1	1.3
Recovery status if sick		
Recovered	6	66.7
Still sick	3	33.3

4.1.4 Distribution of management practices in farms

The majority of the calves, 99% (398/402), were sheltered outdoors, with the type of shelter being predominantly open with no walls and no roof, 99.5% (400/402) (Table 4.4). The major feed type provide for the recruited calves was pasture (grazing), 100% (402/402). In addition to pasture, 68.7% (276/402) of the calves were offered mineral supplements (Table 4.4). Most of the pasture offered was majorly grown within the farm, 100% (402/402), while the mineral supplements being offered were purchased, 61.0% (245/402) (Table 4.4). Majority of the farmers, 73.7% (56/76), sought for veterinary services when there were clinical cases while 5.3% (4/76) reported that they never received veterinary services (Table 4.4).

Majority of the farms, 75.0% (57/76), practiced private grazing, with the others either practicing pastoralism 23.7% (18/76) or communal grazing 25% (19/76) (Table 4.4). About 64.5% (49/76) of the farmers grazed their cattle through herding, while 63.2% (48/76) grazed their cattle through free grazing and 19.7% (15/76) grazed through paddocks (Table 4.4). The mean pasture land size was 58.8 acres with a median of 50 acres. Approximately 7.2% (29/402) of the calves had been previously vaccinated, mainly against anthrax 3.5% (14/402) while about 20.2% (81/402) of the calves had been dewormed in the preceding month to the study (Table 4.4). The predominant dewormer used to deworm calves contained levamisole and oxyclozanide 12.4% (50/402) (Table 4.4). The major shelter materials used were untreated wood 98.5% (396/402) and wire 89.6% (360/402) (Table 4.4).

Table 4.4: Distribution of the management practices in farms in Narok County-Kenya between February and May 2023

Variable	Frequency (n=402)	Percentage (%)
Shelter		
Outdoors	398	99
Indoors	4	1
Shelter type at night		
Open (no walls, no roof)	400	99.5
Closed	1	0.3
Open (no walls, with roof)	1	0.3
Shelter material		
Untreated/ Treated wood	396	98.5
Wire	360	89.6
Iron Sheets	8	2.0
Mud/Earth/ Thatch	6	1.5
Feed type		
Pasture	402	100
Mineral supplements	276	68.7
Hay	39	9.7
Dairy meal	17	4.2
Silage	13	3.2
Cut grass	4	1.0
Source of feed		
Grown within the farm	402	100
Purchased	245	61.0
Dewormed		
No	321	79.9
Yes	81	20.2
Dewormer		
Ivermectin	44	11.0
Levamisole and oxyclozanide	50	12.4
Do not know	4	1.0
Levamisole	3	0.75
Vaccination		
Yes	29	7.2
No	373	92.8
Disease vaccinated against		
Anthrax	14	3.5
Anthrax and L. S. D	8	2.0
Anthrax and Black quarter	1	0.3
Anthrax and R.V. F	1	0.3
E. C. F	5	1.2

Calves fed with adults		
Yes	67	88.2
No	9	11.8
Feeding management		
Private grazing	57	75.0
Communal grazing	19	25.0
Pastoralism	18	23.7
Grazing system		
Herded	49	64.5
Free grazing	48	63.2
Paddocking	15	19.7
Yard	5	6.6
Stall feeding	1	1.3
Wildlife interaction		
Yes	32	42.1
No	44	57.9
Vet distance		
5-10Km	35	46.1
0-5Km	31	40.8
>10Km	10	13.2
Vet services		
Clinical cases	56	73.7
Vaccination`	37	48.7
Regularly	23	30.3
Never	4	5.3
Farm infection history		
No	69	90.8
Yes	7	9.2
Last 4 months infection		
No	71	93.4
Yes	5	6.6
Last 12 months infection		
No	75	98.7
Yes	1	1.3
Infection management		
Nothing	3	60
Consulted AHS	1	20
Self-treated	1	20

4.1.5 Distribution of tick control practices in farms

About 96.0% (386/402) of the calves received tick control treatment during the past one month with spraying of the whole body 95.3% (383/402) being the predominant method of acaricide application (Table 4.5). Acaricide containing Amitraz as the active ingredient 48.0% (193/402) accounted for the most common acaricide used followed by acaricides containing pyrethroids and organophosphates, 34.1% (137/402) (Table 4.5). All the farmers in the study sourced the acaricide from the agrovets.

Approximately 51.3% (39/76) of the farmers applied acaricide four times in a month. About 79.0% (60/76) of the farmers reported to change acaricide use, citing perceived acaricide resistance, 64.5% (49/76) as the main reason for the change (Table 4.5). Presence of ticks on cattle 47.4% (36/76) as well as the cost of acaricide 23.7% (18/76) were also cited as other reasons for the change (Table 4.5).

Table 4.5: Distribution of the tick control practices in farms from Narok County between February and May 2023

Variable	Frequency (n=402)	Percentage (%)
Ectoparasite control		
Yes	386	96.0
No	16	4.0
Acaricide product		
Amitraz	193	48.0
Pyrethroids and Organophosphates	137	34.1
Pyrethroids	52	12.9
Ivermectin	44	11.0
Do not know	4	1.0
Application method		
Whole body spray	383	95.3
Injectables	43	10.7
Specific parts spray	2	0.5
Ectoparasite control frequency monthly		
Four times	39	51.3
Twice	23	30.3
Thrice	7	9.2
Once	6	7.9
No pattern	1	1.3
Dry season ectoparasite control monthly		
Weekly	47	61.8
Every 2 weeks	21	27.6
Monthly	6	7.9
3 times	1	1.3
2 times a week	1	1.3
Wet season ectoparasite control monthly		
Every 2 weeks	33	43.4
Weekly	32	42.1
Monthly	8	10.5
3 times	2	2.6
2 times a week	1	1.3
Acaricide change		
Yes	60	79.0
No	16	21.1
Reason for acaricide change		
Perceived acaricide resistance	49	64.5
Ticks on cattle	36	47.4
Acaricide cost	18	23.7
Others	17	22.4
Season	12	15.8
Friend recommendation	10	13.2
Follow recommendation		
Yes	69	90.8
No	7	9.2

4.2 Prevalence of *Anaplasma* and *Babesia* hemoparasites

4.2.1 Microscopic identification of *Anaplasma* and *Babesia* hemoparasites

A total of 402 blood smears were examined microscopically to screen for *Anaplasma* and *Babesia* parasites in erythrocytes. *Anaplasma* hemoparasites were identified in 32.6% (131/402) of the blood smears while *Babesia* hemoparasites were identified in 22.9% (92/402). Blood smears that had both *Babesia* and *Anaplasma* hemoparasites were 11.4% (46/402).

4.2.2 Seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

The overall estimated seroprevalance of *Babesia bigemina* infection in calves was 60% (241/402) of *Anaplasma marginale* infection was 60% (241/402) and of coinfections of *Babesia bigemina* and *Anaplasma marginale* was 38.1% (153/402). The average seropositivity of *A. marginale* infection was 28.1 with a median of 20.7 while that of *Babesia bigemina* infection was 30.7 with a median of 20 based on percent positivity values.

4.3 Description of farm and calf factors based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

4.3.1 Description of demographic factors based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

There was a higher seroprevalence of *B. bigemina* and *A. marginale* infections in male-headed farms, 60.3% (211/350) and 58.3% (204/350) respectively as compared to female-headed farms 57.7% (30/52) and 71.2% (37/52) respectively (Table 4.6). Farms where the farm head lacked any formal education had a higher seroprevalence for *B. bigemina* and *A. marginale* infections, 55.6% (145/261) and 59.4% (155/261) compared to those where the farm head was formally educated at

either primary, secondary or tertiary level (Table 4.6). There were more seropositive calves for *B. bigemina* and *A. marginale* infections farms where the farm head was a mixed farmer, 61.5 % (150/244) and 59.8% (146/244) compared to those where the farm head was in business, full time employment or doing pastoralist-livestock only (Table 4.6).

Table 4.6: Description of demographic factors based on *Babesia bigemina* and *Anaplasma marginale* seropositivity in Narok County between February and May 2023

Variable	Frequency (n=402)	*B. bigemina* positive (%)	*A. marginale* positive (%)
Gender			
Male	350	211 (60.3)	204 (58.3)
Female	52	30 (57.7)	37 (71.2)
Education			
None	261	145 (55.6)	155 (59.4)
Preschool, Primary	86	61 (70.9)	50 (59.4)
Vocational/ Tertiary	33	22(66.7)	15 (45.5)
Secondary	22	13 (59.1)	21 (95.5)
Marital Status			
Married	380	224 (58.9)	228 (60)
Widowed	20	17 (85)	12 (60)
Single	2	0 (0)	1 (50)
Occupation			
Mixed farmer	244	150 (61.5)	146 (59.8)
Pastoralist-Livestock	107	58 (54.2)	66 (61.7)
Business	26	20 (76.9)	18 (69.2)
Fulltime employment	21	11 (52.4)	9 (42.9)
Other	4	2 (50.0)	2 (50.0)
Animal care			
Wife	300	170 (56.7)	179 (59.7)
Owner	256	156 (60.9)	149 (58.2)
Children	255	160 (62.7)	152 (59.6)
Employee	199	125 (62.8)	121 (60.8
Animals owned			
Sheep	343	206 (60.1)	203 (59.2)
Goats	326	192 (58.9)	187 (57.4)
Chicken	323	199 (61.6)	191 (59.1)
Donkeys	151	97 (64.2)	93 (61.6)

4.3.2 Description of epidemiological factors based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

Majority of *B. bigemina* and *A. marginale* seropositive calves 61.1% (214/350) and 62.6% (219/350) respectively were from farms where the farmers were aware of both babesiosis and anaplasmosis (Table 4.7). Sixty point eight percent, (146/402), of the recruited calves that tested seropositive for *B. bigemina* and *A. marginale* infections, were from farms where the farmers sourced their information on hemoparasites from indigenous knowledge as compared to the other sources such as animal health specialists, extension services and agrovets (Table 4.7). Majority of the seropositive calves, 57.4% (101/176) for *B. bigemina* and 63.1% (111/176) for *A. marginale* infections, were from farms where the owners reported that the two hemoparasites commonly occur in the dry season (Table 4.7).

Table 4.7: Description of epidemiological and knowledge of cattle owners based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in Narok County between February and May 2023

Variable	Frequency (n=402)	*B. bigemina* positive (%)	*A. marginale* positive (%)
Awareness			
Know both infections	350	214 (61.1)	219 (62.6)
Do not know any	41	23 (56.1)	19 (46.3)
Know babesiosis	11	4 (36.4)	3 (27.3)
Information source			
Indigenous knowledge	240	146 (60.8)	146 (60.8)
AHS	68	41 (60.3)	42 (61.8)
Extension services	42	26 (61.9)	29 (69.0)
Agrovets	35	35 (61.4)	35 (61.4)
Others	26	19 (773.1)	13 (50)
Death of an animal	6	3 (50)	5 (83.3)
Cannot recall	4	2 (50)	4 (100)
Anaplasmosis clinical signs			
Anorexia	255	156 (61.2)	157 (61.6)
Lethargy	240	147 (61.3)	152 (63.3)
Rough hair coat	169	106 (62.7)	100 (59.2)
Constipation	109	66 (60.6)	72 (66.1)
Others	66	46 (69.7)	42 (63.6)
None	39	22 (56.4)	25 (64.1)
Weight loss	26	17 (65.4)	14 (53.8)
Babesiosis clinical signs			
Red urine	281	169 (60.1)	174 (61.9)
Lethargy	202	126 (62.4)	129 (63.9)
Anorexia	176	107 (60.8)	106 (60.2)
Movement reluctance	99	57 (57.6)	63 (63.6)
Others	76	45 (59.2)	42 (55.3)
None	41	26 (63.4)	21 (51.2)
Seasonality			
During dry seasons	176	101 (57.4)	111 (63.1)
During rainy seasons	103	61 (59.2)	62 (60.2)
Do not know	96	60 (62.5)	52 (54.2)
Anytime	27	19 (70.4)	16 (59.3)
Tick transmission			
Do not know	47	29 (61.7)	28 (59.6)
Blue tick	17	12 (70.6)	9 (52.9)
Red spotted tick	15	11 (73.3)	10 (66.7)
Transmission route			
Animal movement	186	112 (60.2)	111 (59.7)
Grazing and watering	144	90 (62.5)	95 (66)
Ticks	79	52 (65.8)	47 (59.5)
Do not know	20	15 (75)	16 (80)

4.3.3 Description of calf level factors based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

Majority of the calves under the agropastoral production system had a higher seroprevalence of *B. bigemina* infection, 57.1% (108/189) and *A. marginale* infection, 56.1 % (106/189) respectively compared to the other two livestock production systems (Table 4.8). A larger number of seropositive calves for *B. bigemina* infection were female, 59.7% (123/206) compared to male calves, 60.2% (118/196) (Table 4.8). More male calves were seropositive for *A. marginale* infection, 61.7% (121/196) compared to the female calves, 58.3% (120/206) (Table 4.8). Majority of seropositive calves for *B. bigemina* and *A. marginale* infections were aged between 3-6 months, 52.6% (159/302) and 55.0% (166/302) respectively compared to those aged above 6 months (Table 4.8). Majority of seropositive calves for *B. bigemina* and *A. marginale* infections were still suckling at the time of sampling, 57.6% (197/341) and 57.5% (196/341) respectively compared to those who were not suckling (Table 4.8). A greater number of seropositive calves for *B. bigemina* and *A. marginale* infections had a body condition score defined as 2/5, 64% (128/200) and 61.5% (123/200) respectively compared to those with a body condition of either 1, 3 or 4 on a scale of 1-5 (Table 4.8). Bulk of seropositive calves for *B. bigemina* and *A. marginale* infections had ticks on them, 64.9% (133/205) and 62.9% (129/205) respectively compared to those lacking ticks on them (Table 4.8).

Table 4.8: Description of calf level factors based on *Babesia bigemina* and *Anaplasma marginale* seropositivity in Narok County between February and May 2023

Variable	Frequency (n=402)	*B. bigemina* positive (%)	*A. marginale* positive (%)
Sublocation			
Agropastoral	189	108 (57.1)	106 (56.1)
Mixed	130	87 (66.9)	88 (67.7)
Pastoral	83	46 (55.4)	47 (56.6)
Sex			
Female	206	123 (59.7)	120 (58.3)
Male	196	118 (60.2)	121 (61.7)
Breed			
Boran crosses	388	232 (59.8)	230 (59.3)
Sahiwal	14	9 (64.3)	11 (78.6)
Age			
3-6 months	302	159 (52.6)	166 (55.0)
7-9 months	68	54 (79.4)	51 (75.0)
10-12 months	32	28 (87.5)	24 (75.0)
Suckling status			
Yes	341	197 (57.6)	196 (57.5)
No	61	44 (72.1)	45 (73.8)
Body condition score			
BCS 2	200	128 (64)	123 (61.5)
BCS 1	105	70 (66.7)	61 (58.1)
BCS 3	86	41 (47.7)	51 (59.3)
BCS 4	11	2 (18.2)	6 (54.5)
Tick present			
Yes	205	133 (64.9)	129 (62.9)
No	197	108 (54.8)	112 (56.9)
Tick burden			
0	198	109 (55.1)	113 (57.1)
1-10	153	99 (64.7)	97 (63.4)
11-50	41	26 (63.4)	23 (56.1)
>50	10	7 (70.0)	8 (80.0)
Other ectoparasite identified			
No	376	227 (60.4)	229 (60.9)
Yes	26	14 (53.9)	12 (46.2)
Disease present last 1 month			
Not sick	393	236 (60.1)	234 (59.5)
Sick	9	5 (55.6)	7 (77.8)
Disease known			
No	7	4 (57.1)	5 (71.4)
Yes	2	1 (50.0)	2 (100.0)
Treatment provided for the sick			

Yes	6	3 (50.0)	5 (83.3)
No	3	2 (66.7)	2 (66.7)
Treatment provider			
Self-medication	5	3 (60.0)	5 (100.0)
Self-medication and AHS	1	0 (0)	0 (0)
Recovery status if sick			
Recovered	6	3 (50)	5 (83.3)
Still sick	3	2 (66.7)	2 (66.7)

4.3.4 Description of management practices based on seroprevalence of *Babesia bigemina* and *Anaplasma marginale*

Majority of the recruited calves that were sheltered outdoors 99% (398/402) were the more seropositive for *B. bigemina* and *A. marginale* infections at 59.8% (238/398) and 59.5% (237/398) respectively compared to those sheltered indoors (Table 4.9). Calves that had not received dewormers were showing higher *B. bigemina* and *A. marginale* infections seropositivity at 62.9% (202/321) and 60.4% (194/321) respectively compared to those that had received (Table 4.9). Farms that reported lack of history of the diseases 90.8% (69/76) had a higher number of seropositive calves for *B. bigemina* and *A. marginale* infections 58.3% (214/367) and 57.2% (210/367) respectively (Table 4.9).

Table 4.9: Description of management practices based on *Babesia bigemina* and *Anaplasma marginale* seropositivity in Narok County between February and May 2023

Variable	Frequency (n=402)	*B. bigemina* positive (%)	*A. marginale* positive (%)
Shelter			
Outdoors	398	238 (59.8)	237 (59.5)
Indoors	4	3 (75)	4 (100)
Shelter type			
Open (no walls, no roof)	400	239 (59.8)	239 (59.8)
Closed	1	1 (100)	1 (100)
Open (no walls, with roof)	1	1 (100)	1 (100)
Shelter material			
Untreated/ Treated wood	396	236 (59.6)	235 (59.3)
Wire	360	211 (58.6)	214 (59.4)
Iron Sheets	8	5 (62.5)	5 (62.5)
Mud/Earth/ Thatch	6	4 (66.7)	3 (50.0)
Feed type			
Mineral supplements	276	155 (56.2)	165 (59.8)
Hay	39	23 (59.0)	25 (64.1)
Dairy meal	17	10 (58.8)	8 (47.1)
Silage	13	9 (69.2)	9 (69.2)
Cut grass	4	3 (75.0)	4 (100)
Source of feed			
Grown within farm	402	241 (60)	241 (60)
Purchased	245	134 (54.7)	145 (59.2)
Dewormed			
No	321	202 (62.9)	194 (60.4)
Yes	81	39 (48.1)	47 (58.0)
Vaccination			
No	373	228 (61.1)	219 (58.7)
Yes	29	13 (44.8)	22 (75.9)
Wildlife interaction			
No	213	127 (59.6)	130 (61.0)
Yes	189	114 (60.3)	111 (58.7)
Vet distance			
5-10Km	188	107 (56.9)	105 (55.9)
0-5Km	167	107 (64.1)	107 (64.1)
>10Km	47	27 (57.4)	29 (61.7)
Farm infection history			
No	367	214 (58.3)	210 (57.2)
Yes	35	27 (77.1)	31 (88.6)
Last 4 months infection			
No	377	223 (59.2)	219 (58.1)
Yes	25	18 (72.0)	22 (88.0)

Last 12 months infection			
No	397	236 (59.4)	237 (59.7)
Yes	5	5 (100)	4 (80)
Infection management			
Nothing	11	6 (54.5)	9 (81.8)
AHS and self-treated	10	9 (90)	9 (90)
Self-treated	4	3 (75)	4 (100)
Calves fed with adults			
Yes	352	212 (60.2)	205 (58.2)
No	50	29 (58.0)	36 (72.0)
Feeding management			
Private grazing	303	181 (59.7)	183 (60.4)
Communal grazing	113	72 (63.7)	71 (62.8)
Pastoralism	108	67 (62.0)	68 (63.0)
Grazing system			
Herded	273	163 (59.7)	158 (57.9)
Free grazing	244	150 (61.5)	149 (61.1)
Paddocking	82	49 (59.8)	48 (58.5)
Yard	41	25 (61.0)	30 (73.2)
Stall feeding	9	4 (44.4)	2 (22.2)

4.3.5 Description of ectoparasite control practices based on seroprevalence *Babesia bigemina* and *Anaplasma marginale*

Control of ectoparasites was the major practice on the majority of the study calves, 96% (386/402) (Table 4.10). However, *B. bigemina* and *A. marginale* infections remained high in those calves, 59.1% (228/386) and 58.9% (229/386) respectively (Table 4.10). Acaricide application was reported to be applied weekly by about half of the households 51.3% (39/76) with only one household reporting no regular pattern of acaricide application 1.3% (1/76) (Table 4.10). Despite this, *B. bigemina* and *A. marginale* infections were highest in farms that apply acaricide weekly 56.8% (104/183) and 54.1% (99/183) respectively (Table 4.10).

Table 4.10: Description of ectoparasite control practices based on *Babesia bigemina* and *Anaplasma marginale* seropositivity in Narok County between February and May 2023

Variable	Frequency (n=402)	*B. bigemina* positive (%)	*A. marginale* positive (%)
Ectoparasite control			
Yes	386	228 (59.1)	229 (58.9)
No	16	13 (81.3)	12 (75)
Acaricide product			
Amitraz	193	124 (64.2)	112 (58.0)
Pyrethroids and Ops	137	75 (54.7)	81 (59.1)
Pyrethroids	52	27 (51.9)	33 (63.5)
Ivermectin	44	25 (56.8)	23 (52.3)
Do not know	4	2 (50)	3 (75)
Application method			
Whole body spray	383	226 (59.0)	226 (59.0)
Injectable	43	24 (55.8)	24 (55.8)
Specific parts spray	2	1 (50)	2 (100)
Ectoparasite control frequency			
Four times	183	104 (56.8)	99 (54.1)
Twice	137	89 (65.0)	90 (65.7)
Thrice	44	26 (59.1)	25 (56.8)
Once	29	21 (72.4)	22 (75.9)
No pattern	9	1 (11.1)	5 (55.6)
Dry season ectoparasite control			
Weekly	225	128 (56.9)	116 (51.6)
Every 2 weeks	132	86 (65.2)	96 (72.7)
Monthly	29	21 (72.4)	22 (75.9)
Others	16	6 (37.5)	7 (43.8)
Wet season ectoparasite control			
Every 2 weeks	208	128 (61.5)	135 (64.9)
Weekly	142	81 (57.0)	70 (49.3)
Monthly	35	25 (71.4)	28 (80.0)
Others	17	7 (41.2)	8 (47.1)
Acaricide change			
Yes	334	196 (58.7)	197 (59.0)
No	68	45 (66.2)	44 (64.7)
Reason for acaricide change			
Acaricide resistance	278	169 (60.8)	167 (60.1)
Ticks on cattle	190	109 (57.4)	108 (56.8)
Acaricide cost	101	65 (64.4)	63 (62.4)
Others	99	63 (63.9)	67 (67.7)
Season	72	39 (54.2)	45 (62.5)
Friend recommendation	70	48 (68.6)	43 (61.4)
Follow recommendation			
Yes	382	226 (59.2)	228 (59.7)
No	20	15 (75)	13 (65)

4.4 Univariable mixed effects logistic regression analysis

4.4.1 Risk factors associated with *Babesia bigemina* infections in calves

The factors significantly associated with *B. bigemina* infections at $p \leq 0.1$ were: the age of the calf, the body condition score of the calf, the suckling status of the calf, the presence of mineral supplements in forage, the purchasing of forage, acaricide application, whole body spraying of acaricides, deworming of calves, vaccinating of calves, the weight of the calf, the rectal temperature of the calf, tick infestation and the presence of blue tick (Table 4.11). Four variables had a positive association, while the other 10 had a negative association.

Table 4.11: Factors associated with *Babesia bigemina* infections from the univariable mixed effects logistic regression analysis (p – value = 0.1) of calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Tick infestation	Present	1.561	0.995 - 2.450	0.053
	Absent	Reference	----------	-------
Blue tick presence	Present	1.638	1.015 - 2.644	0.043
	Absent	Reference	----------	-------
Rectal temperature	≥ 39.5	0.824	0.655 - 1.037	0.098
	≤ 39.5	Reference	----------	-------
Purchasing of forage	Yes	0.566	0.358 - 0.896	0.015
	No	Reference	----------	-------
Feeding mineral supplements	Yes	0.541	0.322 - 0.908	0.020
	No	Reference	----------	-------
Dewormed	Yes	0.501	0.278 - 0.901	0.021
	No	Reference	----------	-------
Still suckling	Yes	0.468	0.240 - 0.911	0.025
	No	Reference	----------	-------
Levamisole and oxyclozanide use	Yes	0.404	0.200 - 0.818	0.012
	No	Reference	----------	-------
Vaccinated	Yes	0.404	0.151 - 1.084	0.072
	No	Reference	----------	-------
Whole body acaricide spraying	Yes	0.333	0.096 - 1.150	0.082
	No	Reference	----------	-------
Acaricide application	Yes	0.271	0.065 - 1.124	0.072
	No	Reference	----------	-------
Body condition score	3	0.442	0.232 - 0.842	0.013
	1	Reference	----------	-------
Body condition score	4	0.109	0.021 - 0.573	0.009
	1	Reference	----------	-------
Age	---	1.346	1.204 - 1.504	0.000*
Weight	---	1.027	1.015 - 1.039	0.000*

***These are continuous variables.**

4.4.2 Risk factors associated with *Anaplasma marginale* infections in calves

The factors significantly associated with *A. marginale* infections at $p \leq 0.1$ were: the age of the calf, the suckling status of the calf, the weight of the calf, the whole body spraying of acaricides, the presence of brown ear ticks (Table 4.12). Six variables had a positive association, while the other three had a negative association.

Table 4.12: Factors associated with Anaplasma marginale infections from the univariable mixed effects logistic regression analysis (p-value = 0.1) of among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Age	≥ 5 months	2.780	1.734 - 4.456	0.000
	≤ 5 months	Reference	----------	-------
Weight	≥ 70 kg	2.246	1.393 - 3.621	0.001
	≤ 70 Kg	Reference	----------	-------
Acaricide application	≤ 2 times	1.806	1.013 - 0.220	0.045
	≤ 2 times	Reference	----------	-------
Perceived seasonality	Yes	1.140	0.779 - 1.668	0.500
	No	Reference	----------	-------
Brown tick	Presence	0.602	0.338 - 1.071	0.084
	Absent	Reference	----------	-------
Still suckling status	Yes	0.540	0.272 - 1.073	0.079
	No	Reference	----------	-------
Tick control method	Whole body spray	0.348	0.099 - 1.223	0.100
	Specific parts	Reference	----------	-------

4.4.3 Risk factors associated with co-infections of *Babesia bigemina* and *Anaplasma marginale* in calves

The factors significantly associated with co-infections of *B. bigemina* and *A. marginale* at $p \leq 0.1$ were: acaricide application, spraying acaricide on the whole body, feeding of mineral supplements, purchasing of feed, use of wood as shelter material, the calf weight, the rectal temperature of the calf and the presence of brown ear ticks (Table 4.13). Four variables had a positive association, while the other seven had a negative association.

Table 4.13: Factors associated with B. bigemina and A. marginale coinfections from the univariable mixed effects logistic regression (p – value = 0.1) among calves in Narok County between February and May 2023

Variable	Levels	Odds ratio	Confidence Interval	p-value
Weight	≥ 70kg	3.534	2.230 - 5.600	0.000
	≤ 70 Kg	Reference	----------	------
Age	≥5 months	3.302	2.079 - 5.243	0.000
	≤ 5 months	Reference	----------	------
Month of samples collection	March	2.907	1.259 - 6.712	0.012
	April & May	Reference	----------	------
Acaricide application frequency	≤2 times	2.216	1.297 - 3.787	0.004
	≤ 2 times	Reference	----------	------
Rectal temperature	≥ 39.5	0.193	0.045 - 0.818	0.026
	≤ 39.5	Reference	----------	------
Feeding mineral supplements	Yes	0.603	0.352 - 1.034	0.066
	No	Reference	----------	------
Brown tick	Presence	0.586	0.317 - 1.084	0.088
	Absent	Reference	----------	------
Purchasing of forage	Yes	0.555	0.340 - 0.904	0.018
	No	Reference	----------	------
Wood as shelter material	Yes	0.097	0.009 - 1.094	0.059
	No	Reference	----------	------
Acaricide application	Yes	0.311	0.093 - 1.037	0.057
	No	Reference	----------	------
Whole body spray of acaricides	Yes	0.308	0.102 - 0.925	0.036
	No	Reference	----------	------

4.5 Multivariable mixed effects logistic regression analysis

4.5.1 Risk factors associated with *Babesia bigemina* infections in calves

On assessment of the multivariable model of factors associated with *B. bigemina* infections, suckling status was found to be confounding age. The intra-farm correlation coefficient (ICC) in this model (ICC = 0.014) was negligible; hence, model diagnosis was done without controlling for cluster. Multicollinearity of factors was tested using variance inflation factor and variance covariance estimator tests, revealing a lack of collinear factors. A goodness of fit of the model was done using Pearson Chi square, where the model had a p-value of 0.497, while using the Hosmer–Lemeshow Chi square where the model had a p-value of 0.451, which suggested that the model generally fits the data.

The factors significantly associated with *B. bigemina* infections at $p \leq 0.05$ were: calves aged 7 months and above, calves weighing 70 kg and above, calves of body condition between 3 and 4, the purchasing of forage and the education of the household head (Table 4.14).

The calves that were aged 7 months and above were more likely to test positive for *B. bigemina* antibodies compared to those aged below 7 months (OR = 3.030). The calves that weighed 70 kg and above were more likely to test positive for *B. bigemina* antibodies compared to those that weighed below 70kg (OR = 2.478). The calves being raised on farms where the household head had formal education were more likely to test positive for *B. bigemina* antibodies compared to those without any formal education (OR = 1.907). The calves with a body condition ranging between 3 and 4 were less likely to test positive for *B. bigemina* antibodies compared to those with a body condition of 1 and 2 (OR = 0.467). The calves being raised on farms where forage was purchased were less likely to positive for antibodies compared to those on farms that do not purchase forage (OR = 0.536) (Table 4.14). Suckling status (p-value = 0.163) was confounding

age and that is why it was included in the model. Suckling status, whether present or absent, will affect age and *B. bigemina* antibodies by 27.8%.

Table 4.14: Factors associated with B. bigemina infections from the multivariable mixed effects logistic regression analysis (p – value = 0.05) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Age	≥ 7 months	3.030	1.355 - 6.776	0.007
	≤ 7 months	Reference	----------	-------
Weight	≥ 70 Kg	2.478	1.383 - 4.440	0.002
	≤ 70 Kg	Reference	----------	-------
Education level	Formal education	1.907	1.154 - 3.154	0.012
	No formal education	Reference	----------	-------
Feed source	Purchased feed	0.536	0.337 - 0.853	0.009
	Grown within farm	Reference	----------	-------
Body condition	3 and 4	0.467	0.277 - 0.789	0.004
	1 and 2	Reference	----------	-------

4.5.2 Risk factors associated with *Anaplasma marginale* infections in calves

On assessment of the multivariable model of factors associated with *A. marginale* infections, there was no evidence of any confounding factor. The intra-farm correlation coefficient (ICC) in this model (ICC = 0.027) was negligible; hence, model diagnosis was done without controlling for cluster. Multicollinearity of factors was checked using variance inflation factor and variance covariance estimator tests, revealing a lack of collinear factors. Goodness of fit was checked using Pearson Chi square where the model had a p-value of 0.5276 and using the Hosmer–Lemeshow Chi square where the model had a p-value of 0.4681 which suggested that the model generally fits the data.

The factors associated with *A. marginale* infections at $p \leq 0.05$ were: calves aged 5 months and above and weekly application of acaricide during dry seasons (Table 4.15).

The calves that were aged 5 months and above were more likely to test positive for *A. marginale* antibodies compared to those aged below 5 months (OR = 2.736). The calves that were raised on farms practicing acaricide application weekly in a month during dry seasons were less likely to test positive for *A. marginale* antibodies compared to those on farms applying acaricides less than 4 times in a month (OR = 0.445).

Table 4.15: Factors associated with A. marginale infections from the multivariable mixed effects logistic regression analysis (p – value = 0.05) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence interval	p-value
Age	≥ 5 months	2.736	1.733 - 4.320	0.000
	≤ 5 months	Reference	----------	-------
Acaricide application frequency	4 times a month	0.445	0.275 - 0.722	0.001
	<4 times a month	Reference	----------	-------

4.5.3 Risk factors associated with co-infections of *Babesia bigemina* and *Anaplasma marginale* in calves

Assessment of the multivariable model for factors associated with co-infections of *B. bigemina* and *A. marginale* revealed the lack of any confounders. The intra-farm correlation coefficient (ICC) in this model (ICC = 0.000) was negligible; hence, model diagnosis was done without controlling for cluster. Multicollinearity using variance inflation factor and variance covariance estimator tests, revealed a lack of collinear variables. Checking for goodness of fit using Pearson Chi square, the model had a p-value of 0.073 and using the Hosmer–Lemeshow Chi square, the model had a p-value of 0.755 which suggested that the model fitted the data.

The factors associated with co-infections with *B. bigemina* and *A. marginale* at $p \leq 0.05$ were calves aged 5 months and above, calves weighing 70kg and above, calves with a rectal temperature of 39.5 and above, previous history of the infection on the farm, acaricide application more than twice in a month and the purchasing of feed (Table 4.16).

The calves that were aged 5 months and above were more likely to test seropositive for co-infections with *B. bigemina* and *A. marginale* than those below 5 months (OR = 2.073). The calves that weighed 70 kg and above were more likely to test positive for co-infections of *B. bigemina* and *A. marginale* antibodies than those below 70kg (OR = 2.269). The calves that recorded a rectal temperature of 39.5 and above were less likely to test seropositive for co-infections of *B. bigemina* and *A. marginale* than those with a rectal temperature below 39.5 (OR = 0.231). The calves being raised in farms where acaricide application was being done more than twice in a month were less likely to test seropositive for co-infections of *B. bigemina* and *A. marginale* than those in farms where acaricide application was less than twice in a month (OR = 0.536). The calves being raised in farms where feed was purchased were less likely to test seropositive for co-infections with *B. bigemina* and *A. marginale* than those who did not (OR = 0.449). The calves that were raised on

farms with a history of the two infections were more likely to test seropositive for co-infections of *B. bigemina* and *A. marginale* than those in farms that did not have the history (OR=3.803).

Table 4.16: Factors associated with coinfections of B. bigemina and A. marginale from the multivariable mixed effects logistic regression analysis (p – value = 0.005) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Infections history	Present	3.803	1.683 - 8.591	0.001
	Absent	Reference	----------	-------
Age	≥ 5 months	2.073	1.125 - 3.820	0.019
	≤ 5 months	Reference	----------	-------
Weight	≥ 70 kg	2.269	1.230 - 4.185	0.009
	≤ 70 Kg	Reference	----------	-------
Temperature	≥ 39.5	0.231	0.062 - 0.856	0.028
	≤ 39.5	Reference	----------	-------
Acaricide application	≥ 2 times	0.536	0.342 - 0.842	0.007
	≤ 2 times	Reference	----------	-------
Source of feed	Purchased feed	0.449	0.282 - 0.714	0.001
	Grown within farm	Reference	----------	-------

CHAPTER FIVE: DISCUSSION

5.1 Seroprevalence of *Anaplasma marginale* and *Babesia bigemina* among calves

The purpose of this study was to estimate the prevalence of *Babesia bigemina* and *Anaplasma marginale* infections and determine their associated risk factors among calves aged 3 – 12 months in Narok County-Kenya. The findings of this study confirmed the existence of *Babesia* and *Anaplasma* infections and exposures through the presence of antibodies among calves in Narok County.

The seroprevalence of *B. bigemina* estimated in this study was 60%, which was higher than studies conducted in Kenya such as: 37.1% in rural Western Kenya and 27.5% in periurban Western Kenya (Okuthe and Buyu, 2006); 19% in Mbeere District (Gachohi *et al*., 2010) and 0.5% in the Coastal region of Kenya (Masiga *et al*., 2022). However, this prevalence was closer to the 40.6% prevalence reported in Machakos (Wesonga *et al*., 2017) and 42.2% in Ngong (Moumouni *et al*., 2015).

From this study, the estimated seroprevalence of *A. marginale* was 60% which was higher than previous reports, 15.8% in Machakos (Adjou *et al*., 2015), 32.1% in peri urban Western Kenya (Okuthe and Buyu, 2006), 19.7% in Western Kenya (Chiuya *et al*., 2021), 10.9% in the Coastal region (Masiga *et al*., 2022). This was close to studies in rural Western Kenya at 50.2% (Okuthe and Buyu, 2006), in Mbeere District at 58% (Gachohi *et al*., 2010), Machakos at 53.4% (Wesonga *et al*., 2017). A high seroprevalence for *A. marginale* has been reported in other countries including: 57% in Soroti District, Uganda (Kabi *et al*., 2008), 41.1% in Tanga Region, Tanzania (Swai *et al*., 2009) and 50% in Central Equatorial State, South Sudan (Malak *et al*., 2012).

The high seroprevalence of *A*. *marginale* and *B*. *bigemina* infections reported in this study suggests associated with continuous infected tick challenge, as the study area is considered to be an endemic area. The study area also practices open and communal grazing, which encourages constant exposure to tick vectors and the subsequent constant challenge of the infections, hence the presence of antibodies against the infections.

The seroprevalence of *A*. *marginale* and *B*. *bigemina* infections were similar, possibly because they have the same vector, that is, *R. decoloratus/R. microplus* ticks. These two hemoparasites can be co-acquired and transmitted simultaneously by the same vector tick (Adjou *et al*., 2015). This also explains the presence of co-infections with A. *marginale* and *B. bigemina*. *Anaplasma* species is reported to have broader transmission sources and dynamics including mechanically through iatrogenic route or hematophagous flies. The finding of a similar seroprevalence seems to suggest that the mechanical transmission route is of less importance in the epidemiology for *Anaplasma* species.

From this study, the prevalence of *B. bigemina* on microscopy was 22.9% (92/402) while that of *A. marginale* was 32.6% (131/402). The prevalence of co-infections with *B. bigemina and A. marginale* was 11.4% (46/402) on microscopy. These results varied from the ELISA results of 60%, 60% and 38.1% for *A. marginale* and *B. bigemina*, and co-infections of *A. marginale* and *B. bigemina*, respectively. Microscopic examination is known to have low sensitivity and is inadequate in carrier animals, animals in pre-symptomatic phase of *Babesia and Anaplasma* infections (Böse *et al*., 1995; Almería *et al*., 2001; Salih *et al*., 2015). However, ELISA is a more specific and sensitive test for the diagnosis of *Babesia and Anaplasma* parasites, particularly in carrier animals or the preclinical stage of the infection (Knowles *et al.,* 1996; De Echaide *et al*., 1998; Strik *et al.,* 2007).

There was lack of clinical disease in positive calves, that should be presented with clinical signs such as fever, despite the evidence of disease at both the microscopic level and in serology. On ELISA, *A. marginale* and *B. bigemina* antibodies were present in apparently healthy calves, similar to an observation from another study in peri-urban Nairobi by Peter *et al*., (2020). After recovering from primary or acute infections of *A. marginale* and *B. bigemina*, animals frequently develop subclinical infections that cannot be detected under a microscope. The lack of a clinical infection among cattle with infections may be associated with endemic stability state as previously described (Bock *et al*., 2004; Kocan *et al*., 2010). In enzootically stable areas of bovine babesiosis, the clinical cases are absent along the year, but most of the cattle are *Babesia*-infected. Under those conditions, the cows have high antibodies titers that are able to transfer passively to calves through colostrum. Due to that, calves appear as seropositive, but in fact are false positive in terms of *Babesia*-infection (Osaki *et al*., 2002). Relative resistance to tick infestation and vector-borne diseases has been suggested in indigenous cattle and their crosses raised communally, which constituted the study animals in this study area (Marcellino *et al*., 2011; Bock *et al*., 2004; Tabor *et al*., 2017).

Endemic stability is characterized by >70% seroprevalence while endemic instability by < 30% seroprevalence of vector-borne diseases infections/exposures (Norval *et al*., 1992; Deem *et al*., 1993; Gitau *et al*., 1999; Maloo *et al*., 2001). From this study, the seroprevalence estimated suggests a "near endemic stable state" as the seroprevalence of Anaplasma and Babesia infections/exposures. The probability of endemic status using seroprevalence is only indicated through a single cross-sectional study (Gitau *et al*., 1997; Maloo *et al*., 2001; Rubaire-Akiiki *et al*., 2004; Swai *et al*., 2005; Bazarusanga *et al*., 2007). This prevalence can differ quite considerably with vector tick density, climatic conditions, human-related activities, vector control

programs, habitat modification and host population density (Gitau *et al*., 1999; Maloo *et al*., 2001; Rubaire-Akiiki *et al*., 2006; Olwoch *et al*., 2008).

5.2 Risk factors associated with *Anaplasma marginale* and *Babesia bigemina* seroprevalence

Calves aged 5 months and above had increased odds of *A. marginale* infections, *B. bigemina* infections or coinfections of *A. marginale* and *B. bigemina* than those aged below 5 months. The relationship between age and *B. bigemina* infection seroprevalence has been previously reported (Wray *et al*., 2000; Magona *et al*., 2008; Simuunza *et al*., 2011; Terkawi *et al*., 2011; Hamou *et al*., 2012; Atif *et al*., 2013; M'ghirbi *et al*., 2016; Wesonga *et al*., 2017;). All age groups are susceptible to *B. bigemina* and *A. marginale* infections but the prevalence increases with age (Gachohi *et al*., 2012). The sustained exposure to ticks translates to the risk of exposure to *Babesia* infection increasing as the calves get older. The grazing of older cattle far in the bush also increases their chance of tick exposure, which consequently increases their chance of infection by Vector-borne diseases (Bazarusanga *et al*., 2007).

Calves being raised in farms that were reported to have a history of *A. marginale* and *B. bigemina* infections had a higher odds of testing seropositive for coinfections of *A. marginale* and *B. bigemina*. An animal that survives an acute infection, may develop a persistent infection and serve as a carrier for *A. marginale* and *B. bigemina* infections in the herd (Kocan *et al*., 2003). To be able to comprehend the epidemiology and control of the two infections, it is important to identify the chronically infected animals in order to prevent introducing the source of infections to naïve cattle (Figueroa *et al*., 1993; Calder *et al*., 1996; Goff *et al*., 2008).

Calves that recorded a fever were less likely to test for *B. bigemina* and *A. marginale* coinfections. These two infections are known to cause a fever when the clinical disease develops. The latter shows that the temperature recorded in these animals could be due to other infections and/or

disease conditions or environmental factors. This also shows the possibility of endemic stability to the two infections leading to asymptomatic seropositive animals due to the presence of immunity against the two vector-borne infections (Bock *et al*., 2004; Kocan *et al*., 2010).

Calves with body condition above 2 on a scale of 1-5, were less likely to test seropositive for *B. bigemina* compared to those with body condition of 2 and below. Various studies have reported a similar finding (Sitotaw *et al*., 2014; Admassu *et al*., 2015; Hamsho *et al*., 2015; Wodajnew *et al*., 2015; Adugna and Tamrat, 2022). Lowered immunity can be demonstrated in poorly conditioned cattle, which encourages the establishment of *Babesia* and *Anaplasma* infections. Establishing whether the depreciation in body condition is a result of the disease or other potential risk factors can only be possible through a longitudinal study.

From this study, acaricide application frequency was significantly associated with *A. marginale* infections and coinfections with *A. marginale* and *B. bigemina*. Farms that applied acaricide weekly had lower odds of *A. marginale* infection and those that applied acaricide more than twice in a month had lower odds of *A. marginale* and *B. bigemina* coinfections. Acaricide application has been recommended for the prevention and control of infections of *B. bigemina* and *A. marginale* in cattle (Stachurski, 2000; Adjou, 2012; Adehan *et al*., 2016a). Acaricide application effectiveness is shown through this study, as a high proportion (49.3%) of the study calves had no tick infestation.

The association between higher calf weight relating to a higher likelihood of seropositivity to *B. bigemina* and *B. bigemina* / *A. marginale* coinfections was not explainable. The relationship may have been associated with, for example, the source of forage where such farms may have brought tick-infested fodder from other farms.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

1. A seroprevalance of 60 % for *Babesia bigemina* and *Anaplasma marginale* infections was among calves aged between 3 months and 12 months in Narok County-Kenya is considerably high.
2. Calves aged 5 months to 12 months were reported to have a higher seroprevalence to *Babesia bigemina and Anaplasma marginale* infections compared to the calves aged 3 months to 5 months.
3. The application of acaricide was associated with lower seroprevalence to *Babesia bigemina* and *Anaplasma marginale* infections.

6.2 Recommendations

1. A longitudinal study should be undertaken among the calves in Narok County-Kenya to establish the incidence rate of *Babesia* and *Anplasma* infections/exposure
2. Strategic and holistic acaricide application should be adopted in vector-borne disease control and prevention with particular interest on application methods, frequency of application, dilutions and the type of acaricide to optimize the effectiveness.
3. A study based on advanced molecular tests, which are more sensitive and specific, should conducted to provide more accurate epidemiological data on ticks and tick-borne diseases.

REFERENCES

Abbas, R. Z., Zaman, M. A., Colwell, D. D., Gilleard, J., & Iqbal, Z. (2014). Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Veterinary Parasitology*, *203*(1–2), 6–20.

Adjou Moumouni, P. F., Aboge, G. O., Terkawi, M. A., Masatani, T., Cao, S., Kamyingkird, K., Jirapattharasate, C., Zhou, M., Wang, G., & Liu, M. (2015). Molecular detection and characterization of Babesia bovis, Babesia bigemina, Theileria species and Anaplasma marginale isolated from cattle in Kenya. *Parasites & Vectors*, *8*(1), 1–14.

Admassu, B., Yeneneh, H., Shite, A., Haile, B., & Mohammed, S. (2015). *Prevalence and identification of major Ixodid tick genera of cattle in Dangila district, Awi Zone, North West Ethiopia*.

Adugna, H., & Tamrat, H. (2022). Epidemiological study on Ixodid tick infestation and tick borne haemopathogens on cattle in Awi Zone, northwest Ethiopia. *Veterinary Medicine and Science*, *8*(5), 2194–2205.

Almería, S., Castella, J., Ferrer, D., Ortuno, A., Estrada-Peña, A., & Gutierrez, J. F. (2001). Bovine piroplasms in Minorca (Balearic Islands, Spain): a comparison of PCR-based and light microscopy detection. *Veterinary Parasitology*, *99*(3), 249–259.

Alvarez, J. A., Rojas, C., & Figueroa, J. V. (2019). Diagnostic tools for the identification of Babesia sp. in persistently infected cattle. *Pathogens*, *8*(3), 143.

Anderson, J. M., Moore, I. N., Nagata, B. M., Ribeiro, J. M. C., Valenzuela, J. G., & Sonenshine, D. E. (2017). Ticks, Ixodes scapularis, feed repeatedly on white-footed mice despite strong

inflammatory response: an expanding paradigm for understanding tick–host interactions. *Frontiers in Immunology*, *8*, 1784.

Atif, F. A., Khan, M. S., Muhammad, F., & Ahmad, B. (2013). Sero-epidemiological study of Anaplasma marginale among cattle. *J Anim Plant Sci*, *23*, 740–744.

Aubry, P., & Geale, D. W. (2011). A review of bovine anaplasmosis. *Transboundary and Emerging Diseases*, *58*(1), 1–30.

Barbet, A. F. (2009). Persistence mechanisms in tick-borne diseases: tick-borne diseases. *Onderstepoort Journal of Veterinary Research*, *76*(1), 53–58.

Bazarusanga, T., Vercruysse, J., Marcotty, T., & Geysen, D. (2007). Epidemiological studies on Theileriosis and the dynamics of Theileria parva infections in Rwanda. *Veterinary Parasitology*, *143*(3–4), 214–221.

Beugnet, F., & Moreau, Y. (2015). Babesiosis. *Revue Scientifique et Technique (International Office of Epizootics)*, *34*(2), 627–639.

Billiouw, M., & Berkvens, D. (1999). The current epidemiological status of bovine theileriosis in eastern Zambia. *Tropical Medicine & International Health*, *4*(9), A28–A33.

Bock, R., Jackson, L., De Vos, A., & Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology*, *129*(S1), S247–S269.

Bonsma, J. A. N. (1980). Livestock production--a global approach. *Livestock Production--a Global Approach.*

Böse, R., Jorgensen, W. K., Dalgliesh, R. J., Friedhoff, K. T., & De Vos, A. J. (1995). Current state and future trends in the diagnosis of babesiosis. *Veterinary Parasitology*, *57*(1–3), 61–

74.

Bradway, D. S., de Echaide, S. T., Knowles, D. P., Hennager, S. G., & McElwain, T. F. (2001). Sensitivity and specificity of the complement fixation test for detection of cattle persistently infected with Anaplasma marginale. *Journal of Veterinary Diagnostic Investigation*, *13*(1), 79–81.

Brıgido, C., da Fonseca, I. P., Parreira, R., Fazendeiro, I., do Rosário, V. E., & Centeno-Lima, S. (2004). Molecular and phylogenetic characterization of Theileria spp. parasites in autochthonous bovines (Mirandesa breed) in Portugal. *Veterinary Parasitology*, *123*(1–2), 17–23.

Byaruhanga, C., Collins, N. E., Knobel, D., Chaisi, M. E., Vorster, I., Steyn, H. C., & Oosthuizen, M. C. (2016). Molecular investigation of tick-borne haemoparasite infections among transhumant zebu cattle in Karamoja Region, Uganda. *Veterinary Parasitology: Regional Studies and Reports*, *3*, 27–35.

Byaruhanga, C., Collins, N. E., Knobel, D. L., Khumalo, Z. T. H., Chaisi, M. E., & Oosthuizen, M. C. (2018). Molecular detection and phylogenetic analysis of Anaplasma marginale and Anaplasma centrale amongst transhumant cattle in north-eastern Uganda. *Ticks and Tick-Borne Diseases*, *9*(3), 580–588.

Byaruhanga, C., Oosthuizen, M. C., Collins, N. E., & Knobel, D. (2015). Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases amongst transhumant zebu cattle in Karamoja Region, Uganda. *Preventive Veterinary Medicine*, *122*(3), 287–297.

Calder, J. A., Reddy, G. R., Chieves, L., Courtney, C. H., Littell, R., Livengood, J. R., Norval, R.

A., Smith, C., & Dame, J. B. (1996). Monitoring Babesia bovis infections in cattle by using PCR-based tests. *Journal of Clinical Microbiology*, *34*(11), 2748–2755.

Callow, L. L. (1984). Theileriosis. *Animal Health in Australia*, *5*, 168–173.

Callow, L. L., Rogers, R. J., & De Vos, A. J. (1986). Standard diagnostic techniques for tick-borne diseases (babesiosis and anaplasmosis) of cattle in Australia. *Australian Agricultural Council, Standard Diagnostic Techniques for Animal Diseases*, *29*, 1–29.

Carelli, G., Decaro, N., Lorusso, A., Elia, G., Lorusso, E., Mari, V., Ceci, L., & Buonavoglia, C. (2007). Detection and quantification of Anaplasma marginale DNA in blood samples of cattle by real-time PCR. *Veterinary Microbiology*, *124*(1–2), 107–114.

Chauvin, A., Moreau, E., Bonnet, S., Plantard, O., & Malandrin, L. (2009). Babesia and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. *Veterinary Research*, *40*(2).

Chenyambuga, S. W., Waiswa, C., Saimo, M., Ngumi, P., & Gwakisa, P. S. (2010). *Knowledge and perceptions of traditional livestock keepers on tick-borne diseases and sero-prevalence of Theileria parva around Lake Victoria Basin*.

Childs, J. E., & Paddock, C. D. (2003). The ascendancy of Amblyomma americanum as a vector of pathogens affecting humans in the United States. *Annual Review of Entomology*, *48*(1), 307–337.

Chiuya, T., Villinger, J., Masiga, D. K., Ondifu, D. O., Murungi, M. K., Wambua, L., Bastos, A. D. S., Fèvre, E. M., & Falzon, L. C. (2021). Molecular prevalence and risk factors associated with tick-borne pathogens in cattle in western Kenya. *BMC Veterinary Research*,

17(1), 1–17.

Coetzee, J. F., Apley, M. D., & Kocan, K. M. (2006). Comparison of the efficacy of enrofloxacin, imidocarb, and oxytetracycline for clearance of persistent Anaplasma marginale infections in cattle. *Veterinary Therapeutics*, *7*(4), 347.

Coetzee, J. F., Schmidt, P. L., Apley, M. D., Reinbold, J. B., & Kocan, K. M. (2007). Comparison of the complement fixation test and competitive ELISA for serodiagnosis of Anaplasma marginale infection in experimentally infected steers. *American Journal of Veterinary Research*, *68*(8), 872–878.

Commission, I. O. of E. B. S., & Committee, I. O. of E. I. (2008). *Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees* (Vol. 2). Office international des épizooties.

Costa, S. C. L., de Magalhães, V. C. S., de Oliveira, U. V., Carvalho, F. S., de Almeida, C. P., Machado, R. Z., & Munhoz, A. D. (2016). Transplacental transmission of bovine tick-borne pathogens: frequency, co-infections and fatal neonatal anaplasmosis in a region of enzootic stability in the northeast of Brazil. *Ticks and Tick-Borne Diseases*, *7*(2), 270–275.

Courtney, J. W., Kostelnik, L. M., Zeidner, N. S., & Massung, R. F. (2004). Multiplex real-time PCR for detection of Anaplasma phagocytophilum and Borrelia burgdorferi. *Journal of Clinical Microbiology*, *42*(7), 3164–3168.

Dalgliesh, R. J. (1993). Babesiosis. *Immunology and Molecular Biology of Parasitic Infections.*, *Ed. 3*, 352–383.

De-Whittier, D., Currin, N., & Currin, J. (2007). Anaplasmosis in Beef Cattle. *Virginia Tech*

Cooperative Extension, Blacksburg, 400–465.

de Castro, J. J. (1997). Sustainable tick and tickborne disease control in livestock improvement in developing countries. *Veterinary Parasitology*, *71*(2–3), 77–97.

de Echaide, S. T., Knowles, D. P., McGuire, T. C., Palmer, G. H., Suarez, C. E., & McElwain, T. F. (2001). Detection of Cattle Naturally Infected with Anaplasma marginale in a Region of Endemicity by Nested PCR and a Competitive Enzyme-Linked Immunosorbent Assay Using Recombinant Major Surface Protein 5. *Journal of Clinical Microbiology*, *39*(3), 1207.

De Echaide, S. T., Knowles, D. P., McGuire, T. C., Palmer, G. H., Suarez, C. E., & McElwain, T. F. (1998). Detection of cattle naturally infected with Anaplasma marginale in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. *Journal of Clinical Microbiology*, *36*(3), 777–782.

De La Fuente, J., Rodrıguez, M., Montero, C., Redondo, M., Garcıa-Garcıa, J. C., Méndez, L., Serrano, E., Valdés, M., Enrıquez, A., & Canales, M. (1999). Vaccination against ticks (Boophilus spp.): the experience with the Bm86-based vaccine Gavac^{TM}. *Genetic Analysis: Biomolecular Engineering*, *15*(3–5), 143–148.

de Souza, J. C. P., Soares, C. O., Madruga, C. R., & Massard, C. L. (2001). Prevalence of antibodies against Anaplasma marginale (Rickettsiales: Anaplasmataceae) in cattle in the" Medio Paraiba" mesoregion, Brazil. *Ciencia Rural (Brazil)*.

De Vos, A. J., Dalgliesh, R. J., & McGregor, W. (1986). Effect of imidocarb dipropionate prophylaxis on the infectivity and immunogenicity of a Babesia bovis vaccine in cattle. *Australian Veterinary Journal*, *63*(6), 174–178.

De Vos, A. J., De Waal, D. T., & Jackson, L. A. (2004). Bovine babesiosis. *Infectious Diseases of Livestock, Volume One*, *Ed. 2*, 406–424.

de Vos, A. J., Potgieter, F. T., De Waal, D. T., & Van Heerden, J. (1994). Babesioses. *Infectious Diseases of Livestock with Special Reference to Southern Africa.*, 278–308.

Deem, S. L., Perry, B. D., Katende, J. M., McDermott, J. J., Mahan, S. M., Maloo, S. H., Morzaria, S. P., Musoke, A. J., & Rowlands, G. J. (1993). Variations in prevalence rates of tick-borne diseases in Zebu cattle by agroecological zone: implications for East Coast fever immunization. *Preventive Veterinary Medicine*, *16*(3), 171–187.

Desta, B. (2016). Review on the impact of ticks on livestock health and productivity. *J. Bio. Agr. Health*, *6*, 1–7.

DeVos, A. J., Brock, R., & Molly, J. B. (2006). Tick borne diseases of cattle. *Australian and New Zealand Standard Diagnostic Procedures. Sub Committee on Animal Health Laboratory Standards*, 1–29.

Dipeolu, O. O. (1989). Research on ticks of livestock in Africa: review of the trends, advances and milestones in tick biology and ecology in the decade 1980–1989. *International Journal of Tropical Insect Science*, *10*(6), 723–740.

Dipeolu, O. O., & Amoo, A. (1984). The presence of kinetes of a Babesia species in the haemolymph smears of engorged Hyalomma ticks in Nigeria. *Veterinary Parasitology*, *17*(1), 41–46.

Dohoo, I. R., Martin, W., & Stryhn, H. E. (2003). *Veterinary epidemiologic research*.

Domingos, A., Antunes, S., Borges, L., & Rosario, V. E. do. (2013). Approaches towards tick

and tick-borne diseases control. *Revista Da Sociedade Brasileira de Medicina Tropical*, *46*, 265–269.

Dreher, U. M., Hofmann-Lehmann, R., Meli, M. L., Regula, G., Cagienard, A. Y., Stärk, K. D. C., Doherr, M. G., Filli, F., Hässig, M., & Braun, U. (2005). Seroprevalence of anaplasmosis among cattle in Switzerland in 1998 and 2003: no evidence of an emerging disease. *Veterinary Microbiology*, *107*(1–2), 71–79.

Duguma, B., Kechero, Y., & Janssens, G. P. J. (2012). Survey of major diseases affecting dairy cattle in Jimma town, Oromia, Ethiopia. *Global Veterinaria*, *8*(1), 62–66.

Dumler, J. S., Barbet, A. F., Bekker, C. P., Dasch, G. A., Palmer, G. H., Ray, S. C., Rikihisa, Y., & Rurangirwa, F. R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combi. *International Journal of Systematic and Evolutionary Microbiology*, *51*(6), 2145–2165.

Estrada-Peña, A., Ruiz-Fons, F., Acevedo, P., Gortázar, C., & De la Fuente, J. (2013). Factors driving the circulation and possible expansion of Crimean–Congo haemorrhagic fever virus in the western Palearctic. *Journal of Applied Microbiology*, *114*(1), 278–286.

Ewing, S. A. (1981). Transmission of Anaplasma marginale by arthropods. *Proceedings of the 7th National Anaplasmosis Conference. Mississippi State University, Mississippi State*, *395*, 423.

Figueroa, J. V, Chieves, L. P., Johnson, G. S., & Buening, G. M. (1993). Multiplex polymerase chain reaction based assay for the detection of Babesia bigemina, Babesia bovis and

Anaplasma marginale DNA in bovine blood. *Veterinary Parasitology*, *50*(1–2), 69–81.

Friedhoff, K. T. (2018). Transmission of babesia. In *Babesiosis of domestic animals and man* (pp. 23–52). CRC Press.

Futse, J. E., Ueti, M. W., Knowles Jr, D. P., & Palmer, G. H. (2003). Transmission of Anaplasma marginale by Boophilus microplus: retention of vector competence in the absence of vector-pathogen interaction. *Journal of Clinical Microbiology*, *41*(8), 3829–3834.

Gachohi, J. M., Kitala, P. M., Ngumi, P. N., Skilton, R. A., & Bett, B. (2013). Population attributable fractions of farm vector tick (Rhipicephalus appendiculatus) presence on Theileria parva infection seroprevalence under endemic instability. *Preventive Veterinary Medicine*, *108*(2), 103–113. https://doi.org/https://doi.org/10.1016/j.prevetmed.2012.08.009

Gachohi, J. M., Ngumi, P. N., Kitala, P. M., & Skilton, R. A. (2010). Estimating seroprevalence and variation to four tick-borne infections and determination of associated risk factors in cattle under traditional mixed farming system in Mbeere District, Kenya. *Preventive Veterinary Medicine*, *95*(3–4), 208–223.

Gachohi, J., Skilton, R., Hansen, F., Ngumi, P., & Kitala, P. (2012). Epidemiology of East Coast fever (Theileria parva infection) in Kenya: past, present and the future. *Parasites & Vectors*, *5*(1), 1–13.

García-Sanmartín, J., Nagore, D., García-Pérez, A. L., Juste, R. A., & Hurtado, A. (2006). Molecular diagnosis of Theileria and Babesia species infecting cattle in Northern Spain using reverse line blot macroarrays. *BMC Veterinary Research*, *2*, 1–7.

García-García, J. C., Montero, C., Redondo, M., Vargas, M., Canales, M., Boue, O., Rodríguez,

M., Joglar, M., Machado, H., & González, I. L. (2000). Control of ticks resistant to immunization with Bm86 in cattle vaccinated with the recombinant antigen Bm95 isolated from the cattle tick, Boophilus microplus. *Vaccine*, *18*(21), 2275–2287.

Gasparin, G., Miyata, M., Coutinho, L. L., Martinez, M. L., Teodoro, R. L., Furlong, J., Machado, M. A., Silva, M., Sonstegard, T. S., & Regitano, L. C. A. (2007). Mapping of quantitative trait loci controlling tick [Riphicephalus (Boophilus) microplus] resistance on bovine chromosomes 5, 7 and 14. *Animal Genetics*, *38*(5), 453–459.

Georges, K., Loria, G. R., Riili, S., Greco, A., Caracappa, S., Jongejan, F., & Sparagano, O. (2001). Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. *Veterinary Parasitology*, *99*(4), 273–286.

Gitau, G. K., Perry, B. D., Katende, J. M., McDermott, J. J., Morzaria, S. P., & Young, A. S. (1997). The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya; a cross-sectional study. *Preventive Veterinary Medicine*, *30*(2), 95–107.

Gitau, G. K., Perry, B. D., & McDermott, J. J. (1999). The incidence, calf morbidity and mortality due to Theileria parva infections in smallholder dairy farms in Murang'a District, Kenya. *Preventive Veterinary Medicine*, *39*(1), 65–79.

Goff, W. L., Johnson, W. C., & Kuttler, K. L. (1985). Development of an indirect fluorescent antibody test, using microfluorometry as a diagnostic test for bovine anaplasmosis. *American Journal of Veterinary Research*, *46*(5), 1080–1084.

Goff, W. L., Johnson, W. C., Molloy, J. B., Jorgensen, W. K., Waldron, S. J., Figueroa, J. V, Matthee, O., Adams, D. S., McGuire, T. C., & Pino, I. (2008). Validation of a competitive

enzyme-linked immunosorbent assay for detection of Babesia bigemina antibodies in cattle. *Clinical and Vaccine Immunology*, *15*(9), 1316–1321.

Goff, W. L., Johnson, W. C., Parish, S. M., Barrington, G. M., Tuo, W., & Valdez, R. A. (2001). The age-related immunity in cattle to Babesia bovis infection involves the rapid induction of interleukin-12, interferon-γ and inducible nitric oxide synthase mRNA expression in the spleen. *Parasite Immunology*, *23*(9), 463–471.

GoK. (2015). Kenya National Bureau of Statistics. *Narok County Statistical Abstract*.

Gubbels, J. M., De Vos, A. P., Van der Weide, M., Viseras, J., Schouls, L. M., De Vries, E., & Jongejan, F. (1999). Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. *Journal of Clinical Microbiology*, *37*(6), 1782–1789.

Guswanto, A., Allamanda, P., Mariamah, E. S., Munkjargal, T., Tuvshintulga, B., Takemae, H., Sivakumar, T., AbouLaila, M., Terkawi, M. A., & Ichikawa-Seki, M. (2017). Evaluation of immunochromatographic test (ICT) strips for the serological detection of Babesia bovis and Babesia bigemina infection in cattle from Western Java, Indonesia. *Veterinary Parasitology*, *239*, 76–79.

Hamou, S. A., Rahali, T., Sahibi, H., Belghyti, D., Losson, B., Goff, W., & Rhalem, A. (2012). Molecular and serological prevalence of Anaplasma marginale in cattle of North Central Morocco. *Research in Veterinary Science*, *93*(3), 1318–1323.

Hamsho, A., Tesfamarym, G., Megersa, G., & Megersa, M. (2015). A cross-sectional study of bovine babesiosis in Teltele District, Borena Zone, Southern Ethiopia. *J Veterinar Sci Technol*, *6*(230), 2.

Holman, P. J., Spencer, A. M., TELFORD, S. A. M. R., Goethert, H. K., Allen, A. J., Knowles, D. P., & Goff, W. L. (2005). Comparative infectivity of Babesia divergens and a zoonotic Babesia divergens–like parasite in cattle. *The American Journal of Tropical Medicine and Hygiene*, *73*(5), 865–870.

Homewood, K., Trench, P., Randall, S., Lynen, G., & Bishop, B. (2006). Livestock health and socio-economic impacts of a veterinary intervention in Maasailand: infection-and-treatment vaccine against East Coast fever. *Agricultural Systems*, *89*(2–3), 248–271.

Hunfeld, K.-P., Hildebrandt, A., & Gray, J. S. (2008). Babesiosis: recent insights into an ancient disease. *International Journal for Parasitology*, *38*(11), 1219–1237.

Iseki, H., Alhassan, A., Ohta, N., Thekisoe, O. M. M., Yokoyama, N., Inoue, N., Nambota, A., Yasuda, J., & Igarashi, I. (2007). Development of a multiplex loop-mediated isothermal amplification (mLAMP) method for the simultaneous detection of bovine Babesia parasites. *Journal of Microbiological Methods*, *71*(3), 281–287.

Jabbar, A., Abbas, T., Sandhu, Z.-D., Saddiqi, H. A., Qamar, M. F., & Gasser, R. B. (2015). Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. *Parasites & Vectors*, *8*(1), 1–13.

Jaswal, H., Bal, M. S., Singla, L. D., Amrita, K. P., Mukhopadhyay, C. S., & Juyal, P. D. (2014). Application of msp1β PCR and 16S rRNA semi nested PCR-RFLP for detection of persistent anaplasmosis in tick infested cattle. *Int. J. Adv. Res*, *2*(8), 188–196.

Jilintai, S. N., Hayakawa, D., Suzuki, M., Hata, H., Kondo, S., Matsumoto, K., Yokoyama, N., & Inokuma, H. (2009). Molecular survey for Anaplasma bovis and Anaplasma phagocytophilum infection in cattle in a pastureland where sika deer appear in Hokkaido,

Japan. *Jpn J Infect Dis*, *62*, 73–75.

Johnston, L. A. Y., Leatch, G., & Jones, P. N. (1978). The duration of latent infection and functional immunity in Droughtmaster and Hereford cattle following natural infection with Babesia argentina and Babesia bigemina. *Australian Veterinary Journal*, *54*(1), 14–18.

Jongejan, F., & Uilenberg, G. (1994). Ticks and control methods. *Revue Scientifique et Technique (International Office of Epizootics)*, *13*(4), 1201–1226.

Jonsson, N. N., Bock, R. E., Jorgensen, W. K., Morton, J. M., & Stear, M. J. (2012). Is endemic stability of tick-borne disease in cattle a useful concept? *Trends in Parasitology*, *28*(3), 85–89.

Jonsson, N. N., Piper, E. K., & Constantinoiu, C. C. (2014). Host resistance in cattle to infestation with the cattle tick R hipicephalus microplus. *Parasite Immunology*, *36*(11), 553–559.

Jorgensen, W. K., Bock, R. E., Kingston, T. G., VOS, A. J. D. E., & Waldron, S. J. (1993). Assessment of tetracycline and Babesia culture supernatant as prophylactics for moderating reactions in cattle to live Babesia and Anaplasma vaccines. *Australian Veterinary Journal*, *70*(1), 35–36.

Kabi, F., Magona, J. W., Nasinyama, G. W., & Walubengo, J. (2008). Sero-prevalences of Tick-borne infections among the Nkedi Zebu and Ankole cattle in Soroti district, Uganda. *The Journal of Protozoology Research*, *18*(2), 61–70. https://doi.org/10.32268/jprotozoolres.18.2_61

Kabi, F., Masembe, C., Muwanika, V., Kirunda, H., & Negrini, R. (2014). Geographic

distribution of non-clinical Theileria parva infection among indigenous cattle populations in contrasting agro-ecological zones of Uganda: implications for control strategies. *Parasites & Vectors*, *7*(1), 1–9.

Kaiser, M. N., Sutherst, R. W., & Bourne, A. S. (1982). Relationship between ticks and Zebu cattle in southern Uganda. *Tropical Animal Health and Production*, *14*(2), 63–74.

Kasaija, P. D., Estrada-Peña, A., Contreras, M., Kirunda, H., & de la Fuente, J. (2021). Cattle ticks and tick-borne diseases: a review of Uganda's situation. *Ticks and Tick-Borne Diseases*, *12*(5), 101756.

Katende, J. M., Toye, P., Skilton, R. A., Nene, V., Morzaria, S. P., & Musoke, A. J. (1998). An ELISA for detection of Theileria parva antibodies in cattle using a recombinant polymorphic immunodominant molecule. *Parasitology Research*, *84*(5), 408–416.

Kay, B. H., & Kemp, D. H. (1994). Vaccines against arthropods. *The American Journal of Tropical Medicine and Hygiene*, *50*(6 Suppl), 87–96.

Kerario, I. I., Muleya, W., Chenyambuga, S., Koski, M., Hwang, S.-G., & Simuunza, M. (2017). *Abundance and distribution of Ixodid tick species infesting cattle reared under traditional farming systems in Tanzania*.

Kieser, S. T., Eriks, I. S., & Palmer, G. H. (1990). Cyclic rickettsemia during persistent Anaplasma marginale infection of cattle. *Infection and Immunity*, *58*(4), 1117–1119.

Kipronoh, K. A., Gathuma, J. M., Kitala, P. M., & Kiara, H. K. (2011). Prevalence of tick-borne infections in extensive cattle management system in West Pokot District, Kenya. Prévalence des infections transmises par les tiques dans le système de gestion extensive du bétail dans

le district de West Pokot, au Kenya. *INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES BUREAU INTERAFRICAN DES RESSOURCES ANIMALES PO Box, NAIROBI, KENYA*, *59*(1), 43–52.

Knowles, D., De Echaide, S. T., Palmer, G., McGuire, T., Stiller, D., & McElwain, T. (1996). Antibody against an Anaplasma marginale MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. *Journal of Clinical Microbiology*, *34*(9), 2225.

Kocan, K. M., Blouin, E. F., & Barbet, A. F. (2000). Anaplasmosis control: past, present, and future. *Annals of the New York Academy of Sciences*, *916*(1), 501–509.

Kocan, K. M., de la Fuente, J., Blouin, E. F., Coetzee, J. F., & Ewing, S. A. (2010). The natural history of Anaplasma marginale. *Veterinary Parasitology*, *167*(2–4), 95–107.

Kocan, K. M., De la Fuente, J., Guglielmone, A. A., & Meléndez, R. D. (2003). Antigens and alternatives for control of Anaplasma marginale infection in cattle. *Clinical Microbiology Reviews*, *16*(4), 698–712.

Laha, R., Das, M., & Sen, A. (2015). Morphology, epidemiology, and phylogeny of Babesia: An overview. *Tropical Parasitology*, *5*(2), 94.

Laisser, E. L. K., Chenyambuga, S. W., Karimuribo, E. D., Msalya, G., Kipanyula, M. J., Mwilawa, A. J., Mdegela, R. H., Kusiluka, L. J. M., & Gwakisa, P. S. (2016). *Tick burden and acquisition of immunity to Theileria parva by Tarime cattle in comparison to Sukuma cattle under different tick control regimes in the Lake Zone of Tanzania*.

Latif, A. A., & Pegram, R. G. (1992). Naturally acquired host resistance in tick control in Africa. *International Journal of Tropical Insect Science*, *13*(4), 505–513.

Lew, A. E., Bock, R. E., Minchin, C. M., & Masaka, S. (2002). A msp1α polymerase chain reaction assay for specific detection and differentiation of Anaplasma marginale isolates. *Veterinary Microbiology*, *86*(4), 325–335.

Löhr, C. V, Rurangirwa, F. R., McElwain, T. F., Stiller, D., & Palmer, G. H. (2002). Specific expression of Anaplasma marginale major surface protein 2 salivary gland variants occurs in the midgut and is an early event during tick transmission. *Infection and Immunity*, *70*(1), 114–120.

M'ghirbi, Y., Bèji, M., Oporto, B., Khrouf, F., Hurtado, A., & Bouattour, A. (2016). Anaplasma marginale and A. phagocytophilum in cattle in Tunisia. *Parasites & Vectors*, *9*, 1–8.

Magona, J. W., Walubengo, J., Olaho-Mukani, W., Jonsson, N. N., Welburn, S. C., & Eisler, M. C. (2008). Clinical features associated with seroconversion to Anaplasma marginale, Babesia bigemina and Theileria parva infections in African cattle under natural tick challenge. *Veterinary Parasitology*, *155*(3–4), 273–280.

Mahoney, D. F., & Goodger, B. V. (1969). Babesia argentina: serum changes in infected calves. *Experimental Parasitology*, *24*(3), 375–382.

Mahoney, D. F., & Ross, D. R. (1972). Epizootiological factors in the control of bovine babesiosis. *Australian Veterinary Journal*, *48*(5), 292–298.

Mahoney, D. F., Wright, I. G., & Mirre, G. B. (1973). Bovine babesiasis: the persistence of immunity to Babesia argentina and B. bigemina in calves (Bos taurus) after naturally acquired infection. *Annals of Tropical Medicine & Parasitology*, *67*(2), 197–203.

Malak, A. K., Mpoke, L., Banak, J., Muriuki, S., Skilton, R. A., Odongo, D., Sunter, J., & Kiara,

H. (2012). Prevalence of livestock diseases and their impact on livelihoods in Central Equatoria State, southern Sudan. *Preventive Veterinary Medicine*, *104*(3–4), 216–223.

Maloo, S. H., Ngumi, P., Mbogo, S., Williamson, S., Thorpe, W., Rowlands, G. J., & Perry, B. D. (2001). Identification of a target population for immunisation against East Coast fever in coastal Kenya. *Preventive Veterinary Medicine*, *52*(1), 31–41.

Maloo, S. H., Thorpe, W., Kioo, G., Ngumi, P., Rowlands, G. J., & Perry, B. D. (2001). Seroprevalences of vector-transmitted infections of small-holder dairy cattle in coastal Kenya. *Preventive Veterinary Medicine*, *52*(1), 1–16.

Mandal, S. C. (2006). Veterinary parasitology at glance 1 st edition. *International Book Distribution, UP: Indian Publishing*, 523–526.

Mans, B. J., Pienaar, R., & Latif, A. A. (2015). A review of Theileria diagnostics and epidemiology. *International Journal for Parasitology: Parasites and Wildlife*, *4*(1), 104–118.

Marana, E. R. M., Alfieri, A. A., de Andrade, G. M., Freire, R. L., Garcia, J. L., & Vidotto, O. (2006). Comparação dos testes sorológicos de Imunofluorescência Indireta, Conglutinação Rápida, ELISA indireto e ELISA por competição para a detecção de anticorpos contra o Anaplasma marginale em soros de bovinos de diferentes áreas enzoóticas. *Semina: Ciências Agrárias*, *27*(4), 629–637.

Marana, E. R. M., Dias, J. A., Freire, R. L., Vicentini, J. C., Vidotto, M. C., & Vidotto, O. (2009). Soroprevalência de Anaplasma marginale em bovinos da região Centro-Sul do estado do Paraná, Brasil, por um teste imunoenzimático competitivo utilizando proteína recombinante MSP5-PR1. *Revista Brasileira de Parasitologia Veterinária*, *18*, 20–26.

Marcellino, W. L., Julla, I. I., & Salih, D. A. (2011). Ticks infesting cattle in the Central Equatoria region of South Sudan. *Onderstepoort Journal of Veterinary Research*, *78*(1), 1–5.

Masiga, D., Ten Bosch, Q., Villinger, J., Koenraadt, C. J. M., & Kalayou, S. (2022). Epidemiology of tick-borne pathogens of cattle and tick control practices in coastal Kenya. *Preventive Veterinary Medicine*, *209*, 105777.

McCosker, P. J. (1981). The global importance of babesiosis. *Babesiosis*, 1–24.

Mehlhorn, H., & Schein, E. (1985). The piroplasms: life cycle and sexual stages. *Advances in Parasitology*, *23*, 37–103.

Merino, O., Almazán, C., Canales, M., Villar, M., Moreno-Cid, J. A., Galindo, R. C., & De la Fuente, J. (2011). Targeting the tick protective antigen subolesin reduces vector infestations and pathogen infection by Anaplasma marginale and Babesia bigemina. *Vaccine*, *29*(47), 8575–8579.

Miyama, T. (2020). Problem-oriented field epidemiological study in dairy production medicine using a causal inference approach. *(No Title)*.

Molad, T., Mazuz, M. L., Fleiderovitz, L., Fish, L., Savitsky, I., Krigel, Y., Leibovitz, B., Molloy, J., Jongejan, F., & Shkap, V. (2006). Molecular and serological detection of A. centrale-and A. marginale-infected cattle grazing within an endemic area. *Veterinary Microbiology*, *113*(1–2), 55–62.

Molloy, J. B., Bowles, P. M., Bock, R. E., Turton, J. A., Katsande, T. C., Katende, J. M., Mabikacheche, L. G., Waldron, S. J., Blight, G. W., & Dalgliesh, R. J. (1998). Evaluation

of an ELISA for detection of antibodies to Babesia bovis in cattle in Australia and Zimbabwe. *Preventive Veterinary Medicine*, *33*(1–4), 59–67.

Molloy, J. B., Bowles, P. M., Knowles, D. P., McElwain, T. F., Bock, R. E., Kingston, T. G., Blight, G. W., & Dalgliesh, R. J. (1999). Comparison of a competitive inhibition ELISA and the card agglutination test for detection of antibodies to Anaplasma marginale and Anaplasma centrale in cattle. *Australian Veterinary Journal*, *77*(4), 245–249.

Montenegro-James, S., Guillen, A. T., Ma, S. J., & Ristic, M. (1988). Use of the DOT-ELISA with isolated A. marginale initial bodies for the serodiagnosis of bovine anaplasmosis. *Am J Vet Res*.

Morzaria, S. P., Katende, J., Musoke, A., Nene, V., Skilton, R., & Bishop, R. (1999). Development of sero-diagnostic and molecular tools for the control of important tick-borne pathogens of cattle in Africa. *Parassitologia*, *41*, 73–80.

Mosqueda, J., Olvera-Ramirez, A., Aguilar-Tipacamu, G., & J Canto, G. (2012). Current advances in detection and treatment of babesiosis. *Current Medicinal Chemistry*, *19*(10), 1504–1518.

Mugisha, A., McLeod, A., Percy, R., & Kyewalabye, E. (2008). Socio-economic factors influencing control of vector-borne diseases in the pastoralist system of south western Uganda. *Tropical Animal Health and Production*, *40*, 287–297.

Muhammad, G., Naureen, A., Firyal, S., & Saqib, M. (2008). Tick control strategies in dairy production medicine. *Pakistan Veterinary Journal*, *28*(1), 43.

Muhanguzi, D., Byaruhanga, J., Amanyire, W., Ndekezi, C., Ochwo, S., Nkamwesiga, J.,

Mwiine, F. N., Tweyongyere, R., Fourie, J., & Madder, M. (2020). Invasive cattle ticks in East Africa: morphological and molecular confirmation of the presence of Rhipicephalus microplus in south-eastern Uganda. *Parasites & Vectors*, *13*(1), 1–9.

Muhanguzi, D., Matovu, E., & Waiswa, C. (2010). Prevalence and characterization of Theileria and Babesia species in cattle under different husbandry systems in western Uganda. *Int. J. Anim. Vet. Adv*, *2*(2), 51–58.

Muhanguzi, D., Picozzi, K., Hatendorf, J., Thrusfield, M., Welburn, S. C., Kabasa, J. D., & Waiswa, C. (2014). Prevalence and spatial distribution of Theileria parva in cattle under crop-livestock farming systems in Tororo District, Eastern Uganda. *Parasites & Vectors*, *7*, 1–8.

Mullen, G. R., & Durden, L. A. (2009). *Medical and veterinary entomology*. Academic press.

Nchu, F., Magano, S. R., & Eloff, J. N. (2012). In vitro anti-tick properties of the essential oil of Tagetes minuta L.(Asteraceae) on Hyalomma rufipes (Acari: Ixodidae). *Onderstepoort Journal of Veterinary Research*, *79*(1), 1–5.

Ngeranwa, J. J. N., Shompole, S. P., Venter, E. H., Wambugu, A., Crafford, J. E., & Penzhorn, B. L. (2008). Detection of Anaplasma antibodies in wildlife and domestic species in wildlife-livestock interface areas of Kenya by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, *75*(3), 199–205.

Nielsen, K., Yu, W. L., Kelly, L., Bermudez, R., Renteria, T., Dajer, A., Gutierrez, E., Williams, J., Algire, J., & Torioni de Eschaide, S. (2007). Development of a lateral flow assay for rapid detection of bovine antibody to Anaplasma marginale. *Journal of Immunoassay and*

Immunochemistry, *29*(1), 10–18.

Norval, R. A. I., & Lightfoot, C. J. (1982). Tick problems in wildlife in Zimbabwe. Factors influencing the occurrence and abundance of Rhipicephalus appendiculatus. *Zimbabwe Veterinary Journal*.

Norval, R. A. I., Perry, B. D., & Young, A. S. (1992). *The epidemiology of theileriosis in Africa*. ILRI (aka ILCA and ILRAD).

Okon, O. E., Opara, K. N., Etim, S. E., Arong, G. A., Oku, E. E., & Iboh, C. I. (2011). Experimental transmission of Babesia bigemina by Boophilus decoloratus in cattle. *Research Journal of Parasitology*, *6*(5), 168–175.

Okuthe, O. S., & Buyu, G. E. (2006). Prevalence and incidence of tick-borne diseases in smallholder farming systems in the western-Kenya highlands. *Veterinary Parasitology*, *141*(3–4), 307–312.

Oliveira-Sequeira, T. C. G., Oliveira, M. C. de S., Araujo Jr, J. P., & Amarante, A. F. T. (2005). PCR-based detection of Babesia bovis and Babesia bigemina in their natural host Boophilus microplus and cattle. *International Journal for Parasitology*, *35*(1), 105–111.

Olwoch, J. M., Reyers, B., Engelbrecht, F. A., & Erasmus, B. F. N. (2008). Climate change and the tick-borne disease, Theileriosis (East Coast fever) in sub-Saharan Africa. *Journal of Arid Environments*, *72*(2), 108–120.

Osaki, S. C., Vidotto, O., Marana, E. R. M., Vidotto, M. C., Yoshihara, E., Pacheco, R. C., Igarashi, M., & Minho, A. P. (2002). Occurrence of antibodies against Babesia bovis and studies on natural infection in Nelore cattle. *Umuarama Municipality, Paraná State, Brazil.*

Rev Bras Med Vet, *11*, 77–83.

Otim, C. P. (2000). Advances in disease control of tick and tick-borne diseases. *Uganda Journal of Agricultural Sciences*, *5*(1), 79–83.

Oura, C. A. L., Bishop, R. P., Wampande, E. M., Lubega, G. W., & Tait, A. (2004). Application of a reverse line blot assay to the study of haemoparasites in cattle in Uganda. *International Journal for Parasitology*, *34*(5), 603–613.

Pacheco, I., Prado, E., Artigas-Jerónimo, S., Lima-Barbero, J. F., de la Fuente, G., Antunes, S., Couto, J., Domingos, A., Villar, M., & de la Fuente, J. (2021). Comparative analysis of Rhipicephalus tick salivary gland and cement elementome. *Heliyon*, *7*(4).

Parizi, L. F., Reck Jr, J., Oldiges, D. P., Guizzo, M. G., Seixas, A., Logullo, C., de Oliveira, P. L., Termignoni, C., Martins, J. R., & da Silva Vaz Jr, I. (2012). Multi-antigenic vaccine against the cattle tick Rhipicephalus (Boophilus) microplus: a field evaluation. *Vaccine*, *30*(48), 6912–6917.

Perry, B. D. (1996). *Tick-borne disease control: the role of impact assessment*.

Perry, B. D., Musisi, F. L., Pegram, R. G., & Schels, H. F. (1985). Zambia: assessment of enzootic stability to tickborne diseases. *World Animal Review (FAO)*.

Perry, B. D., & Young, A. S. (1995). The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: the case of theileriosis. *Preventive Veterinary Medicine*, *25*(2), 107–120.

Potgieter, F. T., Sutherland, B., & Biggs, H. C. (1981). *Attempts to transmit Anaplasma marginale with Hippobosca rufipes and Stomoxys calcitrans*.

Punyua, D. K., & Hassan, S. M. (1992). The role of host management in tick population changes on Rusinga Island, Kenya. *Experimental & Applied Acarology*, *14*(1), 61–65.

Rajput, Z. I., Hu, S., Chen, W., Arijo, A. G., & Xiao, C. (2006). Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science B*, *7*(11), 912–921.

Rechav, Y. (1982). Dynamics of tick populations (Acari: Ixodidae) in the eastern Cape Province of South Africa. *Journal of Medical Entomology*, *19*(6), 679–700.

Reinbold, J. B., Coetzee, J. F., Sirigireddy, K. R., & Ganta, R. R. (2010). Detection of Anaplasma marginale and A. phagocytophilum in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. *Journal of Clinical Microbiology*, *48*(7), 2424–2432.

Richey, E. J. (1991). Bovine anaplasmosis. *American Association of Bovine Practitioners Conference Proceedings*, 3–11.

Riek, R. F. (1963). The control of ticks. *The Control of Ticks.*

Riek, R. F. (1966). Life cycle of Babesia argentina (Lignières, 1903)(Sporozoa: Piroplasmidea) in the tick vector Boophilus microplus (Canestrini). *Australian Journal of Agricultural Research*, *17*(2), 247–254.

Robbertse, L., Richards, S. A., & Maritz-Olivier, C. (2017). Bovine immune factors underlying tick resistance: integration and future directions. *Frontiers in Cellular and Infection Microbiology*, *7*, 522.

Romero-Salas, D., Mira, A., Mosqueda, J., García-Vázquez, Z., Hidalgo-Ruiz, M., Vela, N. A.

O., de León, A. A. P., Florin-Christensen, M., & Schnittger, L. (2016). Molecular and serological detection of Babesia bovis-and Babesia bigemina-infection in bovines and water buffaloes raised jointly in an endemic field. *Veterinary Parasitology*, *217*, 101–107.

Ross, J. P. J., & Löhr, K. F. (1968). Serological diagnosis of Babesia bigemina infection in cattle by the indirect fluorescent antibody test. *Research in Veterinary Science*, *9*(6), 557–563.

Rożej-Bielicka, W., Stypułkowska-Misiurewicz, H., & Gołąb, E. (2015). Human babesiosis. *Przegl Epidemiol*, *69*(3), 489–494.

Rubaire-Akiiki, C. M., Okello-Onen, J., Musunga, D., Kabagambe, E. K., Vaarst, M., Okello, D., Opolot, C., Bisagaya, A., Okori, C., & Bisagati, C. (2006). Effect of agro-ecological zone and grazing system on incidence of East Coast Fever in calves in Mbale and Sironko Districts of Eastern Uganda. *Preventive Veterinary Medicine*, *75*(3–4), 251–266.

Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G. W., Vaarst, M., Kabagambe, E. K., Mwayi, W., Musunga, D., & Wandukwa, W. (2004). The prevalence of serum antibodies to tick-borne infections in Mbale District, Uganda: The effect of agro-ecological zone, grazing management and age of cattle. *Journal of Insect Science*, *4*(1), 8.

Rymaszewska, A., & Grenda, S. (2008). Bacteria of the genus Anaplasma–characteristics of Anaplasma and their vectors: a review. *Vet Med*, *53*(11), 573–584.

Sackett, D., Holmes, P., Abbott, K., Jephcott, S., & Barber, M. (2006). Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers. *MLA Report AHW*, *87*.

Salih, D. A., El Hussein, A. M., & Singla, L. D. (2015). Diagnostic approaches for tick-borne

haemoparasitic diseases in livestock. *Journal of Veterinary Medicine and Animal Health*, *7*(2), 45–56.

Samish, M., Ginsberg, H., & Glazer, I. (2004). Biological control of ticks. *Parasitology*, *129*(S1), S389–S403.

Shyma, K. P., Stanley, B., Ray, D. D., & Ghosh, S. (2013). Prevalence of cattle and buffalo ticks in northern Kerala. *Journal of Veterinary Parasitology*, *27*(1), 55–56.

Silaghi, C., Santos, A. S., Gomes, J., Christova, I., Matei, I. A., Walder, G., Domingos, A., Bell-Sakyi, L., Sprong, H., & Von Loewenich, F. D. (2017). Guidelines for the direct detection of Anaplasma spp. in diagnosis and epidemiological studies. *Vector-Borne and Zoonotic Diseases*, *17*(1), 12–22.

Simuunza, M., Weir, W., Courcier, E., Tait, A., & Shiels, B. (2011). Epidemiological analysis of tick-borne diseases in Zambia. *Veterinary Parasitology*, *175*(3–4), 331–342.

Singh, H., Haque, M., Singh, N. K., & Rath, S. S. (2012). Molecular detection of Anaplasma marginale infection in carrier cattle. *Ticks and Tick-Borne Diseases*, *3*(1), 55–58.

Sitotaw, T., Regassa, F., Zeru, F., & Kahsay, A. G. (2014). Epidemiological significance of major hemoparasites of ruminants in and around Debre-Zeit, Central Ethiopia. *Journal of Parasitology and Vector Biology*, *6*(2), 16–22.

Smith, M. W. (1969a). Variations in tick species and populations in the Bugisu district of Uganda. Part I: the tick survey. *Bulletin of Epizootic Diseases of Africa*, *17*(1).

Smith, M. W. (1969b). Variations in tick species and populations in the Bugisu district of Uganda. Part II: the effects of altitude, climate, vegetation and husbandry an tick species

and populations. *Bulletin of Epizootic Diseases of Africa*, *17*(1).

Ssenyonga, G. S. Z., Kakoma, I., MONTENEGRO-JAMES, S., Nyeko, P. J., Nanteza, A., & Buga, R. (1992). Anaplasmosis in Uganda. II. Prevalence of bovine anaplasmosis. *Scandinavian Journal of Immunology*, *36*, 107–109.

Strik, N. I., Alleman, A. R., Barbet, A. F., Sorenson, H. L., Wamsley, H. L., Gaschen, F. P., Luckschander, N., Wong, S., Chu, F., & Foley, J. E. (2007). Characterization of Anaplasma phagocytophilum major surface protein 5 and the extent of its cross-reactivity with A. marginale. *Clinical and Vaccine Immunology*, *14*(3), 262–268.

Suarez, C. E., & Noh, S. (2011). Emerging perspectives in the research of bovine babesiosis and anaplasmosis. *Veterinary Parasitology*, *180*(1–2), 109–125.

Swai, E. S., French, N. P., Karimuribo, E. D., Fitzpatrick, J. L., Bryant, M. J., Brown, P. E., & Ogden, N. H. (2005). Spatial and management factors associated with exposure of smallholder dairy cattle in Tanzania to tick-borne pathogens. *International Journal for Parasitology*, *35*(10), 1085–1096.

Swai, E. S., Karimuribo, E. D., Kambarage, D. M., & Moshy, W. E. (2009). A longitudinal study on morbidity and mortality in youngstock smallholder dairy cattle with special reference to tick borne infections in Tanga region, Tanzania. *Veterinary Parasitology*, *160*(1–2), 34–42.

Tabor, A. E., Ali, A., Rehman, G., Rocha Garcia, G., Zangirolamo, A. F., Malardo, T., & Jonsson, N. N. (2017). Cattle tick Rhipicephalus microplus-host interface: a review of resistant and susceptible host responses. *Frontiers in Cellular and Infection Microbiology*, *7*, 506.

Tayebwa, D. S., Vudriko, P., Tuvshintulga, B., Guswanto, A., Nugraha, A. B., Gantuya, S., Batiha, G. E.-S., Musinguzi, S. P., Komugisha, M., & Bbira, J. S. (2018). Molecular epidemiology of Babesia species, Theileria parva, and Anaplasma marginale infecting cattle and the tick control malpractices in Central and Eastern Uganda. *Ticks and Tick-Borne Diseases*, *9*(6), 1475–1483.

Taylor N, R. J. & M. (1979). Preliminary observations on the combined use of imidocarb and Babesia blood vaccine in cattle. *Journal of the South African Veterinary Association*, *50*(4), 326–329.

Tebele, N., Skilton, R. A., Katende, J., Wells, C. W., Nene, V., McElwain, T., Morzaria, S. P., & Musoke, A. J. (2000). Cloning, characterization, and expression of a 200-kilodalton diagnostic antigen of Babesia bigemina. *Journal of Clinical Microbiology*, *38*(6), 2240–2247.

Terkawi, M. A., Thekisoe, O. M. M., Katsande, C., Latif, A. A., Mans, B. J., Matthee, O., Mkize, N., Mabogoane, N., Marais, F., & Yokoyama, N. (2011). Serological survey of Babesia bovis and Babesia bigemina in cattle in South Africa. *Veterinary Parasitology*, *182*(2–4), 337–342.

Teshale, S., Geysen, D., Ameni, G., Asfaw, Y., & Berkvens, D. (2015). Improved molecular detection of Ehrlichia and Anaplasma species applied to Amblyomma ticks collected from cattle and sheep in Ethiopia. *Ticks and Tick-Borne Diseases*, *6*(1), 1–7.

Thrusfield, M. (2018). *Veterinary epidemiology*. John Wiley & Sons.

Trueman, K. F., & Blight, G. W. (1978). The effect of age on resistance of cattle to Babesia bovis. *Australian Veterinary Journal*, *54*(6), 301–305.

Uzal, F. A., Carrasco, A. E., Nielsen, K., Echaide, S., & Cabrera, R. F. (1996). An indirect ELISA using a monoclonal anti IgG1 enzyme conjugate for the diagnosis of bovine brucellosis. *Veterinary Microbiology*, *52*(1–2), 175–180.

Vial, H. J., & Gorenflot, A. (2006). Chemotherapy against babesiosis. *Veterinary Parasitology*, *138*(1–2), 147–160.

Villar, M., Pacheco, I., Merino, O., Contreras, M., Mateos-Hernández, L., Prado, E., Barros-Picanço, D. K., Lima-Barbero, J. F., Artigas-Jerónimo, S., & Alberdi, P. (2020). Tick and host derived compounds detected in the cement complex substance. *Biomolecules*, *10*(4), 555.

Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D. S., Okech, S. G., Tweyongyere, R., Wampande, E. M., Okurut, A. R. A., Mugabi, K., & Muhindo, J. B. (2018). Chemical tick control practices in southwestern and northwestern Uganda. *Ticks and Tick-Borne Diseases*, *9*(4), 945–955.

Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D. S., Omara, R., Muhindo, J. B., Lagu, C., Umemiya-Shirafuji, R., Xuan, X., & Suzuki, H. (2018). Evidence-based tick acaricide resistance intervention strategy in Uganda: Concept and feedback of farmers and stakeholders. *Ticks and Tick-Borne Diseases*, *9*(2), 254–265.

Vudriko, P., Okwee-Acai, J., Tayebwa, D. S., Byaruhanga, J., Kakooza, S., Wampande, E., Omara, R., Muhindo, J. B., Tweyongyere, R., & Owiny, D. O. (2016). Emergence of multi-acaricide resistant Rhipicephalus ticks and its implication on chemical tick control in Uganda. *Parasites & Vectors*, *9*(1), 1–13.

Wesonga, F. D., Gachohi, J. M., Kitala, P. M., Gathuma, J. M., & Njenga, M. J. (2017).

Seroprevalence of Anaplasma marginale and Babesia bigemina infections and associated risk factors in Machakos County, Kenya. *Tropical Animal Health and Production*, *49*(2), 265–272.

Wesonga, F. D., Kitala, P. M., Gathuma, J. M., Njenga, M. J., & Ngumi, P. N. (2010). *An assessment of tick-borne diseases constraints to livestock production in a smallholder livestock production system in Machakos District, Kenya*.

Willadsen, P., Bird, P., Cobon, G. S., & Hungerford, J. (1995). Commercialisation of a recombinant vaccine against Boophilus microplus. *Parasitology*, *110*(S1), S43–S50.

Wodajnew, B., Disassa, H., Kabeta, T., Zenebe, T., & Kebede, G. (2015). Study on the prevalence of bovine babesiosis and its associated risk factors in and around Assosa Woreda, Benishangul Gumuz regional state, western Ethiopia. *Researcher*, *7*(8), 33–39.

Wray, K., Musuka, G., Trees, A. J., Jongejan, F., Smeenk, I., & Kelly, P. J. (2000). Babesia bovis and B. bigemina DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. *Journal of the South African Veterinary Association*, *71*(1), 21–24.

Wright, I. G., Goodger, B. V, & Clark, I. A. (1988). Immunopathophysiology of Babesia bovis and Plasmodium falciparum infections. *Parasitology Today*, *4*(8), 214–218.

Young, A. S., Groocock, C. M., & Kariuki, D. P. (1988). Integrated control of ticks and tick-borne diseases of cattle in Africa. *Parasitology*, *96*(2), 403–432.

Zaugg, J. L., & Kuttler, K. L. (1984). Bovine anaplasmosis: in utero transmission and the immunologic significance of ingested colostral antibodies. *American Journal of Veterinary Research*, *45*(3), 440–443.

Zintl, A., Gray, J. S., Skerrett, H. E., & Mulcahy, G. (2005). Possible mechanisms underlying age-related resistance to bovine babesiosis. *Parasite Immunology*, *27*(4), 115–120.

APPENDICES

Appendix 1: Closed – ended questionnaires for study of Anaplasma (Mbenek) and Babesia (Nado kulak) infections in Narok County – 2023

Date of interview (DD/MM/YY) _____/_____/_____

Ward_____ Sublocation_____

Village_____ Household ID_____

CALF CODE:_____

Respondent details

1. Is the respondent the household head
   i) Yes ii) No
2. If answer is no, what is the relationship of the respondent of the respondent to the household head?
   i) Spouse ii) Son/daughter iii) Brother/sister iv) Uncle/aunt v) Nephew/niece
   vi) Grandchild vii) Other (specify)_____
3. Age set of respondents (years)
   i) 20-30 31-40 ii) 41-50 iii) 51-60 iv) 61-70 v) >71
4. Gender of respondent
   i) Male ii) Female
5. Marital status of respondent
   i) Single ii) Married iii) Widow iv) Widower v) Divorced vi) Separated
6. Level of education of respondent
   i) None ii) Preschool Primary education iii) Primary (not completed)
   iv) Secondary education v) Secondary (not completed) vi) Technical /vocational
   vii) Tertiary viii) Tertiary (not completed) ix) Adult education
7. What is the religion of the respondent
   i)Christian ii) Muslims iii) Hindu iv) Traditionalist
   v) Other_____
8. Primary occupation of respondent
   i)Livestock keeping only (pastoralist) ii) Mixed farming (crops and livestock)
   iii) Employed full time iv) Employed part time v) Business person
   vi) Tree crop production vii) Fishing viii) Livestock herding ix) Petty trading

x) Student xi) Others_____

Animal ownership

9. Animals present on the farm and indicate the number and in terms of sex and age

	Animal	Age category	Number
i.	Grade/exotic cattle	< 1 year	
		1-2 years	
		>2 years	
ii.	Grade/exotic cross breed	< 1 year	
		1-2 years	
		>2 years	
iii.	Indigenous/local breed cattle	< 1 year	
		1-2 years	
		>2 years	
iv.	Sheep		
v.	Goats		
vi.	Camels		
vii.	Pigs		
viii.	Chicken		
ix.	Donkeys		
x.	Rabbits		
xi.	Ducks		
xii.	Dogs		
xiii.	Cats		

Livestock production system

10. How long have you been involved in livestock keeping farming
    i) <5 years ii) >5-10 years iii) >11-20 years iv) >20 years

11. Who looks after the animals
    i) Owner ii) Wife iii) Children iv) Employee

12. Do animals interact with wildlife
    i) Yes ii) No

13. If yes, list the wildlife animals
    _____

14. Cattle production system management employed
    i) Communal grazing ii) Roadside grazing iii) Zero grazing iv) Private grazing
    iii) Pastoralism iv) Other_____
15. How do you feed your cattle?
    i) Herded ii) Paddock iii) Tethered iv) Stall fed v) Yard vi) Free grazing
    vii) Other_____
16. If you practice private grazing, what is the size in acres of grazing area? _____
17. Are calves grazed/fed together with adult cattle?
    i) Yes ii) No
18. Do cattle, sheep and goats graze together?
    i) Yes ii) No
19. If no, where do you sheep and goats graze?
    i) Within compound ii) Alongside the roadside iii) In a communal grazing field
    iv) On leased grounds v) Other _____
20. Do you graze your animals with other people's animals?
    i) Yes ii) No
21. Source of fodder fed to the calves on the farm
    i) Outsourcing from the roadsides ii) Outsourcing from near water source
    iii) Own grown fodder in farm iv) From neighborhood v) Purchased from far
    vi) Other_____
22. If not grown within the farm, what is the cost per month (in KSh) of purchasing forage for animals? _____
23. If not grown within farm, do you think the "cut and carry" forage introduces ticks to your herd?
    i) Yes ii) Don't know iii) No
24. If yes, what makes you think so?
    i) Animals get ticks afterwards ii) Animals contract anaplasma and babesia infections
    iii) Other _____
25. Type of fodder do you give your cattle
    i) Pasture ii) Napier grass iii) Grass silage iv) Whole plant maize silage
    v) Grass hay vi) Desmodium vii) Sweet potato vines viii) Other high protein forages-
    lucerne, leucana ix) Tree fodders x) Maize stover xi) Banana leaves xii) Dairy

meal xiii) Wheat bran Maize germ Vitamin/mineral powder Banana stems

Other_____

26. How do the calf mainly access drinking water
    i) Water provided in the housing area ii) Water available in pasture/during grazing

    iii) Water available in stream/river away from farm

    iv) Other_____

27. What is the source of animals' drinking water
    i) Borehole ii) Dam/pond iii) River iv) Water well v) Spring

    vi) Piped/municipal connection

    vii) Other _____

28. If not provided in housing area, how far do animals travel for water?
    i) Below 0.5 km ii) 0.5-1 km iii) 1-2 km iv) 2-3 km v) Above 3 km

29. How often are animals provided with drinking water?
    i) Freely available ii) Once a day iii) Twice a day iv) Every other day

    v) Once in 3 days iv) Other _____

30. Type of shelter available for animals
    i) Closed ii) Open (no walls, with a roof) iii) Open (no walls, no roof)

    iv) Other_____

31. What material has been used for animal shelter?
    i) Untreated wood/bush ii) Treated wood iii) Thatch iv) Iron sheets iv) Bricks/stone

    v) Mud/earth vi) Wire vii) Other _____

32. Cowshed cleaning
    i) Present ii) Absent

33. If yes, specify which disinfectant is used for cleaning? _____

Veterinary services

34. What associations are you a member of?
    i) None ii) Kenya livestock marketing council iii) Kenya camel association
    iv) Kenya livestock producers' association v) Kenya poultry farmers association
    vi) Dairy goat association of kenya vii) Dairy cows' association
    viii) Other_____
35. Who provides veterinary interventions for the calves?
    i) Animal health specialist ii) Self iii) Neighbour/another farm iv) Community health worker v) Other_____
36. How far do you live from the nearest veterinary establishment (office, agrovet, vet/paravet)
    i) 0-5 km ii) 5-10 km iii) More than 10 km
37. Frequency of veterinary services
    i) Regularly ii) Only in clinical cases iii) For vaccination iv) For artificial insemination v) Never
38. Where do you source veterinary drugs and products including the acaricides?
    i) Agrovet ii) Distributed by pharmaceutical company iii) Local shop iv) Vet/paravet v) Open- air market vi) Other _____
39. What is the cost/month of accessing veterinary services or purchasing veterinary drugs and products in the last one month? _____
40. What are the sources of information about livestock keeping including advice on tick control?
    i) Veterinary Department ii) NGOs iii) Farmer to farmer training iv) Internet
    v) Veterinarians vi) Peers vii) Researcher viii) Agrovet attendants
    ix) Others (specify) _____
41. Do you perform any ectoparasite control in your herd?
    i) Yes ii) No
42. Type of acaricide used (take a picture of the acaricide product)
    _____
43. What is the cost of parasite control in your head per month in Kenyan shillings?
    _____

44. In the last one month, how many times have you done parasite control?
    i) 1 ii) 2 iii) 3 iv) 4 v) none
45. How often do you do ectoparasite control in the dry season?
    i) Weekly ii) Every 2 weeks iii) Monthly

    iv) Other _____

46. How often do you do ectoparasite control in the wet season?
    i) Weekly ii) Every 2 weeks iii) Monthly

    iv) Other _____

47. Do you normally change the acaricide used on your head?
    i) Yes ii) No

48. If yes, what informs the decision to change the acaricide?
    i) Tick on cattle ii) Season iii) Advertisement of new product iv) Anaplasmosis and babesiosis outbreak v) Cost of acaricide vi) Current acaricide not having 'knockdown' effect on ticks vii) Government directive viii) Advice from animal health specialist ix) Recommendation from neighbor/friend

    x) Other_____

49. Do you follow manufacturer's recommendations on acaricide dilution rate?
    i) Yes ii) No

50. Acaricide application method
    i) Handspray ii) Pour-on iii) Dipping iv) Spraying (specific body parts)

    v) Spraying (whole body) vi) Handpicking vii) Injectables viii) Traditional methods

    ix) Other_____

51. Have you ever used knapsack spraying?
    i) Yes ii) No

52. If yes, how many animals do you spray with a full knapsack sprayer?
    i) 1-5 ii) 6-10 iii) 11-15 iv) More than 15

53. Is there communal dip in your village?
    i) Yes ii) No

54. If yes, do you take your animals to communal dip for dipping?
    i) Yes ii) No

55. If no, why don't you use the communal dip?
    i) Distance ii) High dipping charges iii) Ticks don't fall after dipping iv) Poor dip management v) Risk of animals contracting disease from others vi) No defined stock routes

56. If the reason for not using the communal dip is distance, how far (in minutes) does it take to get to the communal dip from your home? _____

57. Do you vaccinate your animals?
    i) Yes ii) No

58. If yes, what diseases do you vaccinate your animals against?
    _____
    _____
    _____

59. Do you know of Babesia (Nado kulak) and Anaplasma (Mbenek) infections?
    i) Yes ii) No

60. How do you breed your cattle?
    i) Own bull ii) Shared bull iii) Artificial insemination iv) Synchronization v) Other_____

61. If by shared bull, how is this done?
    i) Borrowed bull from other herd ii) Send cow elsewhere for servicing

62. If by artificial insemination, who provides the services?
    i) Government inseminator ii) Private inseminator

Herd health and dynamics

63. In the last 1 month have you had any of the following?
    i) Animal births ii) Animal deaths iii) Animals slaughtered iv) Animals sold

    v) Animals given away vi) Animals lost/stolen vii) Animals purchased

    vii) Animals received (gifts)

64. Introduction process of new animals
    i) Isolate and quarantine ii) Join herd directly

    iii) Other _____

Anaplasma and Babesia infections

65. Are you aware of Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) Yes ii) No

66. If yes, how did you know about Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) Death of animal ii) Media iii) Baraza iv) Neighbour v) Extension services

    vi) Animal health specialist

    vii) Other _____

67. Do you know some of the clinical signs of Anaplasma (Mbenek) and Babesia (Nado kulak) clinical disease?
    i) Red urine ii) Lethargy iii) Reluctance to move iv) Dyspnoea v) Loss of weight

    vi) Anorexia vi) Constipation vii) Hard feces viii) Enlarged gall bladder

    ix) Rough haircoat x) Abortion/still birth

    xi) Others _____

68. Do you know some of the clinical signs of Anaplasma (Mbenek) and Babesia (Nado kulak) clinical disease?
    i) Red urine ii) Lethargy iii) Reluctance to move iv) Dyspnoea v) Loss of weight

    vi) Anorexia vii) Constipation viii) Hard feces ix) Enlarged gall bladder x)

    Rough haircoat xi) Abortion/still birth xii) Others _____

69. How do your animals get infected with Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) Ticks ii) Animal-animal interaction iii) Environment iv) Other_____

70. What type of tick causes anaplasma (Mbenek) and babesia (Nado kulak) infections
    i) Blue tick ii) Brown tick iii) Red spotted tick iv) Don't know

71. Is there a history of anaplasma (Mbenek) and babesia (Nado kulak) infections
    i) Yes ii) No

72. Have you had anaplasma (Mbenek) and babesia (Nado kulak) infections within the herd within the last 4 months?
    i) Yes ii) No

73. If yes, record the number of affected animals:

Animals	Number sick with anaplasma (Mbenek) and babesia (Nado kulak) in the last one month
Local breed adult cattle (> 1 year)	
Local breed juvenile cattle (<1 year)	
Exotic breed adult cattle (>1 year)	
Exotic breed juvenile cattle (< 1 year)	
Mixed breed adult cattle (>1 year)	
Mixed breed juvenile cattle (<1 year)	

74. Have you had anaplasma (Mbenek) and babesia (Nado kulak) infections within the herd within the last 12 months?
    i) Yes ii) No

75. If yes, record the number of affected animals:

Animals	Number sick with anaplasma (Mbenek) and babesia (Nado kulak) in the last 12 months
Local breed adult cattle (> 1 year)	
Local breed juvenile cattle (<1 year)	
Exotic breed adult cattle (>1 year)	
Exotic breed juvenile cattle (< 1 year)	
Mixed breed adult cattle (>1 year)	
Mixed breed juvenile cattle (<1 year)	

76. How many animals died because of Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) <10 ii) 10-20 iii) 20-30 iv) 30-40 v) >50
77. Do you know of the seasonality of Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) Immediately after rainy seasons ii) During rainy seasons iii) During dry seasons

    iv) Immediately after dry season

78. How do you think the two diseases got into your herd?
    i) Neighbour's cattle ii) Wildlife iii) Communal grazing iv) Watering points

    v) Live animal market vi) Other _____

79. What did you try to stop the spread of Anaplasma (Mbenek) and Babesia (Nado kulak) infections?
    i) Separated sick animal ii) Consulted animal health iii) Consulted someone else

    iv) Self-treated v) Slaughtered sick animal vi) Sold sick animal vii) Nothing

    viii) Other _____

Calf ID _____

1. Breed

   i) Boran ii) Sahiwal iii) Friesian iv) Guernsey v) Ayrshire vi) Jersey

   vii) Cross – local and local viii) Cross – exotic and exotic ix) Cross- exotic and indigenous x) Indigenous xi) Zebu xii) Others _____

2. Age (months)_____
3. Sex of calf

   i) Male ii) Female

4. Body condition score

   i) 1 ii) 2 iii) 3 iv) 4 v) 5

5. Is the calf still suckling

   i) Yes ii) No

6. In the last one month, where has the calf been kept at night?

   i) Indoors ii) Outdoors

7. In the last one month, what type of shelter has been available for the calf at night?

   i) Closed ii)Open (no walls, with a roof) iii)Open (no walls, no roof)

   iv) Other (specify)

8. In the last one month, what materials have been used for calf shelter at night?

   i) Untreated wood/bush ii) Treated wood iii) Thatch iv) Iron sheets

   v) Bricks/stone vi) Mud/earth vii) Wire viii) No shelter

   ix) Other (specify)

9. In the last one month, what have you fed the calf with?

   i) Pasture ii) Maize stalks iii) Cut grass iv) Nappier grass

   v) Banana stems vi) Hay vii) Dairy meal viii) Mineral supplements ix) Silage x) Other (specify)

10. What is the source of forage/feed for calf?

    i) Grown within the farm ii) From neighborhood iii) Imported from far

    iv) Purchased v) Other (specify)

11. In the last one month, how has the calf mainly accessed drinking water?

i) Water provided in the housing area ii) Water available in pasture/during grazing iii) Water available in stream/river away from farm iv)Other (specify)

12. In the last one month, have you performed ectoparasite control for calf?

i) Yes ii) No

13. What methods of parasites control did you use? (Select all that apply)

i) Pour on ii) Dipping iii) Spraying (specific body parts) iv) Spraying (whole body) v) Hand picking/dressing vi) Injectables vii) Traditional methods viii) Other (specify)

14. Please specify the name of acaricide _____

15. Where did you source the acaricide? (Select all that apply)

i) Agrovet ii) Distributed by pharmaceutical company iii)Local shop iv) Vet/paravet v) Open-air market vi) Other (specify)

16. In the last one month, has the calf been dewormed?

i) Yes ii) No

17. Please specify the product used for deworming _____

18. In the last one month, has the calf been vaccinated?

i) Yes ii) No

19. If yes, which disease/s has the calf vaccinated against? (Select that apply)

i) Anthrax (Empuruo) ii) Black quarter (enkeya engeju)

iii) Brucellosis iii) Contagious bovine pleuropneumonia iv) East Coast Fever v) Foot and mouth disease (Olkirobi) vi) Lumpy Skin Disease (Olomorooj loo nkishu) vii) Rabies (Ngarasa/kichaa) viii) Rift valley Fever (Enkeeya Sikitoi) ix) Other (specify)

20. In the last one month, has the calf experienced ill health?

i) Yes ii) No

21. If yes, what symptoms were observed in the calf?

i) Decreased appetite /inappetence ii) Loss of body condition

iii) Coughing/nasal discharge /difficulty in breathing iv) Diarrhoea v) Lameness/recumbency/ inability to move vi) Circling/head pressing/ aggression/ incoordination vii) Hair loss/itching/ lumps viii) Bloody urine/abnormal vaginal/ preputial discharges/scrotal swelling ix) Corneal opacity x) Rough haircoat xi) Fever xii) Swollen lymph nodes xiii) Reddened mucus membranes (eye, tongue, gums, vulva) xiv) Other (specify)

22. If yes, do you know the disease the calf suffered from?

i) Yes ii) No

23. If yes, what disease did the calf suffer from?

i) Anaplasmosis (imbenek) ii) Anthrax (empuruo) iii) Babesiosis (nado kulak) iv) Black quarter (enkeya engeju) iv) Bovine ephemeral fever v) Body wounds /resemble LSD (enkeeya omunyi/ engoroto) vi) Brucellosis vii) Contagious Bovine Pleuropneumonia (Olkipei loo nkishu) viii) East coast fever (oltikana) ix) Ephemeral fever (Kububou) x) Foot and mouth disease (Olkirobi) xi) Heartwater (Olmilo) xii) Helminthosis (Enkinyoot) xiii) Hemorrhagic septicemia (osertet) xiv) Lumpy skin disease (Olomorooj loo nkishu) xv) Malignant catarrhal Fever (Emboroto) xvi)Rabies (Ngarasa/kichaa) xvii) Rift Valley Fever (Enkeeya Sikitoi) xviii) Scabies / mange (Oloondapan) xix) Streptothricosis xx) Ticks (Ilmasherr) xxi) Trypanosomiasis xxii) Other (specify)

24. Was the sick calf treated?

i) Yes ii) No

25. If yes, who provided the intervention/s for calf?

i) Animal health specialist ii) Self iii) Neighbour iv) Community health worker v) Other (specify)

26. What is the current status of calf?

i) Recovered ii) Still sick

27. Source of the calf

i) homebred ii) brought-in iii) Other _____

28. If the calf is brought in, what is the source of the calf?

i) Local market ii) Neighborhood iii) Neighboring location/subcounty

iv) N/A v) Other _____

29. In the last one month has the calf's dam experienced ill health?

i) Yes ii) No

30. If yes, do you know the disease the dam suffered from?

i) Yes ii) No

31. If yes, what disease did the dam suffer from? (tick all that apply)

i) Anaplasmosis (imbenek) ii) Anthrax (empuruo) iii) Babesiosis (nado kulak) iv) Black quarter (enkeya engeju) iv) Bovine ephemeral fever v) Body wounds /resemble LSD (enkeeya omunyi/ engoroto) vi) Brucellosis vii) Contagious Bovine Pleuropneumonia (Olkipei loo nkishu) viii) East coast fever (oltikana) ix) Ephemeral fever (Kububou) x) Foot and mouth disease (Olkirobi) xi) Heartwater (Olmilo) xii) Helminthosis (Enkinyoot) xiii) Hemorrhagic septicemia (osertet) xiv) Lumpy skin disease (Olomorooj loo nkishu) xv) Malignant catarrhal Fever (Emboroto) xvi)Rabies (Ngarasa/kichaa) xvii) Rift Valley Fever (Enkeeya Sikitoi) xviii) Scabies / mange (Oloondapan) xix) Streptothricosis xx) Ticks (Ilmasherr) xxi) Trypanosomiasis xxii) Other (specify)

32. Was the dam treated?

i) Yes ii) No

33. If yes, who provided the intervention/s for the dam?

i) Animal health specialist ii) Self iii) Neighbour iv) Community health worker v) Other (specify)

34. What is the current status of the dam?

i) Recovered ii) Still sick

CALF VITAL PARAMETERS

Weight in kgs _____Temperature _____

Respiration _____ Mucus membrane _____

Ticks

Head _____

Neck _____

Flank _____

Perineum _____

Extremities_____

Other ectoparasites _____

Significant physical findings of calf_____

Appendix 2: Ethical approval for the research study

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2023/417

Dr. Naomi Kibet
Dept. of Clinical Studies
University of Nairobi
03/01/2023

Dear Naomi,

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

Prevalence of *Anaplama* and *Babesia* infections in calves and their associated risk factors in Narok County, Kenya

Naomi Kibet J56/41561/2021

We refer to your proposal submitted to our committee for review and your application letter dated 14th Dec 2022. We have reviewed your application for ethical clearance.

The number of calves and protocols that will be used to assess prevalence of *Anaplasma* and *Babesia* infections and their associated risk factors meets the minimum standard of the Faculty of Veterinary medicine ethical regulation guidelines.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi