EPIDEMIOLOGY AND TICK-BORNE HAEMOPARASITE DIVERSITY AMONGST TRANSHUMANT ZEBU CATTLE IN KARAMOJA REGION, UGANDA

By

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Submitted in fulfilment of the requirements for the degree of **Philosophiae Doctor** (Veterinary Science) in the Faculty of Veterinary Science, University of Pretoria

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DECLARATION

I declare that this thesis, which I hereby submit for the degree **Philosophiae Doctor** at the University of Pretoria, is my original research work and has not previously been submitted by me for an award at any other University or institution.

…….

Charles Byaruhanga

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LIST OF ABBREVIATIONS

°C	degrees celsius
µl	microlitre
ANOVA	analysis of variance
APUA	Alliance for the Prudent Use of Antibiotics
ARC-OVI	Agricultural Research Council - Onderstepoort Veterinary Institute
ATAAS	Agricultural Technology and Agribusiness Advisory Services
BLAST	Basic Local Alignment Search Tool
bp	base pair
CAHW	community-based animal health worker
CAT	card agglutination test
CBPP	contagious bovine pleuropneumonia
cELISA	competitive enzyme-linked immunosorbent assay
CI	confidence interval
CTTBD	Centre for Ticks and Tick-borne Diseases
DAFF	Department of Agriculture, Forestry and Fisheries
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
dUTP	deoxyuridine triphosphate
DVO	district veterinary officer
ECF	East Coast fever
FAO	Food and Agriculture Organisation
FITC	fluorescein isothiocyanate
GALVmed	Global Alliance for Livestock Veterinary Medicines
IBM SPSS	International Business Machines - Statistical Package for the Social Sciences
IFAT	indirect fluorescent antibody test
IICD	Institute for International Cooperation and Development
ITM	infection and treatment method
LAMP	loop-mediated isothermal amplification

MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MAP1	microtubule-associated protein 1
MEGA	Molecular Evolutionary Genetics Analysis
ml	millilitre
mm	millimetre
MSP	major surface protein
MPSP	major piroplasm surface protein
NARO	National Agricultural Research Organisation
NGO	non-governmental organisation
OD	optical density
OIE	World Organisation for Animal Health
OR	odds ratio
PAUP	Phylogenetic Analysis Using Parsimony
PCR	polymerase chain reaction
PE	participatory epidemiology
PRA	participatory rural appraisal
qPCR	quantitative real-time polymerase chain reaction
qRT-PCR	quantitative real-time reverse transcriptase polymerase chain reaction
REC	Research Ethics Committee
RLB	reverse line blot
rRNA	ribosomal ribonucleic acid
SNV	Netherlands Development Organisation
SSU	small subunit
TBD	tick-borne disease
UBOS	Uganda Bureau of Statistics
UDG	uracil-DNA glycosylase
UNCST	Uganda National Council for Science and Technology
USA	United States of America
UV	ultraviolet

THESIS SUMMARY

Epidemiology and tick-borne haemoparasite diversity amongst transhumant Zebu cattle in Karamoja region, Uganda

Candidate: Dr Charles Byaruhanga

Supervisor: Prof Marinda Oosthuizen

Co-supervisors: Prof Darryn Knobel and Dr Nicola Collins

Department: Veterinary Tropical Diseases

Degree: PhD

This study was conducted to understand the occurrence, diversity and epidemiology of tick-borne diseases (TBDs) in a transhumant pastoral area of Karamoja Region, Uganda. We used participatory epidemiology (PE), involving focus group discussions (n = 24) with livestock keepers, 30 key informant interviews, review of previous surveillance data, clinical examinations, and laboratory confirmation of cases of TBDs, to define and prioritise cattle diseases, evaluate current control activities, and identify constraints to the control of TBDs. The livestock keepers regarded TBDs, particularly East Coast fever (ECF) and anaplasmosis, as the most important health problems in their cattle, based on morbidity and mortality rates, rates of transmission, treatment costs, difficulty in accessing the correct treatment, difficulty to control, and inadequate knowledge to manage the diseases. The main constraints to the control of TBDs were inadequate knowledge, inadequate veterinary services and limited availability of drugs. Hand picking of ticks was done by all pastoralists while hand spraying was done with under-strength acaricides, often at irregular intervals and with little acaricide wash. We determined the endemic status of TBDs in 20 randomly-selected cattle herds by estimating the proportion of annual ECF and anaplasmosis cases in different age groups of cattle using participatory approaches and clinical examinations, determining the diversity and abundance of ticks (161 cattle in 20 herds), and establishing the seroprevalence of antibodies

to *Theileria parva* and *Anaplasma marginale* among cattle (n = 397 in 20 herds). Clinical examinations and informant interviews showed that TBDs affected all age groups of cattle. Two-thirds of the cattle were infested with moderate (37.3%, 11-50 ticks) to abundant (28.6%, > 50 ticks) numbers of ticks. Out of the 10,923 ticks collected, *Rhipicephalus appendiculatus* (54.4%) was the most abundant species followed by *R. decoloratus* (17.7%), *Amblyomma variegatum* (12%) and *A. lepidum* (11.6%). We collected tick species that are either rare in Uganda (*A. lepidum*, *Hyalomma truncatum*, *A. gemma*) or were not known to be present in the country (*R. pulchellus*). The true seroprevalence of antibodies to *A. marginale* was high (95.1%, 95% confidence interval [CI] 91.3% - 98.5%), while that of *T. parva* was low (16.5%, 95% CI 12.9% - 19.6%). We determined the presence of tick-borne haemoparasites among cattle and the prevalence of infections using reverse line blot (RLB) hybridisation and quantitative real-time polymerase chain reaction (qPCR), and characterised *Theileria* and *Babesia* species by sequence and phylogenetic analyses of the full-length 18S rRNA gene. The RLB hybridisation assay demonstrated the presence of tick-borne haemoparasites in most cattle (99.6%), mostly as mixed infections (97.5%). The most frequently-detected species were *Theileria mutans* (88.3%, 95% CI: 84.6-92.1%), *A. marginale* (73.8%: 68.3-79.2%), *T. velifera* (71.3%: 65.8-76.7%) and *Anaplasma* sp. Omatjenne (63.3%: 57.5-68.8%). Other virulent pathogens, namely *Babesia bigemina* (5.0%) and *T. parva* (2.9%), were also detected with RLB, but *Ehrlichia ruminantium* was not detected. The proportions of qPCR positive samples were 82.9% (*A. marginale*), 12.1% (*A. centrale*), 3.3% (*T. parva*), and 1.7% (*E. ruminantium*). Variations (5 to 9 nucleotides) in the 18S rRNA gene sequences of *B. bigemina* were identified as compared with previously published sequences. We assessed the 16S rRNA gene phylogeny of *Anaplasma* species from cattle and analysed the *msp1α* gene sequences of *A. marginale* to identify genotypes. There was genetic heterogeneity within *A. marginale* in cattle in the study area. Most A. *marginale* sequences (16/19) were closely related (99-100% identities) and clustered with *A. marginale* strain Veld from cattle in South Africa, with strong bootstrap support, while three sequences clustered (100% identity) with *A. marginale* strain from Virginia, USA. The *A. centrale* sequences were closely related (100% identity) and clustered with the Israel vaccine strain. We found four different kinds of MSP1a tandem repeat sequences (UP39-F-M^2-3) that correspond to one *A. marginale* strain unique to Uganda. One tandem repeat (UP39) was unique to Karamoja cattle. The findings from this

study provide knowledge on the diversity and epidemiology of TBDs, which can be used to support diagnosis and strategic control of TBDs, and consequently improve cattle productivity and the livelihoods of pastoralists. More effective control and prevention measures against ticks and TBDs should urgently be implemented in the region. There is need to facilitate and promote immunisation of cattle against ECF. Given the scarcity of veterinarians in the region, the activities of community-based animal health workers [CAHWs] should be strengthened. The local governments in Karamoja Region should facilitate a more favourable business environment to attract private companies, who could scale-up veterinary drug supplies to the region. Pastoralists should be sensitised to improve their ecological knowledge of the seasonal activity of ticks for strategic tick control.

CHAPTER 1

1. General Introduction

1.1 Background

Livestock are the main source of wealth for pastoralists in eastern Africa, in addition to their social and cultural functions (Onono et al., 2015). However, tick-borne diseases (TBDs) inflict substantial economic losses on livestock production and resource use, thereby impacting the livelihoods of pastoralists (Kivaria, 2006; Ocaido et al., 2009a; Marcellino et al., 2011; Kasozi et al., 2014; Laisser et al., 2015). Losses directly attributed to TBDs include mortality, production losses, and costs of diagnosis, treatment and tick control (Kivaria, 2006; Jonsson et al., 2008; Ocaido et al., 2009a). In Tanzania, for example, the total annual national loss due to TBDs was estimated to be US $364 million, including an estimated mortality of 1.3 million cattle (Kivaria, 2006). In Uganda, 75.4% of losses in cattle were attributable to ticks and TBDs (Ocaido et al., 2009b), and the costs for controlling ticks and TBDs in cattle constituted 86% of the total disease control costs (Ocaido et al., 2009a). Tick-borne diseases also constrain the improvement of the local breeds of cattle in Africa because of the high levels of mortality in exotic (*Bos taurus*) and crossbred cattle (Uilenberg, 1995; Minjauw and McLeod, 2003; Muhanguzi et al., 2010b). The most important TBDs that affect cattle in eastern Africa are theileriosis, anaplasmosis, babesiosis and ehrlichiosis (Kivaria, 2006; Gachohi et al., 2010; Kasozi et al., 2014; Laisser et al., 2015).

Theileriosis is caused by parasites of the genus *Theileria*, which are obligate intracellular protozoa that belong to the phylum Apicomplexa, order Piroplasmida and family Theileriidae (Levine et al., 1980; Norval et al., 1992). The most pathogenic and economically important *Theileria* species in cattle are *T. parva* and *T. annulata* (Norval et al., 1992). Benign forms of theileriosis are caused by *T. mutans*, *T. taurotragi* and *T. velifera*, which occur mainly in Africa, while *T. sergenti*, *T. buffeli* and *T. orientalis* occur worldwide (Uilenberg, 1981). *Theileria parva* causes East Coast fever (ECF), Corridor disease and January disease in eastern, central and southern Africa, and is transmitted by the ticks *Rhipicephalus appendiculatus*, *R. zambeziensis*

and *R. duttoni* (Lawrence et al., 2004a; Lawrence et al., 2004b). In eastern Africa, *T. parva* is transmitted between infected and susceptible cattle by *R. appendiculatus*. The infection causes an acute, usually fatal lymphoproliferative disease, ECF (Norval et al., 1992; Oura et al., 2011; Muhanguzi et al., 2014). The disease is more severe in exotic and crossbred cattle, but also in indigenous calves and adult cattle in endemically unstable areas (Perry and Young, 1995). Cattle often become long-term asymptomatic carriers of *T. parva* following treatment or spontaneous recovery, thereby maintaining the parasite population (Dolan, 1986; Kariuki et al., 1995; Kabi et al., 2014).

The genus *Anaplasma* (phylum Proteobacteria, order Rickettsiales, family Anaplasmataceae) contains obligate intracellular organisms that include pathogens of ruminants: *Anaplasma marginale* (the type species), *A. marginale* subsp. *centrale*, *A. bovis* and *A. ovis* (Dumler et al., 2001; Aubry and Geale, 2011). *Anaplasma marginale* subsp. *centrale* is usually considered a separate species and referred to as *A. centrale*. Also included in the genus *Anaplasma* are *A. phagocytophilum*, which infects a wide range of hosts including humans, rodents, birds, dogs and ruminants (Dumler et al., 2001; Hoar et al., 2008; Yang et al., 2013), and *A. platys*, which infects dogs (Dumler et al., 2001). *Anaplasma marginale*, a gram-negative intra-erythrocytic rickettsia, is the main cause of anaplasmosis in cattle (Theiler, 1910; Dumler et al., 2001), and is endemic across much of the globe in tropical and subtropical regions (Kocan et al., 2010). Bovine anaplasmosis is a haemolytic disease that results in considerable economic losses to both dairy and beef industries (Kocan et al., 2010). *Anaplasma marginale* can be transmitted biologically by ticks, mechanically by haematophagous arthropods or infected blood in fomites, and transplacentally from dams to calves (Aubry and Geale, 2011). Infected cattle often remain persistently infected (life-long carriers) regardless of the disease state (Richey and Palmer, 1990; Radostitis et al., 2007; Aubry and Geale, 2011). Although the carrier status provides immunity to clinical disease, it is a source of infection for naïve cattle (Kocan et al., 2010). All cattle are susceptible to infection, but those over two years of age exhibit severe disease with mortality risks of 29% to 49% (Aubry and Geale, 2011). *Anaplasma centrale* (Theiler, 1911) is a less pathogenic organism, and is used as a live vaccine in Israel, South Africa, Australia and South America to provide a certain degree of protection against *A. marginale* (Aubry and Geale, 2011). In eastern Africa, *A. marginale* is transmitted mainly by

the tick *Rhipicephalus decoloratus* (Chenyambuga et al., 2010; Magona et al., 2011a; Magona et al., 2011b). Previous studies in Uganda show that *A. marginale* is distributed in various parts of the country (Oura et al., 2004; Kabi et al., 2008; Muhanguzi et al., 2010a; Magona et al., 2011a; Magona et al., 2011b). World strains of *A. marginale* which vary in genotype, antigenic composition, morphology and infectivity for ticks have been identified using the major surface protein 1a (*msp1α*) gene of the pathogen (de la Fuente et al., 2007; Cabezas-Cruz et al., 2013; Pohl et al., 2013; Mutshembele et al., 2014; Silva et al., 2015). *Anaplasma marginale* surface protein 1a (MSP1a) is a model molecule for classification studies because it serves as a marker for strain identity. The molecule is both an adhesin necessary for infection of cells and an immune-reactive protein, and is also an indicator of the evolution of strain diversity (Cabezas-Cruz and de la Fuente, 2015).

The genus *Ehrlichia* (phylum Proteobacteria, order Rickettsiales, family Anaplasmataceae) includes the obligate rickettsial intracellular pathogen *E. ruminantium* (formerly *Cowdria ruminantium*) that causes heartwater or cowdriosis in some wild, and all domestic, ruminants (Allsopp, 2010). The disease is also a potential emerging zoonosis (Allsopp et al., 2005). The genus also includes *E. canis* and *E. chaffeensis*, which mainly affect canids and humans, respectively, producing a monocytic ehrlichiosis or subclinical infections (Dumler et al., 2001). Other *Ehrlichia* species are *E. ewingi*, *E. muris* and *E. ovina* (Allsopp et al., 2004). Heartwater is severe in small ruminants, exotic breeds of cattle, and stressed or naïve local cattle (Minjauw and McLeod, 2003). The disease is endemic to all of sub-Saharan Africa and several islands in the Caribbean (Maillard and Maillard, 1998; Molia et al., 2008), where it poses a threat of spread to the American mainland (Burridge et al., 2002). Ten species of *Amblyomma* ticks are capable of transmitting *E. ruminantium* in Africa (Allsopp et al., 2004), the most important being *A. variegatum* and *A. hebraeum* (Bezuidenhout, 1987; Walker and Olwage, 1987). *Amblyomma variegatum* is reportedly the tick vector for heartwater in Uganda (Magona et al., 2011b; Nakao et al., 2012).

Babesiosis is caused by obligate intra-erythrocytic and generally host-specific protozoan parasites of the genus *Babesia*, which belongs to phylum Apicomplexa, order Piroplasmida and family Babesiidae (De Vos et al., 2004). The parasites infect a wide range of domestic and

wild animals, and occasionally humans (Bock et al., 2004). In cattle, babesiosis (also known as redwater) is an acute haemolytic and economically important disease, which is distributed across the globe (Suarez and Noh, 2011). The disease is more severe in exotic breeds of cattle, but also occurs in local cattle in endemically unstable situations. Breeds of cattle that are indigenous to *Babesia*-endemic regions often have a certain degree of natural resistance to babesiosis and the consequences of infection are not as serious as when exotic *Bos taurus* breeds are involved. In addition, in tropical areas with a higher vector population, natural exposure usually occurs at an early age and cattle are therefore immune to subsequent challenge as adults (Bock et al., 2004). Animals that recover from babesiosis often become persistent carriers of the infection (Suarez and Noh, 2011). The causes of babesiosis in cattle are *B*. *bovis*, *B*. *bigemina*, *B*. *divergens*, *B*. *ovata* and *B*. *major* (Suarez and Noh, 2011). In Africa, the two most important species in cattle are *B*. *bigemina* and *B. bovis*, transmitted mainly by *Rhipicephalus* (*Boophilus*) ticks (Bock et al., 2004). *Rhipicephalus microplus* is the most important and widespread vector, and in some parts, the tick displaces *R*. *decoloratus*, while *R*. *annulatus* occurs in northern Africa (Bock et al., 2004). *Babesia bovis* is more virulent than *B*. *bigemina*; however, *B. bigemina* is more widespread because of the ability of *R*. *decoloratus* and *R*. *evertsi evertsi* to act as vectors (Friedhoff, 1988). Bovine babesiosis is endemic in Uganda, and is reportedly caused by *B*. *bigemina* and transmitted by *R*. *decoloratus* and *R*. *evertsi evertsi* (Rubaire-Akiiki et al., 2004; Oura et al., 2004; Kabi et al., 2008; Magona et al., 2011b).

Transhumant pastoralism is an adaptive strategy to raise livestock in environments characterised by spatially and temporarily variable pasture and water conditions, and disease hazards (Lengoiboni et al., 2010; Ayantunde et al., 2014; Egeru et al., 2014). The practice is a key coping mechanism for pastoralists to avoid cattle loss and ensure livestock productivity. Because they live in climates that can hardly support cropping, mobile pastoralists depend on livestock as the main source of livelihood (Anderson and Robinson, 2009; Lengoiboni et al., 2010; Mubiru, 2010; Kipronoh et al., 2011). The cattle population in the Karamoja Region of north-eastern Uganda makes up a relatively large percentage (19.76%) of the country's total cattle population of 11,408,750 (MAAIF, 2011). Cattle in Karamoja Region are of the short-horned East African zebu type (*Bos indicus*) (Anderson and Robinson, 2009). The region is a

potential source of beef for Uganda and neighbouring countries (Anderson and Robinson, 2009). The Karamojong often migrate with their animals in search of forage and water, as a result of unpredictable rainfall and frequent droughts (Akabwai and Ateyo, 2007; Egeru et al., 2014).

1.2 Problem Statement and Justification

Livestock diseases are major factors that affect animal production in the Karamoja Region (Jost et al., 1998; Anderson and Robinson, 2009; IICD, 2010). Cattle diseases have increased in the past decades resulting in high mortalities and reduced herd sizes (Anderson and Robinson, 2009; IICD, 2010; FAO, 2013). In Karamoja, cattle are grazed extensively on communal land (Anderson and Robinson, 2009), which regularly exposes the animals to ticks and the infections they transmit (Gachohi et al., 2012). Given the impact of TBDs in pastoral areas, there is need to understand the occurrence, diversity and epidemiology of TBDs, in order to predict disease outbreaks, develop effective control strategies, and increase livestock productivity. However, there is limited data from systematic studies on the situation of TBDs in Karamoja Region.

Participatory epidemiology (PE) involves the use of participatory approaches and methods, with the involvement of communities, in defining and prioritising veterinary-related problems, and in the development of solutions to service delivery, disease control or surveillance (Catley et al., 2012). Pastoralist communities in Africa, including Karamoja Region, live in some of the most underdeveloped and drought-prone environments in the world. In these areas, basic information on the epidemiology of important livestock diseases is limited. Epidemiological research and disease surveillance in pastoralist areas is difficult because human populations are relatively small and highly mobile, and they move their livestock across large areas with few roads or means of modern communication (Catley, 2006). In these situations, conventional approaches to research and disease surveillance require considerable flexibility, commitment and resources. However, government veterinary services are poorly funded and veterinarians tend not to work in remote, underdeveloped and insecure areas. Given the resource and logistical constraints in pastotalist areas,

pastoralists themselves are a valuable source of disease information (Catley, 2006; Catley et al., 2012). Research using participatory methods has led to a better understanding the indigenous technical knowledge developed over many years by rural communities, according to local environmental and socio-cultural conditions (Catley et al., 2012). This indigenous knowledge could make important intellectual contributions to development.

In recent years, PE methods have been adapted by epidemiologists to improve understanding of livestock diseases and impact on pastoralists' livelihoods, for example in Ethiopia and South Sudan (Malak et al., 2012; Catley et al., 2014). Participatory epidemiology provides a relatively cheap and rapid way to generate information by making best use of local knowledge and experience (Malak et al., 2012; Catley et al., 2014). A previous study showed that there is a good agreement between pastoralists' and veterinarians' disease names and diagnostic criteria, showing that pastoralists have a strong ability to diagnose important livestock diseases based on clinical and post-mortem information (Catley, 2006). In Karamoja, communities are clustered in *manyattas* where groups of cattle keepers have similar social, economic and political objectives, and manage and share resources (including animals, water and grazing) together. In such settings, any discussion on livestock is a concern for groups of cattle keepers, with consent of the *manyatta* leader, rather than individuals. When using conventional questionnaires in interviews, local people answer questions posed by outsiders, which may be of limited interest to them. In contrast, PE methods seem to offer lower risk of informants consciously offering incorrect or misleading information because the topic under discussion is often a local priority (Catley et al., 2012).

A more valid approach to epidemiological research requires across-method triangulation where two or more different methods are used to study the same or similar research question. When PE is used, there is need to validate the knowledge and perceptions of the pastoralists by triangulating with the findings from conventional methods of disease investigation. Serological methods for the detection of antibodies and tick vector assessment can provide reliable evidence of exposure to parasites and the likelihood of animals developing immunity to tick-borne infections. Antibody prevalence and other parameters, including disease incidence, case-fatality proportion and age group affected in the herd, are

indicators of endemic stability (Norval et al., 1992). A state of endemic stability implies an ecological balance between cattle, tick, parasite, and the environment where animals are infected with tick-borne parasites with little or no morbidity and mortality in the herds (Norval et al., 1992; Bock et al., 2004). In pastoral areas of eastern Africa, the use of inappropriate practices to control ticks and TBDs is common (Mugisha et al., 2005; Kivaria, 2006; Wesonga et al., 2010). This may affect the patterns of exposure to tick-borne infections in cattle and the establishment of immunity at individual or herd levels. Consequently, endemic instability may occur in indigenous cattle breeds kept by pastoralists (Norval et al., 1992; Perry and Young, 1995). It is, therefore, valuable to assess the indicators of endemic stability in the pastoral areas so as to predict disease outbreaks and mortalities, and provide biological evidence to support decisions around control approaches e.g., dipping, immunisation, enhancement of innate resistance or no intervention (Perry and Young, 1995; Kivaria, 2010).

The development of molecular biological techniques with higher degree of sensitivity has resulted in improved detection, identification and genetic characterisation of previously known and novel tick-borne haemoparasites (Nijhof et al., 2005; Oosthuizen et al., 2008). Whereas serological tests detect exposure to infection (and not necessarily current infections), molecular diagnostic techniques detect current/active infection as well as carrier animals with very low parasitaemias (Bekker et al., 2002; Oosthuizen et al., 2008; Sibeko et al., 2008). The comparison of the 18S rRNA gene and 16S rRNA gene sequences has been recognised as a powerful and precise method for the classification and determination of the phylogenetic relationships of the tick-borne haemoparasites at the family, genus and species levels (Dumler et al., 2001; Chaisi et al., 2013). Although MSP1a tandem repeats of *A. marginale* have been assessed from various parts of the world, no data involving cattle in Uganda is available. Knowledge about *A. marginale* strains, in relationship with geographic and ecological factors, contributes to the development of effective vaccines for the control of bovine anaplasmosis (Cabezas-Cruz and de la Fuente, 2015).

1.3 Objectives of the study

In view of the above, the aim of this study was to determine the epidemiology and diversity of tick-borne haemoparasite infections amongst transhumant Zebu cattle in the Karamoja Region using PE, serological methods, tick vector assessment and molecular techniques.

The specific objectives were to:

1. Investigate the management options, relative importance of tick-borne diseases, and constraints to control of ticks and TBDs using participatory epidemiology.
2. Establish the endemic status of *T. parva* and *A. marginale*, and tick-species diversity among cattle.
3. Determine the occurrence and level of tick-borne haemoparasite infections, and sequence and phylogenetic analyses of the 18S rRNA gene for *Theileria* and *Babesia* spp.
4. Characterise *Anaplasma* spp. using the 16S rRNA gene and sequence analysis of the *msp1α* gene for *A. marginale*.

1.4 Thesis overview

Chapter 1: This provides the general introduction and outlines the rationale and objectives of the study.

Chapter 2: This provides a detailed review of the literature on livestock production in Uganda, transhumant pastoralism and tick-borne haemoparasites of cattle.

Chapter 3: The aim of this chapter was to define and prioritise cattle diseases in the pastoral area of Karamoja, Uganda, evaluate current control activities for TBDs, and identify constraints to the control of TBDs. Participatory epidemiology was used to gather the herders' perceptions of herd health problems. The informants were also facilitated to find solutions to service delivery, disease control and surveillance. Data collection involved focus group discussions in settlement areas (*manyattas*), key informant interviews, direct observation, a

review of surveillance data, clinical examination, and laboratory confirmation of cases of TBDs.

Chapter 4: Information on the endemic status of tick-borne infections may assist in strategic control of TBDs. The indicators of endemic stability include antibody prevalence, disease incidence, case-fatality proportion and age groups affected in the herds. In this chapter, we evaluated the endemic status of TBDs by estimating the proportion of annual ECF and anaplasmosis cases in different age groups of cattle using participatory approaches and clinical examinations, determining the diversity and abundance of ticks, and assessing the seroprevalence of antibodies to *T. parva* and *A. marginale*.

Chapter 5: Molecular biological techniques enable the improved detection, identification and genetic characterisation of many parasites. In this chapter, we used the PCR-based reverse line blot (RLB) hybridisation assay to determine the occurrence and prevalence of *Theileria*, *Babesia*, *Anaplasma* and *Ehrlichia* species. We used quantitative real-time polymerase chain reaction (qPCR) assays for specific detection of *A. marginale*, *A. centrale, T. parva* and *E. ruminantium*. The full-length 18S rRNA genes of samples that hybridised to *Babesia* genus-specific probes and not *Babesia* species-specific probes were amplified, cloned and sequenced. The 18S rRNA sequences obtained in this study were analysed to determine the phylogenetic relationships with known *Theileria* and *Babesia* spp.

Chapter 6: From the qPCR results (samples with threshold cycles 25-29), we characterised *Anaplasma* species by sequence analysis of the 16S rRNA gene and genotyped *Anaplasma marginale* using the *msp1α* gene. The comparison of 16S rRNA gene sequences has been recognised as a valuable method for classification and to determine the phylogenetic relationships of the organisms in the order Rickettsiales and family Anaplasmataceae. The full-length 16S rRNA gene for *Anaplasma* species was amplified, cloned and sequenced. The 16S rRNA sequences obtained were analysed to determine the phylogenetic relationships with known *Anaplasma* spp. We also analysed *msp1α* gene sequences of *A. marginale* to identify tandem repeat structures.

Chapter 7: This provides a general discussion, conclusions and recommendations emanating from this study.

1.5 References

AKABWAI, D., ATEYO, P.E., 2007. The Scramble for Cattle, Power and Guns in Karamoja. Feinstein International Center, Tufts University, 200 Boston Avenue, Suite 4800, Medford, MA 02155, USA, 49 pp. http://fic.tufts.edu/assets/The+Scramble+for+Cattle1.pdf. Accessed October 12, 2015.

ALLSOPP, B.A., 2010. Natural history of *Ehrlichia ruminantium*. Veterinary Parasitology 167, 123-135.

ALLSOPP, B.A., BEZUIDENHOUT, J.D., PROZESKY, L., 2004. Heartwater. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 507-535.

ALLSOPP, M.T.E.P., LOUW, M., MEYER, E.C., 2005. *Ehrlichia ruminantium*: an emerging human pathogen? Annals of the New York Academy of Sciences 1063, 358-360.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

AYANTUNDE, A.A., ASSE, R., SAID, M.Y., FALL, A., 2014. Transhumant pastoralism, sustainable management of natural resources and endemic ruminant livestock in the sub-humid zone of West Africa. Environment, Development and Sustainability 16, 1097-1117.

BEKKER, C.P.J., DE VOS, S., TAOUFIK, A., SPARAGANO, O.A.E., JONGEJAN, F., 2002. Simultenous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Veterinary Microbiology 89, 223-238.

BEZUIDENHOUT, J.D., 1987. Natural transmission of heartwater. The Onderstepoort Journal of Veterinary Research 54, 349-351.

BOCK, R., JACKSON, L., DE VOS, A., JORGENSEN, W., 2004. Babesiosis of cattle. Parasitology 129, S247-S269.

BURRIDGE, M.J., SIMMONS, L., PETER, T.F., MAHAN, S.M., 2002. Increasing risks of introduction of heartwater onto the American mainland associated with animal movements. Annals of the New York Academy of Sciences 969, 269-274.

CABEZAS-CRUZ, A., DE LA FUENTE, J., 2015. *Anaplasma marginale* major surface protein 1a: A marker of strain diversity with implications for control of bovine anaplasmosis. Ticks and Tick-borne Diseases 6, 205-210.

CABEZAS-CRUZ, A., PASSOS, L.M.F., LIS, K., KENNEIL, R., VALDÉS, J.J., FERROLHO, J., TONK, M., POHL, A.E., GRUBHOFFER, L., ZWEYGARTH, E., SHKAP, V., RIBEIRO, M.F.B., ESTRADA-PEÑA, A., KOCAN, K.M., DE LA FUENTE, J., 2013. Functional and immunological relevance of *Anaplasma marginale* major surface protein 1a sequence and structural analysis. PLoS ONE 8 (6), e65243. doi: 10.1371/jounal.pone.0065243.

CATLEY, A., 2006. Use of participatory epidemiology to compare the clinical veterinary knowledge of pastoralists and veterinarians in East Africa. Tropical Animal Health and Production 38, 171-184.

CATLEY, A., ADMASSU, B., BEKELE, G., ABEBE, D., 2014. Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. Disasters 38, 500-516.

CATLEY, A., ALDERS, R.G., WOOD, J.L.N., 2012. Participatory epidemiology: Approaches, methods, experiences. The Veterinary Journal 191, 151-160.

CHAISI, M.E., COLLINS, N.E., POTGIETER, F.T., OOSTHUIZEN, M.C., 2013. Sequence variation identified in the 18S rRNA gene of *Theileria mutans* and *Theileria velifera* from the African buffalo (*Syncerus caffer*). Veterinary Parasitology 191, 132-137.

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. Livestock Research for Rural Development Volume 22 (7), Article # 135. Retrieved May 2, 2013, from http://www.lrrd.org/lrrd22/7/chen22135.htm.

DE LA FUENTE, J., RUYBAL, P., MTSHALI, M.S., NARANJO, V., SHUQING, L., MANGOLD, A.J., RODRÍGUEZ, S.D., JIMÉNEZ, R., VICENTE, J., MORETTA, R., TORINA, A., ALMAZÁN, C., MBATI, P.M., DE ECHAIDE, S.T., FARBER, M., ROSARIO-CRUZ, R., GORTAZAR, C., KOCAN, K.M., 2007. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. Veterinary Microbiology 119, 382-390.

DE VOS, A.J., DE WAAL, D.T., JACKSON, L.A., 2004. Bovine babesiosis. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), Oxford University Press Southern Africa, Cape Town, South Africa, pp. 406-424.

DOLAN, T.T., 1986. Chemotherapy of East Coast fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. Journal of Comparative Pathology 96, 137-146.

DUMLER, J.S., BARBET, A.F., BEKKER, C.P.J., 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 combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. International Journal of Systematic and Evolutionary Microbiology 51, 2145-2165.

EGERU, A., WASONGA, O., KYAGULANYI, J., MAJALIWA, G.J.M., MACOPIYO, L., MBURU, J., 2014. Spatio-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda. Pastoralism: Research, Policy and Practice 4, 6.

FAO, 2013. FAO at work in Karamoja. Food and Agriculture Organization of the United nations (FAO) Representation in Uganda. http://www.fao.org/emergencies/resources/documents/resources-detail/en/c/212585/. Accessed October 23, 2015.

FRIEDHOFF, K.T., 1988. Transmission of *Babesia*. In: Babesiosis of domestic animals and man, RISTIC, M. (Ed.), CRC Press Inc., Boca Raton, Florida, pp. 23-52.

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, 194.

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, 208-223.

HOAR, B.R., NIETO, N.C., RHODES, D.M., FOLEY, J.E., 2008. Evaluation of sequential coinfection with *Anaplasma phagocytophilum* and *Anaplasma marginale* in cattle. American Journal of Veterinary Research 69, 1171-1178.

IICD, 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities, Ruspantini, C. (Ed.), European Commission, Government of Uganda and Institute for International Co-operation and Development (IICD) Uganda, 67 pp. www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/C&DPublicationLivestockDiseaseSurveillance2.pdf. Accessed March 24, 2015.

JONSSON, N.N., BOCK, R.E., JORGENSEN, W.K., 2008. Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses, and the effects of differing intensity of tick control in Australia. Veterinary Parasitology 155, 1-9.

JOST, C., STEM, C., RAMUSHWA, M., TWINAMASIKO, E., MARINER, J., 1998. Comparative ethnoveterinary and serological evaluation of the Karimojong Community Animal Health Worker Program in Uganda. Annals of the New York Academy of Sciences 849, 327-337.

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. Journal of Protozoology Research 18, 61-70.

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, 414.

KARIUKI, D.P., YOUNG, A.S., MORZARIA, S.P., LESAN, A.C., MINING, S.K., OMWOYO, P., WAFULA, J.L.M., MOLYNEUX, D.H., 1995. *Theileria parva* carrier state in naturally infected and artificially immunised cattle. Tropical Animal Health and Production 27, 15-25.

KASOZI, K.I., MATOVU, E., TAYEBWA, D.S., NATUHWERA, J., MUGEZI, I., MAHERO, M., 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. Open Journal of Veterinary Medicine 4, 220-231.

KIPRONOH, K.A., GATHUMA, J.M., KITALA, P.M., KIARA, H.K., 2011. Pastoralists' perception of the impact of East Coast fever on cattle production under extensive management in Northern Rift Valley, Kenya. Livestock Research for Rural Development Volume 23 (6), Article #126. Retrieved May 4, 2014, from http://www.irrd.org/Irrd23/6/Kipr23126.htm.

KIVARIA, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production 38, 291-299.

KIVARIA, F.M., 2010. Climate change and the epidemiology of tick-borne diseases of cattle in Africa. The Veterinary Journal 184, 7-8.

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, 95-107.

LAISSER, E.L.K., CHENYAMBUGA, S.W., MSALYA, G., KIPANYULA, M.J., MDEGELA, R.H., KARIMURIBO, E.D., MWILAWA, A.J., KUSILUKA, L.J.M., 2015. Knowledge and perception on ticks, tick-borne diseases and indigenous cattle tolerance to East Coast fever in agro-pastoral communities of Lake Zone in Tanzania. Livestock Research for Rural Development Volume 27, Article #64. Retrieved June 15, 2015, from http://www.lrrd.org/lrrd27/4/lais27064.htm.

LAWRENCE, J.A., PERRY, B.D., WILLIAMSON, S.M., 2004a. Corridor disease. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 468-471.

LAWRENCE, J.A., PERRY, B.D., WILLIAMSON, S.M., 2004b. East Coast fever. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 448-467.

LENGOIBONI, M., BREGT, A.K., VAN DER MOLEN, P., 2010. Pastoralism within land administration in Kenya-The missing link. Land Use Policy 27, 579-588.

LEVINE, N.D., CORLISS, J.O., COX, F.E.G., DEROUX, G., GRAIN, J., HONIGBERG, B.M., LEEDALE, G.F., LOEBLICH, A.R., LOM, J.I., LYNN, D., MERINFELD, E.G., PAGE, F.C., POLJANSKY, G., SPRAGUE, V., VAVRA, J., WALLACE, F.G., 1980. A newly revised classification of the Protozoa. Journal of Eukaryotic Microbiology 27, 37-58.

MAAIF, 2011. Statistical Abstract, Agricultural Planning Department, Ministry of Agriculture, Animal Industry and Fisheries. www.agriculture.go.ug/userfiles/Statistical Abstract 2011.pdf. Accessed June 7, 2013.

MAGONA, J.W., WALUBENGO, J., KABI, F., 2011a. Response of Nkedi Zebu and Ankole cattle to tick infestation and natural tick-borne, helminth and trypanosome infections in Uganda. Tropical Animal Health and Production 43, 1019-1033.

MAGONA, J.W., WALUBENGO, J., OLAHO-MUKANI, W., JONSSON, N.N., WELBURN, S.W., EISLER, M.C., 2011b. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55, 203-213.

MAILLARD, J.C., MAILLARD, N., 1998. Historique du peuplement bovin et de l'introduction de la tique *Amblyomma variegatum* dans les îles françaises des Antilles: synthése bibliographique. Ethnozootechnie 1, 19-36.

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, 216-223.

MARCELLINO, W.L., SALIH, D.A., JULLA, I.I., EL HUSSEIN, A.M., 2011. Economic impact of East Coast fever in central equatorial state of south Sudan. International Research Journal of Agricultural Science and Soil Science 1, 218-220.

MINJAUW, B., MCLEOD, A., 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, 116 pp.

MOLIA, S., FREBLING, M., VACHIÉRY, N., PINARELLO, V., PETITCLERC, M., ROUSTEAU, A., MARTINEZ, D., LEFRANÇOIS, T., 2008. *Amblyomma variegatum* in cattle in Marie Galante, French Antilles: Prevalence, control measures, and infection by *Ehrlichia ruminantium*. Veterinary Parasitology 153, 338-346.

MUBIRU, D.N., 2010. Climate change and adaptation options in Karamoja. European Commission and Food and Agriculture Organisation of the United Nations, 62 pp. Retrieved May 9, 2014, from www.fao.org/fileadmin/user_upload/drought/docs/Karamoja_Climate_Change_and_Adaptation_Options.pdf.

MUGISHA, A., MCLEOD, A., PERCY, R., KYEWALABYE, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010a. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

MUHANGUZI, D., MATOVU, E., WAISWA, C., 2010b. Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. International Journal of Animal and Veterinary Advances 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, 91.

MUTSHEMBELE, A.M., CABEZAS-CRUZ, A., MTSHALI, M.S., THEKISOE, O.M.M., GALINDO, R.C., DE LA FUENTE, J., 2014. Epidemiology and evolution of the genetic variability of *Anaplasma marginale* in South Africa. Ticks and Tick-borne Diseases 5, 624-631.

NAKAO, R., MORRISON, L.J., ZHOU, L., MAGONA, J.W., JONGEJAN, F., SUGIMOTO, C., 2012. Development of multiple-locus variable-number tandem-repeat analysis for rapid genotyping of *Ehrlichia ruminantium* and its application to infected *Amblyomma variegatum* collected in heartwater endemic areas in Uganda. Parasitology 139, 69-82.

NIJHOF, A.M., PILLAY, V., STEYL, J., PROZESKY, L., STOLTSZ, W.H., LAWRENCE, J.A., PENZHORN, B.L., JONGEJAN, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. Journal of Clinical Microbiology 43, 5907-5911.

NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S., LAWRENCE, J.A., MUKHEBI, A.W., BISHOP, R.P., MCKEEVER, D.J., SHAW, M.K., 1992. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

OCAIDO, M., MUWAZI, R.T., OPUDA, J.A., 2009a. Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. Tropical Animal Health and Production 41, 731-739.

OCAIDO, M., OTIM, C.P., KAKAIRE, D., 2009b. Impact of major diseases and vectors in smallholder cattle production systems in different agro-ecological zones and farming systems in Uganda. Livestock Research for Rural Development Volume 21 (9), Article #155. Retrieved November 29, 2013, from http://www.lrrd.org/lrrd21/9/ocai21155.htm.

ONONO, J.O., AMIMO, J.O., RUSHTON, J., 2015. Constraints and efficiency of cattle marketing in semiarid pastoral system in Kenya. Tropical Animal Health and Production 47, 691-697.

OOSTHUIZEN, M.C., ZWEYGARTH, E., COLLINS, N.E., TROSKIE, M., PENZHORN, B.L., 2008. Identification of a novel *Babesia* sp. from a sable antelope (*Hippotragus niger* Harris, 1838). Journal of Clinical Microbiology 46, 2247-2251.

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, 603-613.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

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, 107-120.

POHL, A.E., CABEZAS-CRUZ, A., RIBEIRO, M.F.B., DA SILVEIRA, J.A.G., SILAGHI, C., PFISTER, K., PASSOS, L.M.F., 2013. Detection of genetic diversity of *Anaplasma marginale* in Minas Gerais, Brazil. Revista Brasileira de Parasitologia Veterinária 22, 129-135.

RADOSTITIS, O.M., GAY, C.C., HINCHCLIFF, K.W., CONSTABLE, P.D., 2007. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th edn, Saunders, USA, 2065 pp.

RICHEY, E.J., PALMER, G.H., 1990. Bovine anaplasmosis. Compendium on Continuing Education for the Practicing Veterinarian 12, 1661-1668.

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, 8.

SIBEKO, K.P., OOSTHUIZEN, M.C., COLLINS, N.E., GEYSEN, D., RAMBRITCH, N.E., LATIF, A.A., GRONEVELD, H.T., POTGIETER, F.T., COETZER, J.A.W., 2008. Development of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. Veterinary Parasitology 155, 37-48.

SILVA, J.B., GONÇALVES, L.R., VARANI, A.M., ANDRÉ, M.R., MACHADO, R.Z., 2015. Genetic diversity and molecular phylogeny of *Anaplasma marginale* studied longitudinally under natural transmission conditions in Rio de Janeiro, Brazil. Ticks and Tick-borne diseases 6, 499-507.

SUAREZ, C.E., NOH, S., 2011. Emerging perspectives in the research of bovine babesiosis and anaplasmosis. Veterinary Parasitology 180, 109-125.

THEILER, A., 1910. *Anaplasma marginale* (gen.and spec. nov.): the marginal points in the blood of cattle suffering from a specific disease. Report of the Government Veterinary Bacteriologist for the year 1908-1909. Department of Agriculture, South Africa, 7-64.

THEILER, A., 1911. Further investigations into anaplasmosis of South African cattle. First report of the Director of Veterinary Research. Department of Agriculture of the Union of South Africa, 7-46.

UILENBERG, G., 1981. Theilerial species of domestic livestock. In: Advances in the control of theileriosis, IRVIN, A.D., CUNNINGHAM, M.P., YOUNG, A.S. (Eds.), Martinus Nijhoff Publishers, The Hague, The Netherlands, pp. 4-37.

UILENBERG, G., 1995. International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Veterinary Parasitology 57, 19-41.

WALKER, J.B., OLWAGE, A., 1987. The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. The Onderstepoort Journal of Veterinary Research 54, 353-379.

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. Livestock Research for Rural Development Volume 22 (6), Article #111. Retrieved December 13, 2013, from http://www.irrd.org/Irrd22/6/weso22111.htm.

YANG, J., LIU, Z., GUAN, G., LIU, Q., LI, Y., CHEN, Z., MA, M., LIU, A., REN, Q., LUO, J., YIN, H., 2013. Prevalence of *Anaplasma phagocytophilum* in ruminants, rodents and ticks in Gansu, north-western China. Journal of Medical Microbiology 62, 254-258.

CHAPTER 2

2. Literature Review

2.1 Geographic and climatic characteristics of Uganda

Uganda is located in East Africa (Figure 2.1), lying astride the Equator, between latitudes 1° 29'S and 4° 12'N and longitudes 29° 34'W and 35° 0'E (UBOS, 2014). The country has a total surface area of 241,550.7 km^2 of which 200,523.2 km^2 are land; the remainder being open water bodies and wetlands (UBOS, 2014). The country is bordered by Kenya to the east, Tanzania and Rwanda to the south, South Sudan to the north, and the Democratic Republic of Congo to the east (Figure 2.1). Uganda is administratively divided into four regions and 112 districts. The temperature ranges from 14 to 32°C. Precipitation varies from 750 mm in Karamoja in the north-east to 1,500 mm in the high rainfall areas on the shores of Lake Victoria, in the highlands around Mountain Elgon in the east, the Rwenzori Mountains in the south-west, and some parts of Masindi and Gulu. The altitude above sea level ranges from 620 metres (Albert Nile) to 5,111 m (Mt. Rwenzori peak) (UBOS, 2014). More than two-thirds of the country is a plateau, lying between 1,000 and 2,500 m above sea level (Mwebaze, 2006). The total human population increased from 24.2 million in 2002 to an estimated 36.6 million by mid-2014 (projection based on the Census 2002 final results) (UBOS, 2014). The vegetation is savannah grassland, woodland, bushland, and tropical rain forest (UBOS, 2014).

Figure 2.1: Map of Africa and parts of neighbouring continents showing the location of Uganda (indicated by the red marker). Source: www.primeugandasafaris.com

Agriculture is the backbone of Uganda's economy: 95% of the population farms (crops and/or livestock). The agriculture sector contributes about 20.9% of the total gross domestic product (GDP) (UBOS, 2014). The country can be conveniently divided into seven broad agro-ecological zones, which have similar economic and social backgrounds and in which ecological conditions (soil types, topography, rainfall), farming systems, and practices are fairly homogenous. These are often further split into subzones usually identified by such factors as similar crop combinations, size of holdings, and average plot sizes and yields (Mwebaze, 2006). The seven agro-ecological zones are:

(i) Banana-coffee system - in this system, rainfall is evenly distributed (1,000 - 1,500 mm) on soils of medium to high productivity. Livestock are generally not integrated into the system, but dairy cattle are gaining prominence.

(ii) Banana-millet-cotton system - rainfall for this system is less stable than for the banana-coffee system, so there is greater reliance on annual food crops (millet, sorghum and maize). In the drier areas, livestock production is a main activity.

(iii) Montane system - this is found at higher elevations of 1,500 - 1,750 m above sea level. The area receives high and effective rainfall and cloud cover. High population intensities and intensive agriculture are the norm, with small holdings of about 1.5 hectares. Feeding crop residues to livestock is a common practice.

(iv) Teso system - this area receives bimodal rainfall on sandy-loam soils of low to medium fertility. The dry season is longer, from December to March. The zone is characterised by short grassland that is ideal for grazing. Mixed agriculture (crops and livestock) is practiced, and cultivation by oxen is the main agricultural technology. Livestock are kept extensively in those areas that are free of tsetse flies.

(v) Northern system - the rainfall in this system is less pronouncedly bimodal with about 800 mm annually. The dry season is so severe that drought tolerant annuals are cultivated. The grassland is short and communal grazing abounds. This area is well-known for its pastoral system with semi-nomadic cattle herding.

(vi) West Nile system - the rainfall pattern resembles that of the northern system, with more rain at higher altitudes. Mixed cropping is common with a wide variety of crops. The vegetation community is moist grassland, and livestock activities are limited by the presence of tsetse flies.

(vii) Pastoral system - this system covers the districts of Karamoja Region in the north-east, and parts of western and central districts of Uganda. Annual rainfall is mostly uni-modal and low (under 1,000 mm). The system is characterised by short grassland and pastoralism prevails with extensive nomadic grazing.

2.2 Livestock production and management practices in Uganda

In Uganda, livestock production is important for economic development and poverty reduction. Livestock and livestock products are a source of income for households and provide a source of protein to many families (MAAIF, 2010). Seventy percent of Uganda's households are engaged in some form of livestock rearing (MAAIF, 2011). Households that include livestock in their enterprise mix tend to be generally less poor (UBOS, 2007). The potential for the export market is high and opportunities exist for the expansion of dairy and meat, hides, skins and leather (MAAIF, 2010). However, current production levels in the livestock sub-sector can still only meet half the domestic and regional demands. The livestock sub-sector is constrained by factors that include: endemic diseases, insufficient research into livestock problems and opportunities, and inadequate advisory and veterinary services (MAAIF, 2010). The livestock production systems in Uganda are (i) crop-livestock farming,

where livestock activity is an integral component of crop production and draught cattle are still used; (ii) extensive pastoral system, mainly found in the arid and semi-arid areas of Uganda, where pastoralists utilise rangelands that would be unproductive for crop production to graze their indigenous breeds of cattle and small ruminants; (iii) and the market-oriented system, found mainly in urban and peri-urban areas, where farmers keep a few animals (1-5) of improved breeds, mainly for milk production (Nalubwama et al., 2011). In the market-oriented system, livestock are kept in an intensive manner (e.g. 'zero grazing'), feeding is normally 'cut and carry', and some concentrates are given to the cows to improve milk production (Nalubwama et al., 2011). The main livestock species in Uganda include cattle, sheep, goats, pigs, rabbits, and poultry. Uganda's populations of cattle, goats, and poultry are among the highest in Africa - generally estimated to be in the top quintile (Benson and Mugarura, 2013). In 2013, the national livestock population was estimated at 13.02 million cattle, 14.6 million goats, 3.9 million sheep, 3.7 million pigs, and 43.2 million for poultry (UBOS, 2014). There were 12,187,100 indigenous cattle (93.6%) compared with 798,920 (6.4%) exotic ones in 2013 (UBOS, 2014). There has been a significant increase in the national cattle population, which stood at 7.5 million in 2005 (UBOS, 2006). Beef and dairy cattle are identified as priority agricultural commodities for the country (Benson and Mugarura, 2013). Pastoralists and communal grazers hold about 95% of all the cattle. Therefore, most of the contribution of the livestock sector to GDP is accounted for by pastoralists (MAAIF, 2010). The Agriculture Sector Development Strategy and Investment Plan (ASDSIP) emphasises the need to increase intervention in pastoral areas of Uganda so as to increase production and productivity of pastoral activities, and to improve food security and household incomes in a sustainable and predictable way (MAAIF, 2010).

2.3 Transhumant pastoralism

Transhumance can be defined as a livestock production system characterised by varying degrees of seasonal and cyclical movement of pastoralists and livestock, between complementary ecological areas. The major drivers of transhumant pastoralism are drought conditions, which result from climatic variability, and consequent seasonal pasture and water scarcity, and animal diseases. The practice is therefore a key coping mechanism for pastoralists to avoid loss and ensure livestock productivity (Goldman and Riosmena, 2013;

Ayantunde et al., 2014; Egeru et al., 2014). Other factors can be socio-economic in nature: land use changes, demographic pressure, and consequent loss of pastureland, social relations and networks (Goldman and Riosmena, 2013; Ayantunde et al., 2014; Egeru et al., 2014). Mobile livestock herding is common among pastoral groups in eastern Africa including the Karamojong of Uganda (Egeru et al., 2014), the Turkana of Kenya (Mathew and Boyd, 2014), the Tigray of Ethiopia (Nyssen et al., 2009), and the Maasai of southern Kenya and northern Tanzania (Swai et al., 2005). Because they live in climatically marginal environments, mobile pastoralists depend on livestock as the only economic activity to sustain livelihoods (Ayantunde et al., 2014; Egeru et al., 2014).

2.4 Karamoja Region and livestock production

Karamoja Region is located in north-eastern Uganda (Figure 2.2), bordered by Kenya to the east and South Sudan to the north (Anderson and Robinson, 2009). Currently, the region comprises seven districts: Abim, Amudat, Kaabong, Kotido, Moroto, Nakapiripirit, and Napak. Each district is, in turn, divided into sub-counties containing a number of parishes and smaller settlement areas or *manyattas* (Mubiru, 2010). The semi-arid region of Karamoja is home to multiple ethnic and territorial groups: the Dodoth, Nyangia, Napore and Ik in the North, the Jie and Tobur, or Acholi Labwor, in the central region, and the Bokora, Matheniko, Pian and the Tepeth in the South (Akabwai and Ateyo, 2007). Due to insecurity from cattle raids, many communities are clustered in *manyattas* to ensure security of person and property (Mubiru, 2010). However, the on-going disarmament exercise has brought relative stability to the region. Karamoja covers 27,511 Km^2 (about 10% of Uganda) and has a human population of approximately 1,455,200 (UBOS, 2014). The region is the least socially and economically developed in Uganda, characterised by high poverty rates, drought, poor infrastructure, illiteracy, and poor health (Anderson and Robinson, 2009; Mubiru, 2010).

Figure 2.2: Map of Uganda showing the location of Karamoja Region.
Source: www.wts.edu/stayinformed/view.html?id=1287

The vegetation of Karamoja is savannah covered with seasonal grasses, thorny plants and occasional small trees. Thickets and patches of gallery forest are found along seasonal rivers (Gradé et al., 2009). The region is characterised by wide plains (1,500 m above sea level). The climate is mostly semi-arid characterised by poor rainfall distribution and reliability (uni-modal rainfall, average 500-600 mm per year), manifested through prolonged dry spells (Anderson and Robinson, 2009; FAO, 2013). The dry season typically lasts for seven months from September to March (Anderson and Robinson, 2009; Mubiru, 2010). Temperatures range from a minimum of 15°C to 18°C to a maximum of 28°C to 32.5°C (Mubiru, 2010). Cycles of drought occur in the region and may last 5-15 months, viewed as signs of climate change. The change in climate is manifested by less rainfall, changes in rainfall patterns and increases in temperature (Anderson and Robinson, 2009; Mubiru, 2010). Due to cycles of drought, transhumant pastoralism is the main livestock management system practiced. The mobile livestock system is characterised by well-organised mobile herding groups, which traditionally move together under the guidance of kraal leaders in search of better grazing and watering

areas (Anderson and Robinson, 2009). In the wet season, the livestock enclosures ("kraals") are closer to the permanent settlement zone, taking advantage of temporary pools for watering. In the dry season, pastoralists move their animals to neighbouring districts and countries in search of pastures and water (Anderson and Robinson, 2009). The livestock species kept include cattle, sheep, goats and a few donkeys, camels and poultry. The animals are extensively grazed on communal land with no supplementary feeding. In addition to their economic importance, cattle are also valued for their social and cultural benefits (Anderson and Robinson, 2009). Cattle in Karamoja are of the short-horned East African Zebu breed. Zebu cattle (*Bos indicus*) are the majority of cattle types in Africa (Mwai et al., 2015). These cattle, with the characteristic thoracic hump on their shoulders and pendulous dewlap, are known to have descended from the secondary cattle domestication in the arid areas of the 'Fertile Crescent' (DAGRIS, 2007). Zebu cattle are known to be better than the humpless cattle in regulating body temperature (hence lower body water requirements). They are adapted to dry environmental conditions and high temperatures and are known to be more resistant to tick infestation than *Bos taurus* cattle (Mattioli et al., 2000). Their hardened hooves and lighter bones enable them to endure long migrations (DAFRIS, 2007). Where appropriate, the Karamojong have incorporated crop production as an opportunistic strategy to complement livestock herding (Mubiru, 2010; Stites and Mitchard, 2011; FAO, 2013). However, the region is threatened by cattle losses mainly due to diseases.

2.5 Important tick-borne diseases of livestock in sub-Saharan Africa

2.5.1 Theileriosis

2.5.1.1 Aetiology

Theileriosis is caused by parasites of the genus *Theileria* which comprises tick-transmitted obligate intracellular protozoa of the phylum Apicomplexa, order Piroplasmida and family Theileriidae (Adl et al., 2012). *Theileria* are currently classified in the class Sporozoa, together with human pathogens including *Plasmodium* and *Toxoplasma* (Bishop et al., 2004). Analysis of 18S rRNA gene sequences demonstrates that the genus *Theileria* is phylogenetically most closely related to *Babesia* (Bishop et al., 2004). The parasites affect a wide range of mammals, including domestic and wild ruminants, in tropical and subtropical regions, causing diseases

with varying degrees of severity (Sivakumar et al., 2014). *Theileria* parasites can be broadly categorised into two groups, based on the parasites' ability to transform host leukocytes in a way that enables the infected cells to proliferate indefinitely along with the parasites occupying them: transforming (*Theileria parva*, *T. annulata*, *T. lestoquardi*, and *T. taurotragi*) and non-transforming (*T. orientalis*, *T. mutans*, *T. velifera*, *T. cervi*) (Sivakumar et al., 2014). A recent study added *Theileria* sp. (buffalo), a benign *Theileria* parasite in African buffaloes, to the list of transforming parasite species (Chaisi et al., 2011). The taxonomy of the benign *Theileria sergenti/buffeli/orientalis* group of non-transforming *Theileria* parasites is controversial and has been debated for many years. *Theileria sergenti* is an invalid name taxonomically as it has been used to previously describe a parasite of sheep (Morel and Uilenberg, 1981). It was therefore concluded that *T. sergenti* is not an appropriate name for the *T. sergenti/buffeli/orientalis* group of parasites, and the name for this group should be either *T. orientalis* or *T. buffeli* (Morel and Uilenberg, 1981). However, in 1985 it was suggested that members of this group of *Theileria* parasites were the same species, based on serological and morphological identities, and it was proposed that the name *T. orientalis* should be used for the group (Uilenberg, 1985). Other studies suggested that *T. sergenti* should be separated from *T. buffeli* and *T. orientalis* on the basis of their serological differences and differences in transmissibility (Fujisaki, 1992; Kawazu et al., 1992). Recently, nucleotide sequencing of SSU rRNA and major piroplasm surface protein (MPSP) genes revealed the presence of four *Theileri*a genotypes: *T. orientalis* (*buffeli*), *T. orientalis* (Ikeda), *T. orientalis* (Chitose) and *T. orientalis* type 4 (MPSP) or type c (SSU rRNA) (Kamau et al., 2011).

Theileria parasites are transmitted transstadially by two-or three-host ixodid ticks, and the diseases they cause are among the most serious constraints to livestock production (Lawrence et al., 2004b). Cattle are particularly susceptible to *Theileria*, and the two most important species are *T. parva* and *T. annulata* (Norval et al., 1992b).

Theileria parva, considered to have originated from African buffalo (*Syncerus caffer*), is the most pathogenic and economically significant *Theileria* spp.in eastern, central and southern Africa where it causes East Coast fever (ECF), Corridor disease and January disease in cattle (Norval et al., 1992b). *Theileria parva* was also found to infect waterbuck (*Kobus defessa*)

under natural conditions, and the Asiatic buffalo (*Bubalus bubalis*) under experimental conditions (Lawrence et al., 2004b). *Theileria annulata*, which originated from water buffaloes (*Bubalus bubalis*), occurs in northern Africa, southern Europe, and western, southern and eastern Asia, and causes tropical theileriosis (also called Mediterranean theileriosis). Other species, *T. mutans*, *T. taurotragi* and *T. velifera*, cause benign theileriosis in cattle in sub-Saharan Africa, particularly as mixed infections with other tick-borne pathogens (Norval et al., 1992b). *Theileria sergenti* (Japan and Korea), *T. buffeli* (Europe, Asia, Australia, and Africa), and *T. orientalis* (East Asia) also cause theileriosis in cattle. *Theileria lestoquardi* (Asia and northern Africa), *T. ovis* (Asia), *T. separata* (Asia) (Bishop et al., 2004), *T. uilenbergi*, *T. luwenshuni*, and *T. capreoli* (Ahmed et al., 2006) are infective to small ruminants. The distribution of ECF in eastern, central and southern Africa is directly related to the distribution of its main tick vector, *Rhipicephalus appendiculatus* (Norval et al., 1992b; Olwoch et al., 2008). The disease was also reported in Comoros between 2003 and 2004 for the first time (De Deken et al., 2007). The latter incidence was suggested to result from importation of immunised cattle from Tanzania, which were fed upon by naïve ticks that subsequently transmitted the infection to a susceptible local cattle population (De Deken et al., 2007). Previous studies in Uganda showed that the seroprevalences for *T. parva* infection among cattle were 75.5% in Kiruhura District, western Uganda (Chenyambuga et al., 2010), about 60% in Mbale District, eastern Uganda (Rubaire-Akiiki et al., 2004), 100% in Soroti District, eastern Uganda (Kabi et al., 2008), and 93.3% in Sembabule District, central Uganda (Otim et al., 2004). Molecular studies were also conducted, and showed prevalences of 24% in Mbarara District in western Uganda (Muhanguzi et al., 2010b), 63% around Lake Victoria in central Uganda (Oura et al., 2004a), 30% in 10 agro-ecological zones of Uganda (Kabi et al., 2014), 5.3% in Tororo District in eastern Uganda (Muhanguzi et al., 2014), and 34% on a single farm in Kayunga District, central Uganda (Asiimwe et al., 2013). Microscopic examination of thin blood smears showed prevalences for *T. parva* of 47.4% in central and western Uganda (Kasozi et al., 2014) and 8.8-25.3% around Lake Mburo National Park in south-western Uganda (Ocaido et al., 2009a). Other *Theileria* species reported in Uganda are *T. mutans*, *T. taurotragi* and *T. velifera* (Oura et al., 2004a; Muhanguzi et al., 2010b; Oura et al., 2011b; Asiimwe et al., 2013). Muhanguzi et al. (2010b) also reported *T. buffeli*, *Theileria* sp. sable, *Theileria* sp. buffalo, and *T. bicornis* among cattle.

2.5.1.2 Transmission

In the field, *T. parva* is transmitted cyclopropagatively and transstadially only through the medium of the tick vector. When the infected stage of the tick vector attaches to the new host, sporogony and maturation of the sporozoites in the salivary glands occur, and transmission takes place by the injection of infected saliva. The tick loses its theilerial infection after having transmitted it. The infection does not persist to the post-transmission stage, let alone the next generation (Uilenberg, 2006). East Coast fever and January disease are caused by cattle-derived *T. parva*, transmitted from sick and recovered carrier cattle to susceptible cattle by the three-host brown ear tick *R. appendiculatus* (Lawrence et al., 2004b; Lawrence et al., 2004c). Corridor disease is caused by buffalo-derived *T. parva* that is transmitted from the asymptomatic carrier, African buffalo (*Syncerus caffer*), to susceptible cattle by *R. appendiculatus* and *R. zambeziensis*, and possibly by *R. duttoni* (Lawrence et al., 2004a). *Theileria annulata* is transmitted by several *Hyalomma* tick species while *T. taurotragi* is transmitted by *R. appendiculatus*, *R. zambesiensis* and *R. pulchellus* ticks (Walker et al., 2013). *Amblyomma* tick species are responsible for the transmission of *T. velifera* and *T. mutans* (Bishop et al., 2004; Walker et al., 2013) while *T. sergenti* and *T. buffeli* are transmitted by *Haemaphysalis* tick species (Bishop et al., 2004).

2.5.1.3 Pathogenesis and clinical signs

East Coast fever is an acute and often fatal disease of cattle (Norval et al., 1992b). Sporozoites from ticks infect both T and B lymphocytes (Baldwin et al., 1988). Rapid multiplication of infected T cells is responsible for the pathogenicity of *T. parva* (Morrison et al., 1996). The appearance of parasitised cells in the lymph nodes coincides with the onset of fever, and stimulates active lymphoid proliferation and the release of large numbers of infected and non-infected lymphoblasts into the efferent lymph (Morrison et al., 1981). After a further 2-3 days, parasitised and non-parasitised lymphoblasts enter the peripheral circulation. These establish in other lymph nodes and lymphoid tissues (thymus and spleen), as well as in many parenchymatous organs (Lawrence et al., 2004b). Four to five days after the initial appearance of schizonts, the process of lymphocyte proliferation is succeeded by a process of lymphocyte destruction, and lymphoid organs and lymphoid foci in parenchymatous organs may show evidence of necrosis and depletion of lymphocytes (Emery et al., 1981). Immunosuppression

may predispose such animals to secondary respiratory infections which may eventually prove fatal (Lawrence et al., 2004b). Lymphocyte disintegration in the lungs results in release of vasoactive components (Shitakha et al., 1983), which may be responsible for the pulmonary oedema (Lawrence et al., 2004b). In non-fatal cases, development of specific protective immune responses terminates schizont proliferation (Emery et al., 1981). A proportion of recovered animals remain unthrifty and unproductive, and foci of infected lymphoid cells may be found persisting on a variety of organs (Dolan, 1986). The invasion of erythrocytes by piroplasms is a striking feature of the disease, but the parasite in this form does not have any consistent pathogenic effect. Non-regenerative anaemia and icterus are sometimes described in ECF, but no evidence of a haemolytic process has been presented (Lawrence et al., 2004b).

The incubation period of ECF is generally about 15 days from the time of attachment of the infected tick, but may range from 8 to 25 days (Jarrett et al., 1969). The clinical signs of the disease include fever, respiratory distress, coughing, nasal and lachrymal discharges, diarrhoea, petechiation of the visible mucous membranes (especially those beneath the tongue and in the vulva) and lymph node enlargement (Irvin and Mwamachi, 1983). The disease progresses for a period of 15-25 days. The fever remains high, although in a small proportion of cases there may be a temporary remission for one or two days. Appetite and rumination become increasingly depressed and there is a severe loss of body condition, increasing weakness and ataxia, and frequent recumbency (Lawrence et al., 2004b). Constipation is succeeded by diarrhoea and there may be blood and mucus in the faeces. Opacity of the cornea may be detected. Pregnant cows may abort (Lawrence et al., 2004b). In the terminal stages of the disease, dyspnoea develops with an increased respiratory rate, a watery cough and a discharge of frothy fluid from the nostrils. The enlarged superficial lymph nodes begin to regress, the rectal temperature falls to subnormal levels, and the animal becomes recumbent and dies in a coma (Lawrence et al., 2004b). Death is usually attributed to massive effusion of fluids into the respiratory tract resulting in pulmonary oedema (Irvin and Mwamachi, 1983). Cattle in endemic or epidemic ECF areas often become long-term asymptomatic carriers of *T. parva* schizonts and piroplasms following treatment or spontaneous recovery, thereby maintaining the parasite population (Dolan, 1986; Kariuki et al., 1995). Because piroplasms of *T. parva* undergo only limited replication (Conrad et al.,

1986), the carrier state probably arises from persistence of small numbers of schizont-infected lymphoblasts.

Corridor disease is an acute, usually fatal disease of cattle. The pathogenesis and pathology of Corridor disease are very similar to those of ECF, although Corridor disease is characterised by low schizont parasitosis and piroplasm parasitaemia. The course of the disease is usually shorter than ECF, with death occurring only 3-4 days after the onset of first clinical signs (Lawrence et al., 2004a). In an outbreak, the mortality rate can exceed 90% (Neitz, 1955; Potgieter et al., 1988). The disease is considered self-limiting, because the parasite cannot adapt to cattle and usually fails to complete its development as most cattle die before the parasite develops to the tick-infective stage, the piroplasm (Neitz, 1955). The buffalo-derived *T. parva* carrier state in cattle is not always continuous, which limits transmission by ticks (Norval et al., 1992b; Latif and Hove, 2011). There is no evidence that Corridor disease occurs in Uganda. A previous study in and around Lake Mburo National park (LMNP) showed no evidence for transmission of buffalo-derived *T. parva* genotypes to the cattle population (Oura et al., 2011b).

January disease is usually a milder form of theileriosis (Lawrence et al., 2004c). The disease emerged in Zimbabwe after the eradication of ECF. The name is attributed to the strict seasonality of the disease occurrence which is between December and March, coinciding with the seasonal activity of the adult tick vector (Matson, 1967). The pathogenesis, pathology and clinical signs of January disease are similar to those of ECF (Lawrence et al., 2004c). The disease occurs regularly in Zimbabwe, causing a significant number of deaths each year. There is no evidence that the disease occurs in Uganda.

2.5.2 Anaplasmosis

2.5.2.1 Aetiology

Anaplasmosis is caused by parasites of the genus *Anaplasma* (phylum Proteobacteria, order Rickettsiales, family Anaplasmataceae), which contains obligate intracellular organisms found exclusively within membrane-bound vacuoles in the cytoplasm of both vertebrate and invertebrate host cells (Dumler et al., 2001). Proteobacteria are a major phylum of Gram-

negative bacteria (Dumler et al., 2001). Other organisms placed in the family Anaplasmataceae, and found within membrane-bound vacuoles are *Ehrlichia*, *Neorickettsia* and *Wolbachia* (de la Fuente et al., 2004a). The genus *Anaplasma* includes pathogens of ruminants, namely *A*. *marginale* (the type species), *A*. *marginale* subsp. *centrale* (referred to as *A*. *centrale*), *A*. *bovis* (formerly *Ehrlichia bovis*), and *A*. *ovis*. Also included in this genus is *A*. *phagocytophilum* (previously recognised as *E*. *equi*, *E*. *phagocytophila*, and the human granulocytic ehrlichiosis [HGE] agent), which infects a wide range of hosts including humans, rodents, birds, dogs and cattle, and *A*. *platys* (formerly *E*. *platys*) which infects dogs (Dumler et al., 2001). *Aegyptianella*, which is infective for birds, was tentatively retained as a generic name but was designated a *genus incertae sedis* due to lack of sequence information. Because of the similarities (based on 16S rRNA and *groEL* gene sequences) to the species comprising *Anaplasma*, *Aegyptianella pullorum* was posited also to belong to the genus (Rikihisa et al., 2003); however, *A. pullorum* has yet to be formally re-assigned as a member of the *Anaplasma* genus (Kocan et al., 2010).

Anaplasma marginale is an obligate intra-erythrocytic pathogen that causes bovine anaplasmosis, formerly known as gall sickness (Theiler, 1910; Dumler et al., 2001; Kocan et al., 2010), and is globally the most prevalent tick-borne pathogen of cattle. The pathogen infects erythrocytes and inclusion bodies are found in a marginal position in red blood cells. *Anaplasma marginale* is distributed in tropical and subtropical regions on six continents (de la Fuente et al., 2007b; Kocan et al., 2010; Aubry and Geale, 2011). The wide and increasing distribution of *A*. *marginale* may be due to increased transportation of cattle with subsequent mechanical or biological transmission from asymptomatic persistently infected animals to susceptible ones (Kocan et al., 2010). Anaplasmosis causes considerable economic losses globally to both dairy and beef industries (Kocan et al., 2010). Although clinical disease is most notable in cattle, other ruminants including water buffalo, bison, African antelopes, and some species of deer can be infected (Aubry and Geale, 2011). The losses due to anaplasmosis in cattle include: control and treatment costs, reduced weight gain and milk production, abortions, and the disease frequently leads to death (Aubry and Geale, 2011). *Anaplasma centrale* is less pathogenic for cattle than *A*. *marginale*, and is only occasionally associated with clinical disease (Kocan et al., 2010). The bacterium is found in the erythrocytes of

ruminants, mainly cattle, and is distributed worldwide. As opposed to *A*. *marginale*, *A*. *centrale* inclusion bodies occur in the central part of the cell (Theiler, 1911; Dumler et al., 2001). *Anaplasma centrale* is presently used as a live vaccine in Israel, Australia, South Africa, and South America in order to provide a certain degree of protection against *A*. *marginale* (Callow and Dalgliesh, 1980; Kocan et al., 2010). *Anaplasma ovis*, the agent of ovine anaplasmosis, may cause mild to severe disease in sheep, deer and goats but is not infectious for cattle (Aubry and Geale, 2011). *Anaplasma phagocytophilum* has been detected in Europe, America (North and South), Asia and Africa, but seems to cause tick-borne fever (animals) and human granulocytic anaplasmosis in Europe and USA only (Atif, 2015). Evidence suggests that subclinical persistent infections of *A*. *phagocytophilum* occur in domestic and wild ruminants, including white-tailed deer, red deer, and roe deer (Dumler et al., 2001; Polin et al., 2004; de la Fuente et al., 2004b). The amino acid sequences of the major surface proteins (MSPs) of *A*. *phagocytophilum* are similar to those of *A. marginale* (Aubry and Geale, 2011). *Anaplasma bovis* causes bovine monocytic anaplasmosis, and has been identified in cattle, wildlife and ticks, in Asia (Kim et al., 2003; Kawahara et al., 2006; Ooshiro et al., 2008) and Africa (Muhanguzi et al., 2010a; Fyumagwa et al., 2013). The species *A*. *phagocytophilum*, *A*. *bovis*, and *A*. *platys* invade blood cells other than erythrocytes of their respective mammalian hosts (Aubry and Geale, 2011).

In Uganda, the seroprevalences of anaplasmosis among cattle were about 60% in Mbale District (Rubaire-Akiiki et al., 2004), 58% in Soroti District (Kabi et al., 2008), and 6.6-14% in Tororo and Busia Districts (Magona et al., 2011b) in eastern Uganda. In molecular studies, the prevalence of *A*. *marginale* was 18% on farms around Lake Victoria, central Uganda (Oura et al., 2004a), 8.7% on a single farm in Kayunga District, central Uganda (Asiimwe et al., 2013), and 3.7% in Mbarara District, western Uganda (Muhanguzi et al., 2010a). Studies in Uganda have also shown other *Anaplasma* species in cattle as follows: *A*. *centrale*, *A*. *bovis*, *Anaplasma* sp. Omatjenne, and *A*. *phagocytophilum* (Muhanguzi et al., 2010a); *A*. *bovis* (Oura et al., 2004a; Oura et al., 2011b); and *A*. *centrale* and *A*. *bovis* (Asiimwe et al., 2013).

2.5.2.2 Transmission

Biological transmission of *A*. *marginale* is effected by ticks, and at least 20 species of ticks have been reported as vectors worldwide (Kocan et al., 2010). The tick vectors of *A. marginale* include *Rhipicephalus* (*Boophilus*) spp., *Rhipicephalus* spp., *Dermacentor* spp. and *Ixodes ricinus* (Kocan et al., 2004a). Within the tick, *A*. *marginale* replicates both within the gut epithelium and the salivary gland acini (Scoles et al., 2005a). Tick transmission can occur from one life stage of the tick to another (interstadial or transstadial) or within a stage (intrastadial), while transovarial transmission from one tick generation to the next does not appear to occur (Stich et al., 1989; Samish et al., 1993). The one-host ticks *Rhipicephalus* (*Boophilus*) spp. are the most common vectors of *Anaplasma* spp. in many parts of the world (Tay et al., 2014; Castañeda-Ortiz et al., 2015; Pesquera et al., 2015). In Uganda, the tick vectors for *A*. *marginale* are reportedly *R*. *decoloratus* and *R*. *evertsi evertsi* (Magona et al., 2008; Magona et al., 2011a; Magona et al., 2011b). Vector competence for *A*. *marginale* may vary among or within tick species (Scoles et al., 2005b; Shkap et al., 2009), and among *A*. *marginale* strains (Ueti et al., 2007; Shkap et al., 2009). Intrastadial transmission of *A*. *marginale* has been shown to be effected by male ticks. Intrastadial transmissions occur when male ticks acquire infection, then detach to search for mates on another animal, during which process the pathogen is transmitted from one host to another by the same tick in the same stage (Kocan et al., 1992). Once male ticks become infected, they remain carriers for life and serve as reservoirs for repetitive transmission of *A*. *marginale* when they transfer between cattle (Kocan et al., 1992; Palmer et al., 2001). Therefore, infected male ticks may be an important mechanism of transmission of *A*. *marginale* by one-host ticks including *Rhipicephalus* (*Boophilus*) spp. and *Dermacentor* spp. (Kocan et al., 1992). Transmission of *A*. *marginale* directly from infected to uninfected ticks when they feed together at the same site (co-feeding) does not occur (Kocan and de la Fuente, 2003). Mechanical transmission of *A*. *marginale* occurs when infected blood is transferred to susceptible cattle by bloodsucking diptera of the genera *Tabanus*, *Stomoxys*, and various mosquito species (Scoles et al., 2005a) or blood-contaminated fomites (e.g. needles, dehorning saws, nose tongs, ear tagging devices, castration instruments, tattooing equipment) (Reeves and Swift, 1977). In addition to biological and mechanical transmission, *A*. *marginale* can be transmitted transplacentally from cow to calf (Potgieter and van Rensburg, 1987a). Vertical transmission is an important

factor in early calf mortality and the occurrence of outbreaks of the disease in growing calves, and contributes considerably to the infection of vector ticks and the perpetuation of the disease (Kocan et al., 2003). *Anaplasma centrale* is transmitted by *Rhipicephalus simus*, a strictly African tick species (Potgieter and van Rensburg, 1987b), but also by *Ixodes* spp. and *Haemaphysalis* spp. (Rymaszewska and Grenda, 2008). *A. phagocytophilum* is transmitted by ticks of the genus *Ixodes* (Atif, 2015). Other *Anaplasma* spp. are transmitted by *Haemaphysalis* spp., *Rhipicephalus* spp., and *Ambylomma* spp. (*A. bovis*), *Dermacentor* spp. (*A. ovis*), and *R. sanguineus* (*A. platys*) (Rymaszewska and Grenda, 2008).

2.5.2.3 Pathogenesis and clinical signs

The reticulated form of *Anaplasma* in the tick changes into the dense form. Cattle become infected with *A. marginale* when the dense form is transmitted via the salivary glands during feeding (Kocan et al., 1992; Ge et al., 1996). *A. marginale* parasites invade mature erythrocytes and replicate intracellularly by binary fission (Palmer et al., 2000). Major surface proteins (MSPs) play a crucial role in the interaction of *A. marginale* with host cells, and include adhesion proteins and MSPs from multigene families (Kocan et al., 2010). The incubation period of infection (prepatent period) varies with the infective dose and ranges from 7 to 60 days, with an average of 28 days (Kocan et al., 2003). During that time, the number of infected blood cells increases geometrically (Richey and Palmer, 1990). Depending upon the strain of *Anaplasma* and the susceptibility of the host, from 10% to 90% of the erythrocytes may be parasitised in the acute stage of the infection. At least 15% of the erythrocytes have to be parasitised before there is clinical disease (Aubry and Geale, 2011). During this acute stage of infection, the number of infected erythrocytes may be as high as 10^9 cells per millilitre of blood (Palmer et al., 1999). Infected erythrocytes are removed by the reticulo-endothelial system by phagocytosis, which leads to anaemia and icterus without haemoglobinaemia and haemoglobinuria. The clinical picture of the disease also includes fever, anorexia, lethargy, and often death, from severe anaemia, in animals over 2-years old (Potgieter and Stoltsz, 2004; Aubry and Geale, 2011). There are increased heart and respiratory rates. Erythrocyte count, haemoglobin values, and packed cell volume significantly decrease, coincidental with increasing parasitaemia (Potgieter and Stoltsz, 2004). Milk production drops in lactating cows and pregnant cows may abort (Aubry and Geale,

2011). Bulls may develop temporary infertility (Potgieter and Stoltsz, 2004). With advanced disease, cattle develop gastrointestinal atony, rumen stasis and constipation, which are associated with dehydration and weight loss. Some animals may experience neurological deficits, which have been attributed to episodes of cerebral anoxia (Potgieter and Stoltsz, 2004). Regardless of the age of an animal at the time of infection, once cattle become infected with *A*. *marginale*, they remain persistent carriers for life, whether or not they develop clinical disease (Aubry and Geale, 2011). Persistently-infected cattle have cyclic low-level rickettsaemia and are reservoirs for mechanical and biological transmission (Aubry and Geale, 2011). The immune response allows cattle to recover from acute anaplasmosis and to maintain persistent infection (Palmer et al., 1989). Due to the removal of infected erythrocytes from circulation, and given that the mean lifespan of a bovine red blood cell is 160 days, new erythrocytes need to be re-infected so as to maintain persistent infection. This is accomplished by evasion of the persistently infected carrier's immune response, followed by emergence and replication of antigenic variants of *A*. *marginale* (Palmer and Brayton, 2013). Each cycle reflects the emergence of one or, more commonly, multiple clones that express a unique, central hypervariable region (HVR) of major surface protein (MSP) 2 (French et al., 1999) and MSP3 (Brayton et al., 2003). The HVR encodes variant B-cell epitopes (French et al., 1999). This results in 'escape variants' which are not recognised by antibody present at the time of emergence (Palmer et al., 2000).

Necropsy findings are predictable from the clinical signs and include severe anaemia, icterus, splenomegaly, and hepatomegaly. Petechial haemorrhages are frequently observed on serosal surfaces especially over the heart and pericardium; the heart is often pale and flabby (Potgieter and Stoltsz, 2004).

Infection by *A*. *bovis* can be peracute, acute, sub-acute or chronic (Sreekumar et al., 1996; Kawahara et al., 2006). The clinical symptoms include fever of 39 to 41°C, anorexia, dyspnoea, sometimes a dry cough, tachycardia, diarrhoea, nervous disorders (hyper-excitability, transient paralysis of larynx, epileptic forms and paralysis), incoordination, and enlargement of the lymph nodes (Sreekumar et al., 1996; Kawahara et al., 2006). Anaplasmosis caused by

A. bovis is rarely reported because it affects the monocytes, which are often overlooked in routine field diagnosis (Fyumagwa et al., 2013).

2.5.2.4 Genetic diversity of Anaplasma marginale

Three out of six major surface proteins (MSPs) - MSP5, MSP4 and MSP1a - have been extensively used for the molecular characterisation of *A. marginale* (Aubry and Geale, 2011). The *msp*5 gene is highly conserved among *Anaplasma* spp. and therefore can only be used to establish that organisms are in the genus *Anaplasma*, but not for identification of the species. Furthermore, MSP5-based serologic tests result in cross-reactions between *Anaplasma* spp. (de la Fuente et al., 2005). However, previous analysis of the *msp*4 gene from *A. marginale* isolates demonstrated sufficient sequence variation to support its use in phylogeographic studies (de la Fuente et al., 2003). Analyses of *msp1α* (major surface protein 1 alpha) gene sequences have allowed the identification of *A. marginale* strains worldwide (Cabezas-Cruz et al., 2013). In the genome of *A. marginale*, the *msp1α* is a single copy gene that encodes a 70-100 kDa protein (MSP1a) which is conserved during acute and persistent rickettsaemia in cattle and also during multiplication in ticks (Kocan et al., 2003). MSP1a contains variable numbers of 23-31 amino acid serine-rich tandem repeats located in the N-terminal region of the protein, which is continuous with a highly conserved C-terminal region and the 5'-UTR microsatellite (Allred et al., 1990; Palmer et al., 2001; de la Fuente et al., 2005). Due to its diversity, and the fact that the number and sequence of tandem repeats remains the same in a given strain, the *msp1α* gene has been recognised as a stable marker to identify geographic strains of *A. marginale* that differ in biology, protein sequence and antigenicity (de la Fuente et al., 2007b; Cabezas-Cruz et al., 2013; Mutshembele et al., 2014; Cabezas-Cruz and de la Fuente, 2015; Silva et al., 2015).

Furthermore, MSP1a is an adhesin for bovine erythrocytes and tick cells. The MSP1a tandem repeats contain functional domains that bind to bovine erythrocytes and tick cells, and MSP1a is therefore a determinant of infection for cattle and transmission of *A. marginale* by ticks (McGarey et al., 1994; de la Fuente et al., 2001; Cabezas-Cruz and de la Fuente, 2015). MSP1a is also a good candidate for inclusion in vaccines for the control of anaplasmosis because the protein induces strong T cell responses (Brown et al., 2001; Brown et al., 2002). The B-cell

epitope within the MSP1a tandem repeat ((Q/E) ASTSS) was recognised by a monoclonal antibody that neutralised *A*. *marginale in vitro* (Allred et al., 1990). The neutralisation epitope was found to be conserved among heterologous *A*. *marginale* strains (Palmer et al., 1987). An additional linear B-cell epitope (SSAGGQQQESS) is recognised by immunised and protected cattle, and is therefore immunodominant (Brown et al., 2002; Garcia-Garcia et al., 2004). Recombinant MSP1a has been used in immunisation trials in cattle against *A*. *marginale* (Almazán et al., 2012; Torina et al., 2014) and laboratory models (Santos et al., 2013; Silvestre et al., 2014) and has demonstrated promising results. These results provide fundamental information for the design of MSP1a structure-based vaccines, which would be cross-protective against multiple *A*. *marginale* strains, and for the development of serodiagnostic methods based on differential B-cell epitopes, for epidemiological characterisation of field strains (Cabezas-Cruz et al., 2013).

2.5.3 Heartwater

2.5.3.1 Aetiology

Heartwater (cowdriosis) is a rickettsial disease of some wild, and all domestic, ruminants caused by *Ehrlichia* (formerly *Cowdria*) *ruminantium* (Dumler et al., 2001). *Ehrlichia ruminantium* is an obligate intracellular bacterium that belongs to the phylum Proteobacteria, order Rickettsiales, and family Anaplasmataceae (Dumler et al., 2001). Other species in the genus *Ehrlichia* include *E. canis* and *E. chaffeensis*, which mainly affect canids and humans respectively producing a monocytic ehrlichiosis or subclinical infections (Dumler et al., 2001), and *E*. *ewingi*, *E*. *muris* and *E*. *ovina* (Allsopp et al., 2004). Heartwater is of serious economic importance across an area which includes almost all of sub-Saharan Africa as well as Madagascar, Mauritius, Reunion, Grande Comore and São Tomé, and several islands in the Caribbean (Provost and Bezuidenhout, 1987; Allsopp, 2010). The disease poses a threat of spread to the American mainland due to animal movements, and the presence of a competent tick vector (Mahan et al., 2000; Burridge et al., 2002). The economic impact of heartwater in endemic areas in Africa is difficult to quantify because the definitive diagnosis for the disease is not often performed (Allsopp, 2010). However, estimates that have been made indicate that losses are enormous, and these include death, decreased meat and milk production, unthriftness, decreased draught power and manure, in addition to high cost of

control measures (Mukhebi et al., 1999). Approximately 150 million animals are at risk of heartwater in sub-Saharan Africa (Allsopp, 2010). Small ruminants are particularly at risk from the disease. In susceptible animals in the tropics and subtropics, mortality rates range from 20% to 90% (Uilenberg, 1983; Allsopp et al., 2004). Heartwater is also a major obstacle to the introduction of more productive exotic breeds of ruminants into sub-Saharan Africa to upgrade local stock (Kanyari and Kagira, 2000; Allsopp, 2010), and has been suggested to be a potential emerging zoonosis (Allsopp et al., 2005). Previous molecular studies in Uganda showed low prevalences of *E. ruminantium* among cattle: 0% on farms around Lake Victoria, central Uganda (Oura et al., 2004a), and 4.5% in Mbarara District, western Uganda (Muhanguzi et al., 2010a).

2.5.3.2 Transmission

Ehrlichia ruminantium is transmitted transstadially by three-host ixodid ticks of the genus *Amblyomma* (Allsopp et al., 2004). The distribution of heartwater, therefore, coincides with that of the *Amblyomma* ticks (Walker and Olwage, 1987). Ten *Amblyomma* spp. that are capable of transmitting the organism occur in Africa. The most important vectors are *A. variegatum* and *A. hebraeum* (Bezuidenhout, 1987; Walker and Olwage, 1987) but *A. pomposum*, *A. lepidum*, *A. astrion*, *A. cohaerens*, *A. gemma* (Walker and Olwage, 1987) and *A. marmoreum* (Peter et al., 2000) can also be significant vectors. *A. variegatum* is the most significant in Africa because of its wide distribution, close adaptation to domestic ruminants, and efficacy as a vector (Walker and Olwage, 1987; Camus and Barré, 1992; Walker et al., 2013). The tick has been found in the field in various studies in Uganda (Otim et al., 2004; Rubaire-Akiiki et al., 2004; Rubaire-Akiiki et al., 2006; Magona et al., 2011a). *Ehrlichia ruminantium* was also demonstrated in *A. variegatum* ticks from eastern Uganda by PCR (Nakao et al., 2012). Adults and nymphs of ticks become infected with *E. ruminantium* within 2-4 days of feeding on domestic ruminants (Bezuidenhout, 1988; Camus and Barré, 1992). Intrastadial transmission by male *A. hebraeum* ticks moving from infected to susceptible animals also occurs, and this is important in the epidemiology of the disease (Andrew and Norval, 1989b). Vertical transmission of heartwater from dams to their calves was demonstrated in cattle (Deem et al., 1996b) and in lambs and kids one week old in The Gambia (Faburay et al., 2007b). Infected cells in the colostrum are thought to be responsible for

vertical transmission (Allsopp et al., 2004). Vertical transmission has an important effect on the maintenance of heartwater (Allsopp, 2010).

2.5.3.3 Pathogenesis and clinical signs

Infection of the vertebrate hosts takes place after two days if nymphs are involved and four days in the case of adult ticks (Bezuidenhout, 1987; Kocan et al., 1987). The lifecycle of *E. ruminantium* is characterised by two distinct developmental forms that are found within mammalian host cells (Jongejan et al., 1991). Initially, the infectious forms of the bacterium (elementary bodies) adhere to host target cells and are internalised. Then, inside intracytoplasmic vacuoles, the elementary bodies differentiate into non-infectious reticulate bodies, which replicate by binary fission (Prozesky and Du Plessis, 1985; Prozesky et al., 1986). After 5 to 6 days of intracellular multiplication, disruption of host cells leads to the release of numerous elementary bodies, initiating a new infectious cycle (Allsopp, 2010). Initial replication of the organisms takes place in reticulo-endothelial cells and macrophages in the regional lymphnodes, after which they are disseminated via the bloodstream and invade endothelial cells of blood vessels in various organs and tissues, where further multiplication occurs (Allsopp et al., 2004). In domestic ruminants, *E. ruminantium* most readily infects endothelial cells of the brain, and this coincides with the onset of the febrile reaction (Allsopp et al., 2004). Increased vascular permeability is responsible for effusion into body cavities and tissue oedema, and this is particularly noticeable in the lungs, pericardial sac and pleural cavity. Oedema of the brain is responsible for the nervous signs, while hydropericardium contributes to cardiac dysfunction during the terminal stages of the disease (Allsopp et al., 2004). Progressive pulmonary oedema and hydrothorax result in eventual asphyxiation (Allsopp et al., 2004). The effusion of fluid into tissues and body cavities also results in a drastic reduction in blood volume (Allsopp et al., 2004).

The incubation period of heartwater in susceptible animals is on average less than two weeks (Van de Pypekamp and Prozesky, 1987). In a herd or flock, the morbidity and mortality rates are influenced by the species, breed and age of the animals affected, the virulence of the *E. ruminantium* stock, the effectiveness of immunisation, tick control and specific chemotherapy programmes applied on the farm, and, in some instances, the season (Allsopp et al., 2004).

The peracute form of the disease is seen in exotic animals and in 6-to-18 month-old animals of different breeds (Allsopp et al., 2004). Peracutely affected animals die within a few hours after the initial development of fever, either without any clinical signs having been manifested, or having shown terminal, paroxysmal convulsions and marked respiratory distress (Allsopp et al., 2004). Acute heartwater is most common and affects cattle between the ages of three and 18 months. It is characterised by a fever of 40^{o}C or higher, which usually persists for 3-6 days, showing only small fluctuations before the body temperature falls to subnormal shortly before death (Allsopp et al., 2004). During the later stages of acute heartwater, nervous signs occur which range from mild incoordination to pronounced convulsions. The animals are hypersensitive to stimuli. They display abnormal gait, show constant chewing movements, push against objects, and may wander around aimlessly. In the later stages, the animals fall down suddenly and assume a position of lateral recumbency. In most cases, the animals weaken rapidly and death usually follows soon after the commencement of a convulsive attack (Allsopp et al., 2004). Case fatality rate in adult cattle goes up to 82% (Du Plessis and Malan, 1987c). The subacute form of heartwater is characterised by a fever which may remain high for 10 days or longer. The clinical signs are similar to those described for the acute form of heartwater, but less pronounced (Allsopp et al., 2004).

2.5.3.4 Carrier state

Apparently healthy ruminant hosts carrying *E. ruminantium* organisms, at very low levels, can be infective to ticks for long periods, at least 361 days for cattle (Andrew and Norval, 1989a) and 11 months for goats (Allsopp, 2010). This demonstrates the danger that is posed by the movement of heartwater carrier animals to areas free of the disease.

2.5.4 Babesiosis

2.5.4.1 Aetiology

Babesiosis is caused by obligate intra-erythrocytic and generally host-specific protozoan parasites of the genus *Babesia*, which belongs to the phylum Apicomplexa, order Piroplasmida and family Babesiidae (Bock et al., 2004; Adl et al., 2012). *Babesia* parasites infect a wide range of domestic and wild animals and occasionally humans, and are

distributed worldwide (Allsopp et al., 1994; Bock et al., 2004; Schnittger et al., 2012). Babesias are considered to be the second most commonly-found parasites in the blood of mammals after trypanosomes (Schnittger et al., 2012). Bovine babesiosis, or redwater fever, is an important disease with an estimated 400 million cattle at risk worldwide (Zintl et al., 2005). The costs of bovine babesiosis are connected with mortalities, decreased milk and meat production, loss of drought power, abortions, control measures (acaricides, vaccines, and therapeutics), as well as losses of production and cattle trade restrictions (Bock et al., 2004; Suarez and Noh, 2011; Schnittger et al., 2012).

The most important *Babesia* species in cattle are *B. bovis*, *B. bigemina* and *B. divergens*, while others include *B. major*, *B. ovata*, *B. occultans*, and *Babesia* sp. Kashi. *Babesia ovis* and *B. motasi* infect sheep and goats (Bock et al., 2004; Uilenberg, 2006; Schnittger et al., 2012). The distribution of *Babesia* species of cattle includes Africa, Asia, Australia, Central and South America, and southern Europe (*B. bigemina* and *B. bovis*); north-west Europe, Spain, Great Britain, and Ireland (*B. divergens*); Europe, north-west Africa, and Asia (*B. major*); and eastern Asia (*B. ovata*). *Babesia occultans* is distributed in Africa while *Babesia* sp. Kashi is found in China (Bock et al., 2004; Schnittger et al., 2012). *Babesia divergens* is also important as a human pathogen (Uilenberg, 2006; Schnittger et al., 2012). In Africa, the two most important species that cause babesiosis in cattle are *B. bovis* and *B. bigemina* (De Vos et al., 2004). *Babesia bovis* is classically known as a 'small' *Babesia* measuring up to 2 µm in diameter, while *B. bigemina* is larger and can extend to the full diameter of an erythrocyte (Potgieter, 1977); however, large forms of *B. bovis* are quite common (Potgieter, 1977). In Uganda, cattle are reportedly exposed only to *B. bigemina* (Rubaire-Akiiki et al., 2004; Kabi et al., 2008; Magona et al., 2011b). Epidemiological studies on *Babesia* spp. in Uganda showed that the seroconversion rates to *B. bigemina* were 4.9-16% in Busia and Tororo Districts (Magona et al., 2011b), 60% in Mbale District (Rubaire-Akiiki et al., 2004), and 100% in Soroti District (Kabi et al., 2008) in eastern Uganda. A molecular study showed a low prevalence (2%) of *B. bigemina* around Lake Victoria, central Uganda (Oura et al., 2004a). The other species of *Babesia* reported in Uganda is *B. vogelli* (0.6%) in Mbarara District in the western part of the country (Muhanguzi et al., 2010b).

2.5.4.2 Transmission

The geographical distribution of *Babesia* parasites corresponds with that of their tick vectors (Suarez and Noh, 2011). In Africa, transmission of *B. bigemina* and *B. bovis* is by the ticks *Rhipicephalus microplus*, *R. annulatus* and *R. geigyi*. In addition, *B. bigemina* is transmitted by *R. decoloratus* and *R. evertsi evertsi* (Büscher, 1988; Bock et al., 2004; Walker et al., 2013). *Babesia bovis* is the more virulent of the two parasites; however, *B. bigemina* is more widespread than *B. bovis* in Africa because of the ability of both *R. decoloratus* and *R. evertsi evertsi* to act as vectors (Friedhoff, 1988). In Uganda, *B. bigemina* is known to be transmitted by *R. decoloratus* and *R. evertsi evertsi* (Rubaire-Akiiki et al., 2004; Ocaido et al., 2005; Rubaire-Akiiki et al., 2006; Magona et al., 2011a). *Babesia bovis* and *B. bigemina* exhibit a typical apicomplexan life cycle characterised by merogony, gametogony, and sporogony, have erythrocytes as the only single cell target in the bovine host, and are transovarially transmitted (Mehlhorn and Schein, 1985; Hunfeld et al., 2008; Chauvin et al., 2009). Cattle are infected by the injection of sporozoites with saliva of the vector tick. The sporozoites invade and replicate inside erythrocytes (Uilenberg, 2006; Hutchings et al., 2007; Chauvin et al., 2009). In the tick, motile club-shaped kinetes escape into the haemolymph to infect a variety of cell types and tissues, including the ovaries (Uilenberg, 2006). In all species, sporozoite development usually only begins when the infected tick attaches to the vertebrate host (Bock et al., 2004). In *B. bigemina*, some development takes place in the feeding larvae, but infective sporozoites take about nine days to appear and therefore only occur in the nymphal and adult stages of the tick (Bock et al., 2004). Transmission of *B. bigemina* can then occur throughout the rest of the nymphal stage and by adult females and males (Bock et al., 2004). In the case of *B. bovis*, the formation of infective sporozoites usually occurs within two or three days of larval tick attachment (Bock et al., 2004). Therefore, *B. bovis* is transmitted during the larval stage, and infection does not persist beyond the larval stage (Mahoney and Mirre, 1979). Transovarial transmission increases transmission efficiency in the field, when only the adult tick feeds on cattle. This transmission is also considered as a major adaptation for long-lasting persistence, because some ticks remain infected and infective for several generations without needing to again feed on infected animals (Uilenberg, 2006; Chauvin et al., 2009). Therefore, *Babesia* spp. can be considered to be vertically-transmitted parasites (Chauvin et al., 2009). In addition, larvae and adult males of *Rhipicephalus* (*Boophilus*) spp.

are known to migrate from infested to uninfested cattle, and may play a part in the transmission of *Babesia* (Mason and Norval, 1981). The transmission of *B*. *bigemina* by *R*. *evertsi evertsi* is transovarial, and only the nymphal stage infects the bovine host (Büscher, 1988).

2.5.4.3 Pathogenesis, clinical signs and pathology

Babesia bovis kills more than half of the susceptible cattle that it infects (Zintl et al., 2005). The prepatent period following inoculation of *B*. *bovis* by tick larva is generally between six and 12 days, with peak parasitaemia and the manifestation of other clinical signs three to five days thereafter (Bock et al., 2004). The overproduction of cytokines and other pharmacologically active agents during the immune response to *B*. *bovis* contributes to disease progress (Ahmed, 2002; Bock et al., 2004). However, *B*. *bovis* parasitaemia remains low (0.2-0.04%) in acutely infected cattle, as infected erythrocytes undergo sequestration by attachment to capillary endothelium, resulting in allergic reactions and organ damage, which may result in neurological signs and respiratory distress syndrome (Bock et al., 2004; Zintl et al., 2005; Suarez and Noh, 2011; Schnittger et al., 2012). An initial fall in haematocrit is largely due to haemodilution associated with circulatory stasis rather than erythrocyte destruction, and acute cases rapidly emerge showing severe hypotensive shock (Zintl et al., 2005). Fever (> 40°C) is usually present for several days before signs become obvious. This is followed by inappetence, depression, increased respiratory rate, weakness and a reluctance to move. Anaemia and jaundice develop especially in more protracted cases. Muscle wasting, tremors, and recumbency develop in advanced cases followed by coma (De Vos et al., 2004). Diarrhoea is common. The fever may cause pregnant cattle to abort (Callow, 1984) and surviving bulls to show reduced fertility lasting six to eight weeks (Bock et al., 2004). Acute cases will show haemoglobinuria, but this may be absent in subacute or chronic cases (Bock et al., 2004; De Vos et al., 2004). Clinical pathology centres on a haemolytic anaemia, which is characteristically macrocytic and hypochromic (Bock et al., 2004; De Vos et al., 2004). Failure to provide early diagnosis and treatment may lead to deaths among affected animals (Bock et al., 2004). In sub-acute infections, clinical signs are less pronounced and sometimes difficult to detect (De Vos et al., 2004). Lesions include an enlarged soft and pulpy spleen, a swollen liver, a distended gall bladder with thick granular bile, congested dark-coloured kidneys and

generalised anaemia and jaundice. Other organs may show congestion or petechial haemorrhages, and occasionally there will be pulmonary oedema. The grey matter of the brain can appear pink (Bock et al., 2004; De Vos et al., 2004). Infections by *B*. *bigemina*, on the other hand, result in high parasitaemias, and in these cases pathogenicity is mainly associated with massive erythrocyte destruction, leading to severe anaemia, icterus and death (Callow, 1984; Bock et al., 2004; Schnittger et al., 2012). The clinical signs occur on a continuum ranging from subclinical to acute disease, the severity of the disease being determined by the degree of parasitaemia. In acutely-infected animals, more than 40% of erythrocytes may be infected, causing anaemic anoxia. However, only a relatively small proportion of cases are fatal, although the disease can develop very rapidly (Bock et al., 2004; De Vos et al., 2004; Zintl et al., 2005). Haemoglobinuria is present earlier and more consistently than in *B*. *bovis* infections and fever is less of a feature. Common causes leading to death in acutely infected cattle are shock and respiratory distress (Suarez and Noh, 2011).

The signs of bovine babesiosis occur in mature naïve animals. In contrast, calves of less than about nine months of age possess an innate resistance to disease (Callow, 1984; Zintl et al., 2005). The protection in neonates is partially due to antibodies acquired from colostrum of cows in endemic areas, if sufficiently exposed to infection; however, young animals remain resistant longer than passively transferred antibodies persist (Zintl et al., 2005). Recovering animals develop resistance to reinfection (concomitant immunity) due to the persistence of the original infection (Chauvin et al., 2009). The immunity lasts for a number of years with *B*. *bovis* and for a few months in *B*. *bigemina* (Zintl et al., 2003). However, the protection after infection can be broken down by stress factors such as starvation or concurrent disease, and clinical signs may reappear, while repeated infections result in permanent immunity. It was reported that infection with *B*. *bigemina* can lead to a degree of cross-protection against subsequent *B*. *bovis* infection (Wright et al., 1987).

2.5.4.4 Carrier state

Often, especially in cattle younger than a year old, acute babesiosis resolves into low-level parasitaemias without apparent ill-effects (Trueman and Blight, 1978; Smith et al., 2000), and the animals become carriers of the parasites for several months or several years, thus

ensuring transmission by competent ticks (Allred and Al-Khedery, 2006; Chauvin et al., 2009; Suarez and Noh, 2011). For *B*. *bigemina* infection, recovering animals remain infective for ticks for 4-7 weeks and carriers for only a few months (Bock et al., 2004). Parasite survival in *B*. *bovis* infection is aided by evasion of host adaptive immune responses by the sequential expansion of antigenically variant populations during an infection, a phenomenon referred to as 'antigenic variation' (Allred, 2001; Allred and Al-Khedery, 2006). Antigenic variation in *B*. *bovis* is intimately linked to cytoadhesion, where mature parasite-containing erythrocytes bind to the capillary endothelium (Allred and Al-Khedery, 2006). *Babesia bovis*-infected erythrocytes that remain sequestered do not circulate in the blood and avoid phagocytosis by spleen macrophages, favouring the establishment of persistent infections (Allred, 2001; Allred and Al-Khedery, 2006; Hutchings et al., 2007). The mechanism of sequestration in *B. bovis* infection involves remodeling of the surface of the erythrocyte resulting in a change of its mechanical and adhesive properties to the advantage of the parasite (Hutchings et al., 2007; Gohil et al., 2010). For *B*. *bigemina*, the mechanisms involved in the persistence of the parasites in cattle have been less explored (Suarez and Noh, 2011). In general, carrier animals are protected from babesiosis but not from infection (*B*. *bovis* and *B*. *bigemina*) as they bear extremely low numbers of parasites in the blood (Chauvin et al., 2009).

2.6 Epidemiology of tick-borne diseases

Knowledge of the epidemiology of tick-borne diseases (TBDs) is important because it greatly influences the choice of approaches for control in an area or production system (Gachohi et al., 2013). The epidemiology of TBDs is complex and the disease distribution, prevalence, and impact are determined by factors that include environment (e.g. co-grazing with other animal species, temperature, rainfall, humidity, vegetation cover, and management practices), host characteristics (e.g. population dynamics, susceptibility to ticks and pathogens, acquired immunity, level of parasitaemia and carrier state), the tick vector (e.g. vector competence, abundance, and seasonality), and the pathogen (virulence, antigenic variation and infection rate in the tick). Global changes in climate and resistance to chemotherapeutics and acaricides are also important factors (Norval et al., 1992b; Kivaria, 2010; Kocan et al., 2010; Marufu et al., 2010).

2.6.1 Livestock-wildlife interface and tick-borne diseases

In most pastoral areas of sub-Saharan Africa, free-ranging wildlife and cattle share common grazing grounds. This wildlife-livestock interface can facilitate transmission of tick-borne parasites between wild animals and cattle (Oura et al., 2011a; Oura et al., 2011b; Kabuusu et al., 2013; Walker et al., 2014). Moreover, various tick vectors feed on wild animals, increasing the risk for occurrence, abundance and distribution of ticks among cattle at the interface (Smith and Parker, 2010; Fyumagwa et al., 2013; Walker et al., 2013). African buffalo (*Syncerus caffer*), in particular, are considered to be an important reservoir for various tick-borne haemoparasites (Oura et al., 2011a; Oura et al., 2011b; Gachohi et al., 2012; Fyumagwa et al., 2013; Walker et al., 2014). *Anaplasma marginale* can persistently infect wild animals including the African buffalo (Oura et al., 2011a; Oura et al., 2011b), bison (*Bison bison*) (Zaugg and Kuttler, 1985; de la Fuente et al., 2003; Kocan et al., 2004b), water buffalo (*Bubalus bubalis*), various species of deer, and Rocky Mountain elk (Kuttler, 1984); these species may then be potentially important in the epidemiology and spread of anaplasmosis through mechanical and biological transmission (Kuttler, 1984; Zaugg and Kuttler, 1985; Kocan et al., 2010; Oura et al., 2011a; Oura et al., 2011b). However, there are limited substantial field studies that demonstrate the transmission of *A. marginale* between cattle and wild ruminants (Aubry and Geale, 2011). Nevertheless, in Uganda, it was demonstrated that the prevalence of *A. marginale* and *T. parva* in cattle significantly increased with closer proximity to a wildlife-livestock interface, but not for *B. bigemina* (Kabuusu et al., 2013). Molecular studies in four national parks in Uganda showed that a high proportion of buffaloes were carriers of *A. marginale* (40-74%) and *A. centrale* (56-84%) (Oura et al., 2011a; Oura et al., 2011b). Buffaloes in the national parks in Uganda were also carriers of *T. parva* (0-95%), *T. mutans*, *T. velifera* and *Theileria* sp. buffalo (Oura et al., 2011a; Oura et al., 2011b). Impala (*A. centrale*, unidentified *Babesia*/*Theileria*), eland (*T. taurotragi*, *T. buffeli*), and bushbuck (*T. taurotragi*, *T. buffeli*, *A. bovis*) were also carriers of tick-borne parasites (Oura et al., 2011b). However, the wild animals were negative for *E. ruminantium, B. bigemina* and *B. bovis* (Oura et al., 2011a; Oura et al., 2011b). The *T. parva* carrier status of buffalo indicates a risk of spread of Corridor disease from buffalo to cattle grazing at the wildlife-livestock interface. The interface is also likely to have implications for the control of TBDs; for example, a study in Kenya showed that elimination of *T. parva* infection from cattle was unlikely to be accomplished solely by

frequent acaricide use on cattle when grazing land is shared with buffalo (Walker et al., 2014). In Tanzania, the African buffalo was reported to be an important reservoir for *A*. *bovis*, and the animals are favourite hosts of the vector *Rhipicephalus evertsi evertsi* (Fyumagwa et al., 2013). Twenty-five species of wild ruminants, of which 15 are found in Africa (including the African buffalo), have been shown to be susceptible to *E*. *ruminantium*, either naturally or experimentally (Allsopp, 2010). However, the existence of a wild ruminant reservoir is not essential for maintenance of heartwater, which can be maintained entirely in a domestic stock population (Allsopp, 2010). *Babesia bovis* and *B*. *bigemina* were identified in white-tailed deer in northern Mexico (Cantu et al., 2007), and in brown brocket deer and marsh deer in Brazil (da Silveira et al., 2011). White-tailed deer (*Odocoileus virginianus*) were also identified as suitable hosts for *Rhipicephalus* (*Boophilus*) ticks, the vectors of *Babesia* spp. (Cantu et al., 2007). In other studies, water buffaloes were reported to bear subclinical infections of *B*. *bovis* and *B*. *bigemina* (Uilenberg, 2006; Ferreri et al., 2008), while *B. orientalis* was associated with clinical disease in this host in China (Liu et al., 1997).

2.6.2 Climatic factors, seasonal changes, vegetation and ecology

Ticks are very vulnerable to environmental conditions during the free-living phase of their life cycle, often requiring very specific conditions of humidity and temperatures (Léger et al., 2013). Therefore, climatic variability influences the suitability of vector habitats, thereby impacting the distribution and abundance of ticks and the prevalence and incidence of TBDs (Rubaire-Akiiki et al., 2006; Gachohi et al., 2012; Gachohi et al., 2013; Leta et al., 2013). Highly suitable areas for *R*. *appendiculatus* occur in areas that have moderate temperature and high precipitation (Leta et al., 2013). Therefore, *R*. *appendiculatus* is distributed in eastern, central and southern Africa, where the climate is warmer and more humid, but is not found in the horn of Africa (Norval et al., 1992b; Leta et al., 2013). One of the reasons for higher risk of ECF in eastern Africa, compared to southern Africa, is the bimodal rainfall in the region, which allows up to three generations of *R*. *appendiculatus* to occur each year in some areas, with all instars occurring simultaneously on cattle (Perry and Young, 1995). A unimodal rainfall pattern results in restricted activity of *R*. *appendiculatus* instars and year-to-year variation in rainfall causes fluctuations in tick phenology and *T*. *parva* transmission (Norval et al., 1992b).

Climate change affects the biology of the pathogen itself, as well as vectors, farming practice and use, zoological and environmental factors, and thus influences the occurrence, distribution and prevalence of livestock diseases (Speranza, 2010). Climate change may also influence the movement of tick hosts, which may result in changes in host population and/or diversity, leading to disruption in the life cycles of ticks and the distribution of TBDs (Olwoch et al., 2008; Kocan et al., 2010; Hassan and Salih, 2013). It has been predicted that the distribution of ECF is likely to extend to other parts of Africa, where climatic factors favour the survival of *R. appendiculatus* (Leta et al., 2013). Climate change is likely to increase atmospheric concentrations of carbondioxide which leads to increased biomass in plants, which will provide more shelter for free-living stages of parasites including ticks (Sutherst, 2001). On the other hand, desertification and habitat deterioration may lead to local extinctions of some wildlife species, which act as important means of dispersion of ticks (Hassan and Salih, 2013). The distribution of ticks and TBDs may also vary within a defined climatic zone. Rainfall leads to peak number of ticks and increased incidence of TBDs (Billiouw and Berkvens, 1999; Olwoch et al., 2008; Allsopp, 2010). Clinical outbreaks of anaplasmosis occur most frequent during warm-wet seasons when vector-borne transmission is more prevalent (Kocan et al., 2010), and higher risks of *T. parva* infection were reported in rainy seasons as compared to drought conditions (Billiouw and Berkvens, 1999). Seasonal variation in the abundance of *R. appendiculatus* and *R. decoloratus* ticks was reported in eastern Uganda (Magona et al., 2011b). In another study, *R. appendiculatus* counts increased with rainfall in western Uganda (Kivaria et al., 2004), and the tick thrives best with annual rainfall of over 500 mm (Norval et al., 1992b; Ochanda, 2006). A general increase in minimum temperature shortens the generation time of ticks, and may allow populations to pass through additional generations, leading to higher tick populations (Olwoch et al., 2008; Léger et al., 2013). However, arid and semi-arid areas are clearly unsuitable for *R. appendiculatus* tick survival and development (Olwoch et al., 2008; Marufu et al., 2010; Gachohi et al., 2012; Leta et al., 2013), and are mainly characterised by low levels of ticks, very low infection challenge and/or low tick infection rates, which affects the endemic status of TBDs (Fandamu et al., 2005).

The vegetation cover, which influences the microclimate of the habitat of the vector, also determines the epidemiology of tick-borne infections. The vegetation requirements for *R. appendiculatus* include grass-covered savannah and savannah woodland habitats, and the tick is usually absent from regions where overgrazing, deforestation and environmental degradation have occurred, and in extensive and heavily forested zones (Norval et al., 1991; Madder et al., 2002; Gachohi et al., 2012; Hassan and Salih, 2013). High densities of plants like *Lantana camara* and *Ocimum suave*, which have anti-tick properties, also create a microclimate which is not favourable for tick survival (Magona et al., 2011b).

The occurrence and abundance of ticks can be influenced by ecological factors. The ecological suitability of some tick species is at high altitude (Léger et al., 2013) and others, for example *R. appendiculatus*, in lowlands (Matthysse and Colbo, 1987; Rubaire-Akiiki et al., 2006). *Rhipicephalus appendiculatus* is rare at elevations higher than 1200 m above sea level (Matthysse and Colbo, 1987). Therefore, the risk of infection with *T. parva* was reported to be higher in lower elevation zones compared to the upland zones in Kenya and Uganda (Rubaire-Akiiki et al., 2004; Rubaire-Akiiki et al., 2006; Gachohi et al., 2012). The highest number of *R. decoloratus* and a subsequent higher *B. bigemina* seroprevalence was observed in the upland agro-ecological zone compared with lower zones in Mbale and Sironko Districts in Uganda (Rubaire-Akiiki et al., 2004; Rubaire-Akiiki et al., 2006).

2.6.3 Production system, management practices and control strategies

The livestock production system, grazing and management practices, and control strategies have an important influence on the exposure of cattle to ticks and incidence of TBDs. Open grazing systems are characterised by unrestricted tick exposure, and consequently higher incidence of infection, while animals under confinement, as in zero-grazing units, are less exposed to ticks (Rubaire-Akiiki et al., 2006; Swai et al., 2009; Muhanguzi et al., 2010b; Gachohi et al., 2012). In eastern Africa, extensive movement of livestock among the Maasai in northern Tanzania, and across the international border with Kenya, was thought to contribute to high tick diversity (Fyumagwa et al., 2007). However, continuous exposure of cattle, especially in indigenous breeds, to infected ticks under open grazing in endemic areas may facilitate the development of immunity, decreasing susceptibility to disease in cattle

(Gachohi et al., 2012). Intensive acaricide application in commercial farms disrupts the transmission of tick-borne pathogens, which leaves cattle naïve and susceptible to infection (Simuunza et al., 2011; Gachohi et al., 2012). On the other hand, production systems with appropriate acaricide application and good management practices lead to the disruption in the transmission of TBDs by ticks; and even where transmission occurs, clinical cases are recognised early, and the animals are treated accordingly (Perry and Young, 1995). Management factors like differences in acaricide application may differently control tick species depending on the tick life cycle (Gachohi et al., 2010). Therefore, one-host ticks such as *R*. *decoloratus* are easily killed because they require less frequent application of acaricides (Muhanguzi et al., 2010b), unlike the three-host ticks like *R*. *appendiculatus* which requires management systems that involve more frequent application of acaricides.

2.6.4 Breed of cattle

Purebred taurine cattle show higher susceptibility to TBDs than *Bos indicus* cattle (Perry and Young, 1995; Bock et al., 1999; Gachohi et al., 2012). The relative resistance to TBDs between breeds of cattle was attributed to differences in levels of immune responses mounted by the cattle (Mattioli et al., 2000). Indigenous breeds of cattle acquire immunity to TBDs through natural exposure as calves at an early age (Norval, 1994). However, the resistance in local breeds does not prevent the establishment of infection but reduces the severity of clinical disease (Allsopp et al., 2004). Indigenous breeds of cattle in Africa include the Zebu, Sanga and Nguni. Zebu cattle are found mainly in eastern Africa while Nguni and Sanga cattle are found in southern Africa (Zwane et al., 2016). Sanga cattle originated from eastern and northern Africa, and are possibly crossbreeds between the indigenous humpless cattle (*Bos taurus*) and the Zebu. The Zebu, Sanga and Nguni (*Bos indicus*) breeds are recognised for their ability to survive when exposed to high temperatures and low-quality grass (Mapholi et al., 2014; Zwane et al., 2016). The three indigenous African breeds have also evolved a relatively stable relationship with ticks and, except in unusual circumstances, are less affected by continued exposure to ticks than the imported and local crossbred cattle (De Vos et al., 2004; Lamy et al., 2012; Mapholi et al., 2014). To the best of our knowledge, there is no data from a systematic study on the difference between Sanga, Nguni and Zebu cattle in relation to ticks and TBDs. Other indigenous *Bos indicus* cattle breeds such as the N'Dama (Mattioli et al.,

2000) and Brahman (Mapholi et al., 2014) are also relatively more adapted and resistant to ticks as compared with European breeds (Mattioli et al., 2000). The Ankole Longhorn breed is an ancient breed belonging to the Sanga group and has similar resistance to ticks and TBDs as the Zebu, Sanga and Nguni (Peterson et al., 2004). The breed is indigenous to the central and eastern regions of Africa and is found in the western and south-western parts of Uganda (Peterson et al., 2004). It is classified as an intermediate *Bos indicus* (lateral horned Zebu) and *Bos taurus* (Hamitic longhorn) breed type (Peterson et al., 2004). The resistance to tick infestation involves a local skin reaction that is mediated by histamine, which is elicited by the tick bite and tick saliva components (Mattioli et al., 2000). However, TBDs can be a problem in indigenous cattle especially in situations where endemic instability exists, mainly due to intensive tick control or when cattle are moved from disease-free to endemic areas (Norval et al., 1992b; Okello-Onen et al., 1994). The relative susceptibility to tick-borne infections of *Bos taurus* cattle and their crosses with indigenous cattle, as compared with *Bos indicus* breeds, has been demonstrated in various studies in Uganda (Oura et al., 2004a; Muhanguzi et al., 2010b; Kasozi et al., 2014). Due to the relative resistance of the indigenous African breed types to ticks and TBDs, these cattle require minimal tick control, ranging from total absence of control in dry areas to strategic control during the wet season when reared in humid areas (Norval, 1994). Natural immunity, developed by cattle in environments where ticks are endemic, shows promise for genetic tick control strategies which will reduce expenditure on acaricides and other control methods (Mapholi et al., 2014). In beef cattle production, problems with low resistance in exotic breeds can be avoided by crossing them with high-resistance indigenous breeds. However, there is concern that productivity traits are not increased to the same level as resistance in such crossbred cattle. The major challenge in dairy systems is that semen is obtained from donor bulls in temperate countries. These bulls have not been subject to either artificial or natural selection for resistance to tick infestation. This results in crossbred progeny with only moderate resistance to ticks. It is likely to be a serious challenge in crossbreeding with Holstein to achieve resistance to ticks without sacrificing milk production.

2.6.5 Age of cattle

In indigenous breeds of cattle, bred in ECF-endemic areas, mortality in cattle due to ECF is higher in calves of 3-6 months as compared to older calves and adults (Muraguri et al., 2005; Oura et al., 2005; Bazarusanga et al., 2008; Swai et al., 2009). Calves less than three months of age, born of cows that are kept under open grazing system, are protected from ECF by antibodies passively transferred from colostrum (Norval et al., 1992b; Kivaria et al., 2004; Jonsson et al., 2012). Under open grazing system, cows are constantly exposed to *T. parva*-infected ticks; therefore, the colostrum has sufficiently high antibody levels. The antibody levels wane by four months of age (Gitau et al., 2000; Kivaria et al., 2004) after which any antibodies in animals are in response to *T. parva* infection (Gitau et al., 2000; Kivaria et al., 2004; Rubaire-Akiiki et al., 2006; Gachohi et al., 2012). Increasing age is associated with increased *T. parva* seroprevalence. This is because age is a proxy for exposure time, and antibodies for *T. parva* persist in the circulation for as long as six months (Gachohi et al., 2012). However, under certain instances, lower infection rates in calves as compared to adults may occur, attributed to restriction of calf movement by keeping them indoors, with less or no exposure to infection (Rubaire-Akiiki et al., 2006; Muhanguzi et al., 2010b). For babesiosis and anaplasmosis, inverse age immunity is a common feature (Chauvin et al., 2009; Jonsson et al., 2012). Although cattle of all ages can become infected with *A. marginale*, calves are less susceptible to clinical disease. Under six months of age, the illness is rare. Animals between six and 12 months of age usually develop mild disease. Animals between one and two years of age suffer from acute but rarely fatal disease. On the other hand, in adult cattle over two years of age, the disease is acute and often fatal with mortality risks between 29% and 49% (Aubry and Geale, 2011). A study in Kwale District, Kenya, showed that the incidence of anaplasmosis increased with age from six to 15 months of age (Muraguri et al., 2005). Babesiosis is more severe in adult cattle when they become exposed for the first time, causing high mortality (Suarez and Noh, 2011). In neonates, passive protection is partially due to maternal antibodies acquired from colostrum, which lasts two months, but is followed by innate immunity from 3-9 months of age (Mahoney and Ross, 1972; Zintl et al., 2005; Jonsson et al., 2012). Therefore, clinical symptoms of babesiosis are rare in calves exposed during the first 6-9 months, and these animals develop a solid long-lasting immunity (Mahoney and Ross, 1972; Zintl et al., 2005; Suarez and Noh, 2011; Jonsson et al., 2012). Studies in Uganda showed

that the number of cattle infected with *Babesia* species generally increased with age (Muhanguzi et al., 2010b) and seroconversion rates for babesiosis decreased from young to adult cattle (Magona et al., 2011b). On the other hand, there is less documentary support for inverse age immunity in heartwater caused by *E. ruminantium*, although the protective role of colostrum has been shown (Deem et al., 1996a), and vertical transmission has been proposed (Norval et al., 1995). Vertical transmission of heartwater from dams to their calves was demonstrated in cattle in Zimbabwe (Deem et al., 1996b), and in lambs and kids of one week old in The Gambia (Faburay et al., 2007b). Vertical transmission may play an important role in the maintenance of the disease and the initial establishment of endemic stability to heartwater in animals exposed to continuous field tick challenge (Faburay et al., 2007b).

Young animals have a lower tick burden than older animals, due to continuous selective grooming behaviour in the young animals (Mapholi et al., 2014).

2.6.6 Cattle movements and population dynamics

Animal movement for trade, nomadism, or migration due to social and civil unrest is a means of introduction of ticks into new ecosystems, as happened in Ethiopia and Sudan (Norval et al., 1991; Hassan and Salih, 2013), and may contribute to high tick diversity, as demonstrated in Tanzania (Fyumagwa et al., 2007). The introduction of ECF in Comoros between 2003 and 2004 was attributed to importation of immunised cattle from Tanzania, which were fed upon by naïve ticks that subsequently transmitted the infection to a susceptible local cattle population (De Deken et al., 2007). The transmission cycle of *T. parva* involves ticks that have dropped from infected cattle during the preceding stage of the life cycle, and this facilitates spatial spread of infection for migrating animals during grazing (Billiouw and Berkvens, 1999). Changes in vertebrate host populations may also have significant impacts on the ecology and evolution of ticks at different biological scales (individual, population and community). Therefore, an increase in the population size of a reservoir host species, or of an amplifying host species, can result in a significant increase in tick density and in the prevalence of the diseases they transmit (Léger et al., 2013). African cattle egrets (*Bubulcus ibis*) are commonly infested with *A. variegatum* larvae, and carry small numbers of nymphs, and are therefore likely to be important agents for disseminating the tick and *E. ruminantium* (Allsopp, 2010).

Free movement of cattle may also encourage the spread of ticks that are resistant to acaricides (George et al., 2004; SNV, 2013b).

2.6.7 Physiological state

Heifers and cows are thought to be more resistant to ticks than bulls, and this could be attributed to testosterone in bulls, which reduces both innate and acquired resistance to tick feeding (Hughes and Randolph, 2001). Pregnant cows are more susceptible to ticks than non-pregnant cows due to the immunosuppressive effects of gestational hormones in the former (Mapholi et al., 2014). Carrier cows in advanced pregnancy and/or lactation may relapse and develop signs of acute anaplasmosis, most likely due to immunosuppression associated with the periparturient period (Kehrli et al., 1989; Kocan et al., 2010). High-producing exotic dairy breeds of cattle suffer a more severe form of anaplasmosis as compared to the low-producing indigenous breeds (Kocan et al., 2010). Cattle on a higher plane of nutrition tend to develop more severe anaplasmosis than animals kept on a lower energy plane (Kocan et al., 2010).

2.6.8 Morphological and behavioural traits

Tick infestation is affected by several innate morphological coat traits, most of which have high heritability (Regitano and Prayaga, 2010). Exhibition of coat characteristics that are unfavourable for tick attachment is an important mechanism of resistance to tick infestation in cattle. Cattle with short, smooth, and light-coloured coats tend to have lower tick counts compared to those with long, rough and dark-coloured coats (Mapholi et al., 2014). Short and smooth hairs make it difficult for ticks to attach and easier for animals to groom themselves while dark-coloured hairs act as camouflage thus protecting ticks against predators such as birds (Mapholi et al., 2014). Host body size affects tick abundance, with larger-bodied animals being more heavily infested with ticks than smaller-bodied ones, due to a greater surface area for tick infestation in the former (Mapholi et al., 2014). Preferential grazing in areas with shorter grass and less bush is a form of tick avoidance behaviour which may help to increase resistance to ticks in cattle (Meltzer, 1996).

2.6.9 Endemic stability

The epidemiological state of tick-borne infections is described by the concept of endemic stability (Norval et al., 1992b; Perry and Young, 1995). A state of endemic stability implies an ecological balance between cattle, tick, parasite, and the environment where animals are infected with tick-borne parasites with little or no morbidity and mortality in the herds (Norval et al., 1992b; Perry and Young, 1995; Bock et al., 2004). Endemic stability is common in areas of suitable environment (rainfall, humidity, temperature and vegetation cover) capable of sustaining a sufficient population of vectors and hosts at a given threshold (Norval et al., 1992b), with minimal tick control, and where seasonal fluctuations in vector abundance are minimal (Norval et al., 1992b; Jonsson et al., 2012; Gachohi et al., 2013). Endemic stability occurs due to the low innate susceptibility of cattle, acquired colostral antibodies, the rapid and effective development of acquired immunity, and the low infection rates in ticks acquired from low parasitaemias in immune carrier cattle (Norval et al., 1992b; Perry and Young, 1995; Uilenberg, 2006; Magona et al., 2008). This situation is likely to occur in certain areas in eastern Africa where indigenous Zebu cattle are kept under extensive management conditions, which expose them to high and continuous challenge with infected ticks (Norval et al., 1992b; Perry and Young, 1995). The calves are challenged by infected ticks when they are still protected by innate and/or colostral immunity. There is, therefore, absence of clinical disease in calves despite infection, and a high level of immunity in adult cattle with consequent low incidence of clinical disease and low case-fatality of the few infections (Perry and Young, 1995; Suarez and Noh, 2011; Jonsson et al., 2012; Gachohi et al., 2013). On the other hand, endemic instability occurs in marginal (e.g. drought conditions) and uninfected areas where periodic unsuitability of the climate, and inconsistent tick control programs hamper the development and survival of ticks, which results in the reduction of the inoculation rates and low degrees of immunity in herds (Norval et al., 1992b; Perry and Young, 1995; Lawrence et al., 2004b; Suarez and Noh, 2011; Gachohi et al., 2012). In this case, only a small proportion (< 30%) of cattle in the population become infected and immune by six months of age. This leads to a build-up of susceptible individuals and consequently clinical disease is expected across all age groups (Gachohi et al., 2013). The endemic status is a determinant in choosing approaches to control TBDs, for example, dipping, immunisation, the enhancement of innate resistance, or no intervention (Perry and Young, 1995; Kivaria, 2010).

Therefore, endemic stability is an important epidemiological consideration to control TBDs, especially in societies where intensive tick control is not sustainable due to climate and resource challenges among farmers (Perry and Young, 1995; Perry and Randolph, 1999; Kivaria, 2010). Endemic stability was reported as an important factor for the success of the vaccination strategy with attenuated vaccines, e.g., *Babesia* parasites in areas at risk (Uilenberg, 2006).

Endemic stability is assessed based on a combination of indicators, namely (1) antibody prevalence, (2) disease incidence, (3) case-fatality proportion, and (4) age group affected in the herds (Norval et al., 1992b). Proportions of infected ticks are also considered helpful (Perry and Young, 1995). Antibody prevalence has been used as an indicator of the existence of endemic stability for babesiosis and anaplasmosis (Perry and Young, 1995; Jonsson et al., 2008). In broad terms, there is a strong correlation between high antibody prevalence and endemic stability, and low antibody prevalence and endemic instability (Perry and Young, 1995). This phenomenon also exists for *T. parva*, but may not be absolute, complicated by other factors (Perry and Young, 1995). For example, in production systems with intensive tick control, ECF incidence is low and this is generally accompanied by low antibody prevalence. However, the case-fatality rate of the few infections that occur is high, as all animals in this population are highly susceptible (Perry and Young, 1995). On the other hand, in the indigenous Zebu cattle kept in endemic areas under extensive management with little or no acaricide application, ECF incidence is also low and antibody prevalence is high. This results in a high population immunity, and the case-fatality rate of the few infections that become clinically apparent is low (Perry and Young, 1995). Endemic stability to ECF (74% seroprevalence, and low mortality of 0-5.4%) was reported in calves of up to 12 months of age in western Uganda (Kivaria et al., 2004).

2.6.10 Co-infection with tick-borne haemoparasites

The simultaneous infection of cattle with different tick-transmitted pathogens provides an opportunity for interaction that could generate a positive or negative outcome among pathogen populations (Simuunza et al., 2011). In Zambia, for example, a negative association was observed between the number of co-infecting pathogens and the erythrocyte packed cell

volume of carrier cattle (Simuunza et al., 2011). Concurrent infections of cattle with *T. mutans*, *T. velifera* and *T. parva* infections are common (Oura et al., 2004a; Oura et al., 2011b; Asiimwe et al., 2013). A protective effect of benign *Theileria* co-infections against *T. parva* infection may occur. Recently, a study in western Kenya showed that co-current infections with the less pathogenic theilerias at first infection with *T. parva* were associated with a reduction (89%) in mortality associated with *T. parva* in a population of East African short-horn zebu cattle (Woolhouse et al., 2015). However, pathogenic effects of *T. mutans* and *T. velifera* may also occur, leading to ECF-like clinical signs in cattle. A study on a farm in Uganda showed that 14 out of the 17 cattle that showed high levels of piroplasm parasitaemias in Giemsa-stained blood smears and showed signs of ECF were positive for *T. mutans* and *T. velifera* but negative for *T. parva* on RLB; only two animals had *A. marginale* and no other tick-borne haemoparasites (Oura et al., 2004a). Another study among Maasai zebu cattle in Kenya demonstrated clinical episodes (febrile responses, enlarged lymph nodes, anaemia and weight losses) of *T. mutans* infections (Moll et al., 1986), and in Zambia, *T. mutans* caused anaemia in experimental calves (Musisi et al., 1984). Therefore, *T. mutans* and *T. velifera* play an important role in the pathology, diagnosis, and control of theileriosis (Oura et al., 2011b). Misdiagnosis of ECF is mostly likely because it is difficult to differentiate between *T. mutans* and *T. parva* piroplasms. This can be a problem in resource poor countries where blood smears are the most common means of diagnosis (Oura et al., 2004a).

2.6.11 Vector and pathogen factors

Important considerations relating to the vector are the population and infection rates in the ticks, seasonal changes influencing tick abundance and activity, dissemination of infected ticks and the intensity of tick control. The mechanisms operating during tick-host transmission are also important. *Amblyomma variegatum* ticks differ in their infection rates with different genotypes of *E. ruminantium*, and have been shown to be less susceptible to two southern African isolates than to a western African and a Caribbean isolate (Mahan et al., 1995). On the other hand, a similar level of susceptibility of *A. hebraeum* to infection has been demonstrated in all the isolates tested, which may explain why heartwater is generally a more serious problem in those areas where *A. hebraeum* is the principal vector (Allsopp et al., 2004). Ticks in the field in heartwater endemic areas exhibit low *E. ruminantium* infection

levels (Allsopp, 2010). However, a relatively small numbers of infected ticks can maintain heartwater infection in a particular area. This is because, while, infected larvae or nymphs only become infective after moulting to the next instar, they then remain infective for life (Camus and Barré, 1992). Furthermore, *E. ruminantium*-infected ticks in the field present a highly virulent disease challenge as compared to infected blood needle challenge (Pretorius et al., 2008). Strains of *E. ruminantium* differ considerably in infectivity, pathogenicity and antigenicity, which is a major constraint to the control of heartwater (Allsopp, 2010). This affects the stimulation of immunological cross-protection in ruminants (Allsopp et al., 2004; Allsopp, 2010). Moreover, extensive recombination occurs naturally between different genotypes of *E. ruminantium*, suggesting that newly-generated strains are continuously arising in the field (Allsopp and Allsopp, 2007; Allsopp, 2010). Genetic and antigenic diversity occurs among *T. parva* isolates (Oura et al., 2005; Odongo et al., 2006; Katzer et al., 2010; Asiimwe et al., 2013; Mwega et al., 2015; Muwanika et al., 2016). The common strains of *T. parva* in eastern Africa differ from those in southern Africa in that they result in persistent infections of erythrocytes in cattle, which thus form a virtually continual source of infection to ticks (Perry and Young, 1995). Recombination in tick vectors during sexual reproduction generates genetic diversity in *T. parva* (Asiimwe et al., 2013; Sivakumar et al., 2014). Many strains of *A. marginale* have been identified from various geographical areas (de la Fuente et al., 2007b; Mutshembele et al., 2014; Cabezas-Cruz and de la Fuente, 2015; Silva et al., 2015). The strains differ in biology, morphology, protein sequences, antigenic characteristics, and infectivity for ticks (de la Fuente et al., 2007b). The genome of *A. marginale* is characterised by the diversity of alleles encoding the immunodominant outer membrane proteins, designated MSP2 and MSP3 (Brayton et al., 2005), which underlies the mechanism of persistent infection (Palmer and Brayton, 2013). The msp2/msp3 allelic content determines strain characteristics of *A. marginale*, and is responsible for the capacity of the pathogen to evade the existing strain-specific immunity. This principle of 'strain superinfection', the ability of a second strain to establish infection in a host that has already been infected and mounted an immune response to a primary strain of the same pathogen, clearly affects the epidemiology of *A. marginale* infection (Palmer and Brayton, 2013). Strain superinfection commonly occurs in tropical and subtropical regions where infection is highly endemic, with most animals becoming infected early in life (Palmer and Brayton, 2013; Castañeda-Ortiz et

al., 2015). This is due to consequent immunity against primary strains and thus greater selective pressure for superinfection with antigenically distinct strains (Ueti et al., 2012; Palmer and Brayton, 2013). *Babesia* species exhibit adaptive strategies for transmission by ticks, which lead to long-lasting interactions between the parasite and its hosts (Chauvin et al., 2009). Antigenic differences occur among isolates and selected strains of *B. bovis* and *B. bigemina* (Bock et al., 2004; Tattiyapong et al., 2014). Moreover, *Babesia* strains are polyclonal and often contain subpopulations that differ in virulence (Dalrymple et al., 1992; Nevils et al., 2000). Therefore, recovered cattle are more resistant to challenge with the homologous isolates than with heterologous ones (Bock et al., 2004). Due to antigenic variation in *Babesia* spp., cattle that recover from *B. bovis* and *B. bigemina* infections retain latent infections which vary from six months to several years, with detectable parasitaemia occurring at irregular intervals during the latent phase of the infection (Bock et al., 2004). Chronic low parasitaemia in the vertebrate host, and transovarial and transstadial transmission in the tick host, results in a dynamic equilibrium in *Babesia*-host interactions which enables each partner to survive at the lowest possible cost. This allows the long-term persistence of *Babesia* spp. in the ecosystem, since both its hosts serve as reservoirs (Chauvin et al., 2009). Tick infection rates with *B. bigemina* are usually higher than *B. bovis* (e.g. 0.23% Vs 0.04%) (Bock et al., 2004). Therefore, transmission rates in *B. bigemina* are higher than *B. bovis* and endemic stability is more likely to develop to *B. bigemina* than *B. bovis* in regions where both species are present (Bock et al., 2004).

2.6.12 Life cycle of ticks

Tick lifecycle have an influence on the epidemiology and control of TBDs. Most ixodid species have a three-host life cycle: each active stage (larva, nymph and adult) seeks and completes a blood meal on a particular host before dropping off and moulting (Prine et al., 2013), as shown in Figure 2.3. *Rhipicephalus appendiculatus* and most *Amblyomma* spp. have a three-host life cycle. This lifecycle also predominates in the genus *Hyalomma*. The hosts for each stage may or may not be the same species (Prine et al., 2013). The females generally do not attach to the hosts until males have been present for at least three days (Barre and Garris, 1990). The males secrete pheromones to attract females for sexual reproduction. The male may take several small feeds, mate and then die. Once mated, the females attach themselves

to the host where they remain for approximately two weeks (Barre and Garris, 1990). The females feed once and then detach from the host and lay eggs on the ground (Figure 2.3). The depleted female then dies. Larvae develop in the eggs until ready to hatch. Hatching time depends on temperature and ranges from 50 to 100 days, with fewer days required in warmer weather (Pegram and Banda, 1990). Ticks that have recently hatched from eggs or that have recently moulted have soft bodies and are inactive for one to two weeks until the external body wall hardens (Walker et al., 2013). After hatching, the larvae have been shown to survive in the environment for a couple of months during cooler periods (Pegram and Banda, 1990) and congregate on vegetation in search of hosts (Barre and Garris, 1990). Larvae feed once on a host, then detach from the host and hide in sites such as soil or vegetation (Walker et al., 2013) where they moult to nymphs. Nymphs feed once and moult in the same way as larvae. Larval and nymphal periods also vary with temperature and moulting usually occurs on an average of 45 days (Pegram and Banda, 1990). From the nymphal moult either a female or male emerges. For *A*. *variegatum*, after the final moult into the adult stage, the tick remains dormant for four weeks before any host seeking occurs (Pegram and Banda, 1990). Adult ticks of the genera *Amblyomma* and *Hyalomma* are active hunters and will run across the ground to seek hosts that are resting nearby. In *R*. *appendiculatus*, larvae, nymphs and adults crawl onto vegetation and wait for their hosts to pass by (questing). The ticks grab onto the hosts using their front legs and crawl over the skin to find a suitable place to attach and feed (Latif and Walker, 2004). The life cycle of three-host ticks is slow, from six months to several years (Walker et al., 2013). Feeding times vary based on seasons with the adults feeding during the rainy season and the immature stages feeding during the dry. *Ambylomma variegatum* spends very little time on the host feeding, a maximum of 15 days for each stage of development. Most of the life cycle occurs on the ground and in the vegetation which makes the tick sensitive to extremes in temperature (Prine et al., 2013). Due to the ability of three-host ticks to survive in the environment for extended periods, it is recommended that livestock are not reintroduced into pasture that has been vacated in an attempt to eradicate for a period of not less than 46 months (Barre and Garris, 1990). Intensive treatment to achieve very high levels of control of three-host ticks aims to have constant availability of residual acaricide on the livestock sufficient to kill all ticks present or attempting to attach. Acaricide may be applied once per week or longer, depending on the residual effect of the

acaricide used. However, this treatment is likely to reduce tick infestations to such low levels that immunity to TBDs is lost in the livestock resulting in endemic instability (Latif and Walker, 2004).

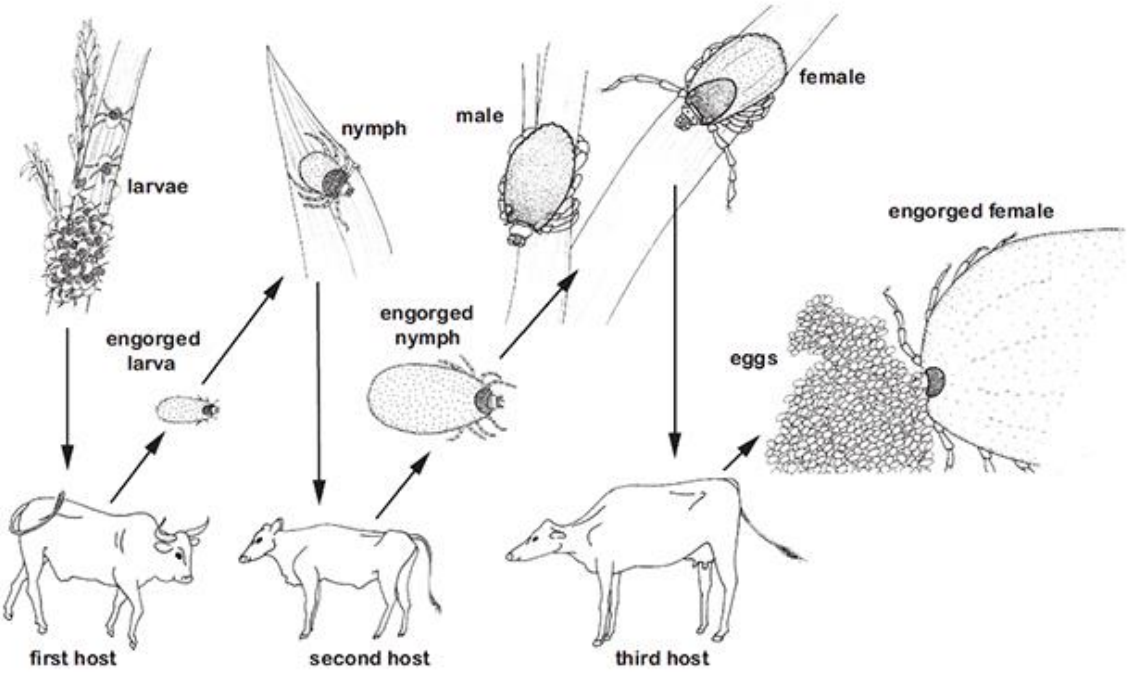

Figure 2.3: An illustration of the life cycle of the three-host tick *Rhipicephalus appendiculatus*. From 'Ticks of Domestic Animals in Africa: a Guide to Identification of species' (Walker et al., 2013).

In a few species, fed juveniles remain and develop on the host, shortening the life cycle (two-host and one-host ticks). The one-host life cycle occurs in all ticks of the *Boophilus* sub-genus of the *Rhipicephalus* genus. Eggs are laid on soil. Larvae hatch after several weeks of development and crawl onto vegetation to quest for a host (Figure 2.4). When they have completed feeding, they remain attached to the host and moulting occurs there. The nymphs then feed on the same host and also remain attached (Walker et al., 2013). After another moult, the adults emerge and then feed on the same host (Figure 2.4). The adults will change position on the same host for moulting. Thus all the three feedings of any individual tick occur on the same individual host. The life cycle of one-host ticks is usually rapid, for *Rhipicephalus* (*Boophilus*) spp., it takes three weeks for the feedings on one host and two months for egg laying and larval development. The two-host life cycle is similar but only the larvae and

nymphs feed on the same individual host, and the adults will feed on another host. *Hyalomma truncatum* and *R. evertsi evertsi* have two-host life cycles (Walker et al., 2013).

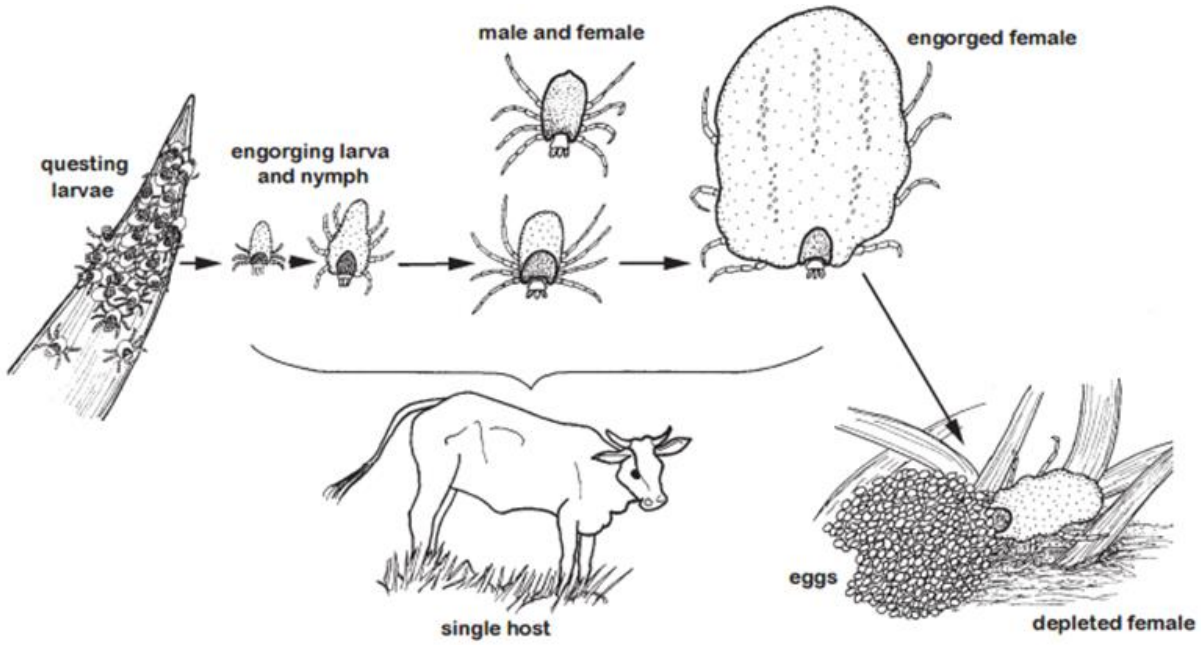

Figure 2.4: An illustration of the life cycle of one-host tick *Rhipicephalus decoloratus*. From 'Ticks of Domestic Animals in Africa: a Guide to Identification of species' (Walker et al., 2013).

The survival of a population of ticks depends on the presence of hosts that are suitable for reproduction by the adults. These hosts are known as maintenance hosts. Maintenance hosts are more limited in variety for one-host and two-host ticks than the hosts on which larvae and nymphs of three-host ticks can survive. They are also more limited than those on which adults may attempt to feed but not necessarily survive. To use information of tick hosts for identification, it is important to realise that a species of tick has a characteristic range of host species but may be found much less commonly on many other kinds of host species (Latif and Walker, 2004).

The activity of many species of tick is adapted to seasonal variations in climate. In the tropics, this is usually to overcome the adverse effects of a long dry season. The survival of many species is improved if they have a seasonal cycle which reduces these risks. For example, *R. appendiculatus* in southern Africa has a mechanism, known as diapause, which delays the questing of adults so that their feeding and reproduction start at the beginning of the single

rainy season. This is followed by peak numbers of larvae toward the end of the rainy season when humidity is highest. Knowledge of the time of year when adults of a species are likely to be found on their hosts is thus an aid to identification and control of ticks (Latif and Walker, 2004).

Treatment before the natural peak of adults will suppress their numbers, reduce greatly the number of eggs, and result in many fewer adults and nymphs for the rest of the year. This reduces acaricide use and other treatment expenses. The remaining ticks are likely to continue transmitting tick-borne pathogens at a low level. Endemic stability is thus more likely to develop resulting in lower mortality in the cattle population due to TBDs (Latif and Walker, 2004). Use of acaricides should coincide with the on-host phases of the ticks and focus on areas of aggregation (Barre and Garris, 1990). One-host ticks like *Rhipicephalus* (*Boophilus*) spp. are easier to control by application of acaricides because they spend most of their life cycle on the host and therefore require less frequent application of acaricides. On the other hand, three-host ticks like *A*. *variegatum* and *R*. *appendiculatus* spend less time on the host; therefore, control requires more frequent application of acaricides. Footbaths containing acaricides have been used to control *A*. *variegatum* with some success, as the ticks often will attach between the hooves (Prine et al., 2013).

2.7 Participatory epidemiology

In most livestock dependent areas of sub-Saharan Africa, Karamoja included, ticks and TBDs are major health impediments to improved livestock production, causing considerable economic losses to livestock keepers as they negatively affect growth, milk production, draft power, fertility, quality of hides and survival of their animals (Ocaido et al., 2009; Laisser et al., 2015). However, pastoralist areas are underdeveloped, physically remote and drought-prone. Therefore, epidemiological research and disease surveillance is difficult because human populations are relatively small and largely mobile, and they move their livestock across large areas with few roads or means of modern communication (Catley, 2006). When combined with the apparent reluctance of herders to provide accurate information on herd size to outsiders, disease estimates (e.g. mortality) determined by conventional research

methods may be questionable due to invalid denominators (Catley et al., 2014). In spite of the technical advances in veterinary diagnostic tests, such as the emergence of molecular techniques, severe limitations in laboratory services in many developing areas, especially in Africa, continue to limit the extent to which these tests are used. In many rural areas, district-level veterinary services often lack basic laboratory and sampling equipment and reagents, and there are few resources to transport samples to more well-resourced central laboratories. In this context, much research and surveillance in these areas will continue to rely heavily on information provided by livestock keepers. The philosophy, approaches and methods of participatory approaches began to emerge in the late 1970s. Participatory approaches can be seen to have had three main origins. The first was dissatisfaction with the biases, especially the anti-poverty biases, of rural development tourism – the phenomenon of the brief rural visit by the urban-based professional. The second origin of participatory approaches was disillusionment with the normal processes of questionnaire surveys and their results. The experience had been that large-scale surveys with long questionnaires tended to be drawnout, tedious, a headache to administer, a nightmare to process and write up, inaccurate and unreliable in data obtained, leading to reports, if any, which are long, late, boring, misleading, difficult to use, and anyway ignored. The third origin was more positive. More cost-effective methods of learning were sought. Research using participatory techniques has led us to understand better that rural people have valuable technical knowledge on many subjects which touch their lives, and which they have developed over many years, according to local environmental and socio-cultural conditions (Chambers, 1994; Mariner and Paskin, 2000; Mariner et al., 2011; Catley et al., 2012). This indigenous technical knowledge is essential in understanding disease epidemiological scenarios (Barnard et al., 1994) and in disease diagnosis (Plowright, 1998). Therefore, over the last few decades, collection of indigenous veterinary knowledge through surveys has become an important method for the identification and prioritisation of animal health problems within communities (McCorkle and Mathias-Mundy, 1992). This has contributed significantly to empowering pastoralist communities, enabling them to use their knowledge and skills to solve their own problems (Mariner and Paskin, 2000; Mariner et al., 2011). The participation of livestock owners and other stakeholders in animal health investigation systems ensures adequate representation of their views and perspectives (Pretty, 1995). Therefore, the

participatory system is responsive to stakeholders' needs, increases efficiency, and leads to more appropriate and potentially more acceptable control activities. This increases the community sense of ownership of and support of the interventions to their problems or needs (Pretty, 1995; Mariner et al., 2011). Participatory methods are usually cheaper and more feasible than full-scale randomised surveys, and the results are usually available very rapidly. The methods also offer more flexibility and they are effective for the design of more conventional studies through better identification of breadth, depth and prioirity issues that may merit quantitative study. A participatory approach has been explained by reference to 'bottom-up' development, which requires a participatory and joint analysis, planning and monitoring with local people (Catley et al., 2012).

Participatory epidemiology (PE) is the systematic use of participatory approaches and methods to obtain epidemiological information contained within communities so as improve understanding of diseases and options for animal disease control (Mariner and Paskin, 2000; Catley et al., 2012). The term 'participatory' in PE is used to refer to the essential involvement of communities in defining and prioritising veterinary-related problems, and in the development of solutions to service delivery, disease control or surveillance (Catley et al., 2012).

Veterinarians began using participatory methods in the 1980s, particularly in community-based livestock projects in Africa and Asia (Catley et al., 2012). Participatory epidemiology was first developed to enhance the effectiveness of rinderpest surveillance in pastoralist systems (Mariner et al., 2011). By the late 1990s, there was increasing use of participatory methods and the term 'participatory epidemiology' became more commonly used to describe veterinary applications of participatory rural appraisal (PRA)-type approaches and methods. Although much of the early development of PE occurred in remote and conflict-affected pastoralist areas of Horn of Africa, there has been considerable increase in the use of participatory methods in veterinary research in pastoral, mixed farming, peri-urban and urban systems (Mariner et al., 2011; Catley et al., 2014). An important concept behind the development of PRA was that professionals needed to change their attitudes and behaviour when working with poor people in developing countries. The need for attitudinal change

emerged from the realisation that despite limited formal education, communities in marginal rural areas were able to conduct their own investigations and analyses, and could design, plan and enact initiatives to solve local problems. Therefore, the role of the typical educator was changed to facilitate applied research and learning among co-learners rather than prescriptive instruction (Catley et al., 2012).

The first main group of participatory methods involves the application of semi-structured interviews. In these methods, interviewers phrase and rephrase questions, and follow-up interesting and unexpected responses. The use of open rather than closed questions is central to this group of participatory methods (Catley et al., 2012). Responses are agreed upon, and suggested solutions will therefore represent an accommodation between different conflicting views (Pretty, 1995). Groups can be powerful when they are interviewed together, as responses and solutions are likely to be greater than the sum of its individual members (Pretty, 1995). The second group involves visualisation methods, which recognise that certain types of information cannot easily be expressed verbally or in writing. The construction of diagrams does not necessarily require informants to be literate because objects and signs can be used to depict important features on the diagram. Diagrams can be constructed on the ground with local materials, and usually involves groups of informants working together. Examples of visualisation methods in PE are: participatory mapping, seasonal calendars, proportional piling, and radar diagrams. Activities under visualisation methods can be standardised and repeated with individual informants or groups of informants, and data can be summarised and analysed using conventional statistical tests (Catley et al., 2014). In Ethiopia, for example, proportional piling was employed retrospectively to estimate the specific causes of excess livestock mortality during drought (Catley et al., 2014). Ranking and scoring methods are the third main group of participatory methods and usually require informants to compare different variables using either ranks or scores. Typically, piles of counters such as seeds or stones are used for ranking and scoring, and as with interviewing and visualisation methods, illiterate informants can participate. Scoring methods are more sensitive than ranking, allowing a weighting of responses. The numerical nature of ranking and scoring methods makes these methods easy to standardise and repeat, with the data being analysed using conventional statistical tests (Catley et al., 2012). In Kenya, Onono et al.

(2013) employed ranking and scoring methods to identify and rank cattle diseases and production constraints that impact livelihood.

In general, PE is conducted in local languages using trained researchers and facilitators. Local disease names are used, and in some diagnostic or exploratory studies, specific attention is directed at the correct translation of these local names into scientific terms, being either specific diseases or disease syndromes. This is designed specifically to reduce on non-sampling errors (Catley et al., 2012).

Participatory epidemiology makes use of two important principles, namely triangulation and flexibility, to improve the quality, validity and reliability of the information gained (Mariner and Paskin, 2000). Triangulation involves obtaining information within the same study from various intentionally different perspectives [diverse sources through a variety of methods] (Mariner and Paskin, 2000; Mariner et al., 2011; Catley et al., 2012). The two types of methodological triangulation that have been of particular importance in PE are 'within-method' and 'across-method' triangulation. Within-method triangulation takes place within the method; for example, the researcher cross-checks information provided by an informant during the interview itself. A response during the early stage of an interview might be checked later on using a re-phrased question. The within-method triangulation therefore depends on the skill and experience of the researcher. Across-method triangulation uses two or more different methods to study the same research question. Participatory epidemiology has used methodological triangulation by cross-checking information within specific participatory methods, by comparing the findings of different participatory methods, and by comparing findings of participatory and conventional veterinary diagnostic methods. In South Sudan (Catley et al., 2001) and Tanzania (Catley et al., 2004), triangulation was achieved by combining clinical and post-mortem examinations, laboratory detection of disease agents, and reference to textbook descriptions of diseases. In South Sudan, Malak et al. (2012) employed a combination of tick collection and identification, laboratory analyses for diseases, and participatory methods (proportional piling, impact matrix scoring, relative incidence scoring, seasonal calendars and participatory mapping) to assess the impact of livestock diseases on livelihoods. When PE is used, there is a need to improve the systematic use of

simple and inexpensive clinical and post-mortem examinations as a means to triangulate information derived from PE methods (Catley et al., 2012). Flexibility with regard to PE methods implies that rapid appraisals are not rigidly preplanned and executed without deviation, but the techniques used and questions asked can be changed at any point during the investigation (Mariner and Paskin, 2000). Flexible approaches that allow for the discovery of new, un-anticipated information or issues, and place partial control of the information collection process into the hands of the stakeholders, are an important component of successful epidemiological research and surveillance, and lead to more effective control and prevention of diseases (Mariner et al., 2011). In applying the principles of flexibility and triangulation, a number of data sources must be tapped. These may be classified either as primary or secondary, depending on their closeness to the actual subjects of study. Primary sources are those within the communities studied and these include direct observation, group and individual interviews of livestock keepers, interviews with key informants such as village elders, local religious leaders and government officials familiar with the area. The secondary sources include previous studies and reports, government statistics and records, maps of the area in question, research papers and even historical texts (Mariner and Paskin, 2000).

Participatory epidemiological studies need to be designed so as to minimise bias. The six sources of bias which may affect PE studies (Chambers, 1994; Mariner et al., 2011) and which must be addressed are:

(i) Spatial biases – the investigators understandably often travel on better roads and the livestock keepers they are able to reach are determined by their proximity to roads and villages, leaving the livestock keepers in more remote areas (who are often the poorest) out of the picture.

(ii) Project bias – visitors and researchers are often channelled to areas where projects have been active, and most of the work will then concentrate in these places.

(iii) Person bias – influential persons interviewed (particularly key informants) are often either biased against poor people, or ignorant of their needs. The ‘rural elite’, while not at all representative of the cross-section of farmers, is often the most outspoken at group interviews, and can give the wrong impression. Thus, it is essential to include rural poor as key informants and ensure that they are interviewed in settings where they feel comfortable

enough to express their views. In addition, there is a tendency for both interviewers and interviewees to be men; the needs of women, and their contribution to farming, are often missed.

(iv) Dry season bias – malnutrition, morbidity and mortality all tend to be highest at the end of the dry season; surveys carried out at other times of the year will miss these phenomena.

(v) Diplomatic biases – for many communities, poverty is the subject of shame, and the needs of the poorest are sometimes concealed, either by the poor themselves or by officials working with them. Therefore, the social context of a disease and the stakeholders involved should be taken into consideration. Participatory approaches offer specific tools, such as wealth ranking to help define the social strata within a community as well as each group's needs, views and interactions.

(vi) Professional biases – professional training may itself be an obstacle, making it difficult for the researcher to understand the linkages in the system they are trying to observe, or leading them only to realise the richer segment of rural society. There is therefore need to seek training or assistance in the application of participatory methods at the onset of the activities. Even after acquiring the training and experience, technical experts cannot anticipate all the issues, opportunities and threats that are important in understanding field situations (Mariner et al., 2011).

Given the previously published reports from participatory studies, it is clear that PE has helped government veterinarians and epidemiologists in some countries to re-engage livestock rearing communities with new attitudes and new methods, leading to better understanding of how and why livestock keepers prioritise diseases. However, a very important issue that needs to be directly addressed at the institutional level is the capacity of government veterinary services and international bodies to respond to PE-based research findings which challenge existing disease control or eradication policies. In order to make the findings of participatory research more useful to the communities, PE emphasises the importance of a genuine participatory, gender-sensitive approach, with professionals responding to local priorities, co-ownership of information and analysis with communities, and joint action to solve local problems (Catley et al., 2012).

A more valid approach to epidemiological research involves across-method triangulation where two or more different methods are used to study the same or similar research questions. Therefore, in this study we used a combination of PE methods, serological and molecular techniques, clinical examination, environmental assessment and tick vector analysis to understand the epidemiology and diversity of tick-borne infections amongst cattle in Karamoja Region, Uganda.

2.8 Diagnosis of tick-borne diseases

2.8.1 Clinical examination, the use of epidemiological information and xenodiagnosis

Diagnostic methods are used alone or in combination to diagnose diseases. The diagnosis of TBDs may be made tentatively based on geographic features, environmental factors, and presenting clinical signs and/or necropsy findings observed in infected animals (Lawrence et al., 2004b). However, clinical signs and pathology may not be reliable since many of the symptoms associated with the diseases are not pathognomonic, with many other infectious and non-infectious conditions exhibiting signs similar to those of TBDs (Allsopp et al., 2004; Lawrence et al., 2004b). The distribution of tick vectors provides an indication of the potential distribution of the corresponding disease. However, tick infestation does not provide exact information on the level of risk, and some regions may be infested with a particular vector without actually experiencing the disease (Minjauw and McLeod, 2003). Furthermore, the ticks may have detached by the time the animal is examined (Lawrence et al., 2004b). Xenodiagnosis (tick pickup and transmission linked with clinical disease) as indicated in Koch's postulate was used to confirm disease-causing species and tick vectors (Skilton et al., 2002; Mans et al., 2015). Direct injection of infected blood may also distinguish species amenable to proliferation in the piroplasm stage (Theiler, 1906). Xenodiagnosis remains an important approach in basic parasite research and epidemiological investigations and may be the only means to confirm tick vector designation and clinical pathology of specific tick-borne strains or as first step in parasite isolation (Mbizeni et al., 2013; Mans et al., 2015). It is, however, not suitable for high-throughput or routine analysis and remains in the domain of specialised research groups (Mans et al., 2015).

Laboratory diagnostic tools and methods are therefore desirable to confirm the diagnosis of TBDs from clinical symptoms, post-mortem findings and epidemiological information (Minjauw and McLeod, 2003). Depending on the quality of laboratory, direct (microscopic identification of parasites) or indirect (serology and molecular techniques) methods can be used for diagnosis. In developing countries, most veterinary laboratories are able to perform direct diagnosis, but few are equipped for the more sophisticated indirect methods (Minjauw and McLeod, 2003).

2.8.2 Microscopic examination

Microscopic examination involves identifying the parasite in Giemsa-stained blood smears or lymphnode biopsy smears. This method is relatively cheap, requiring only a light microscope, and is quick and easy to perform in simple field laboratories (Minjauw and McLeod, 2003). Microscopy allows the detection of all major tick-borne infections and is the method of choice for early treatment of their associated diseases (Minjauw and McLeod, 2003). The major problem associated with direct microscopy is its low sensitivity when parasitaemia is low (in pre-symptomatic or recovered carrier animals) (Minjauw and McLeod, 2003; Carelli et al., 2007; Sibeko et al., 2008; Mans et al., 2015). Therefore, although microscopic examination is good for clinical diagnosis, the method is less useful for determining the prevalence of premunity and/or for epidemiological surveys (Minjauw and McLeod, 2003). Furthermore, differentiating species on the basis of morphology requires a level of expertise (Minjauw and McLeod, 2003), for example, the schizonts and piroplasms of *Theileria parva* are difficult to differentiate from those of *T. taurotragi*, *T. mutans*, *T. buffeli*, and *T. velifera* using light microscopy (Norval et al., 1992b). The diagnosis of classical ECF is based on the microscopic demonstration of schizonts in lymphocytes (early stages) in Giemsa-stained lymph node smears and piroplasms in erythrocytes (later stages) in stained blood smears (Norval et al., 1992b). For *Babesia* spp., blood films should be prepared from capillary blood for best results. Blood from the general circulation should preferably not be used as these specimens may contain up to 20 times fewer *B. bovis* than capillary blood (Callow et al., 1993). For *B. bigemina* infections, parasitised cells are more evenly distributed throughout the blood circulation. Mixed infections of the two *Babesia* spp. are uncommon (De Vos et al., 2004). Species differentiation is good in thin films but poor in the more sensitive thick films. Thick blood films

are especially useful for the detection of low level *B. bovis* infections (De Vos et al., 2004). Diagnosis for babesiosis at necropsy is possible if decomposition is not too advanced. The specimens should include thin films of blood from the peripheral and general circulation, a squash preparation of a small piece of cerebral cortex and impression smears of, in order of preference, kidney, liver, lung and spleen (De Vos et al., 2004). Microscopic diagnosis of heartwater relies on the demonstration of *Ehrlichia ruminantium* in the cytoplasm of endothelial cells of brain capillaries (Allsopp, 2010), where colonies of the organism are generally more numerous than in other tissues. While the characteristic colonies of *E. ruminantium* are easy to detect, at times, it is not possible to distinguish them from other species of *Ehrlichia*, and they may also be confused with *Chlamydia psittaci* (Allsopp, 2010). Given the short comings of microscopic methods, a more suitable solution would be to improve existing methods of parasite detection (Minjauw and McLeod, 2003).

2.8.3 Serological methods

Serological methods have been widely used in epidemiological studies for tick-borne infections because they can detect exposure to infection (Chenyambuga et al., 2010; Gachohi et al., 2010; Magona et al., 2011a; Malak et al., 2012). The most common serological tests used to detect antibodies to tick-borne infections are: indirect fluorescent antibody test [IFAT] (Burridge and Kimber, 1972; Du Plessis and Malan, 1987b), enzyme-linked immunosorbent assay [ELISA] (Neitz et al., 1986; Katende et al., 1998; OIE, 2015a) and card agglutination test [CAT] (OIE, 2015a). Other tests include complement fixation test (CFT) and capillary agglutination assay. However, serological tests have limitations which vary from one test to another and from one type of infection to another. The tests, based on detection of antibodies, will not give a positive reaction on the day of the infection as the host immune system needs a few weeks to produce these antibodies (Sparagano, 2005). The detection of antibodies to tick-borne infections is also unsuitable for determining carrier status since serology is unable to differentiate between previous and current infections (Young et al., 1986; Oura et al., 2004a; Salih et al., 2010).

2.8.3.1 Indirect fluorescent antibody test

The IFAT remains the gold standard assay recommended by the OIE for diagnosis of most economically important parasites (OIE, 2015b). IFAT is used for the diagnosis of *Theileria, Babesia* and *Ehrlichia* spp. (Burridge and Kimber, 1972; De Vos et al., 1982; Du Plessis and Malan, 1987b). However, IFAT has drawbacks which include difficulty in standardisation, subjectivity in interpretation of the results, and the low sample throughput (Norval et al., 1992b; Mans et al., 2015). The biggest problem with IFAT is the significant cross-reactivity observed between closely related species. Cross-reactions between *T. parva* and *T. taurotragi* antigen and anti-sera (Jongejan et al., 1986) and between *T. parva* and *T. annulata* (Burridge et al., 1974) have been observed. A similar observation has been made in regard to *T. parva* and *Theileria* sp. (buffalo) (Pienaar et al., 2014). An IFAT assay for *T. lestoquardi* showed significant cross-reactivity with *T. annulata* and *T. parva* antisera and vice versa (Leemans et al., 1997). Cross-reactions with antibodies to *B. bovis* in the *B. bigemina* IFAT are a particular problem in areas where the two parasites co-exist (Bessenger and Schoeman, 1983). Cross-reactions with antibodies against related *Ehrlichia* and *Anaplasma* spp. also occur (Du Plessis and Malan, 1987a). The IFAT was originally developed to detect *T. parva* antibodies using piroplasm antigens but was later adapted for use with schizont antigens because of the longer duration of the serological response against the latter (Burridge and Kimber, 1972; Goddeeris et al., 1982). However, a drawback in using this test is that antibodies tend to disappear in long-term carriers, whereas *Theileria* piroplasms persist. Therefore, animals with a negative serological test can still infect ticks, and thus IFAT results cannot be independently used to determine the *Theileria* infection status of carrier animals. In this way, antibodies may not be detected if the animal is not subject to a continuous challenge (Burridge and Kimber, 1972). However, IFAT is still useful in epidemiological studies, or where certain species are absent in specific carrier hosts (Mans et al., 2015). The assay has been a main tool in many assessments of endemic stability of *Theileria* infections (Billiouw et al., 2005; Kalume et al., 2013).

2.8.3.2 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) has the advantage over IFAT of being less laborious, and many samples can be tested in a short time (Salih et al., 2010). The *T. parva* ELISA uses the polymorphic immunodominant molecule (PIM) recombinant antigens to

detect *T. parva* antibodies (Katende et al., 1998). The assay does not cross-react with *T. taurotragi*, *T. mutans*, *T. annulata* or *T. buffeli*. However, ELISA may lack sufficient sensitivity to detect evidence of infection in samples from cattle harbouring low parasite burdens. This has important implications for disease control since outbreaks may occur when carrier cattle, which have been incorrectly diagnosed as being clear of infection, are transported to non-endemic areas (Bilgiç et al., 2013). The ELISA assay for *T. parva* has a sensitivity of > 99% and a specificity of 94% to 98% (Katende et al., 1998). The competitive ELISA [cELISA] (Knowles et al., 1996) is the most accurate serological test currently available for identifying *Anaplasma*-infected cattle. The assay uses a monoclonal antibody (MAb) ANAF16C1 (*Anaplasma* Antibody Test Kit; VMRD Inc., Pullman, WA, USA). This assay specifically detects the presence of serum antibodies that target a surface protein, MSP5, of *Anaplasma* spp. (Visser et al., 1992). It has proven very sensitive and specific for the detection of *Anaplasma* spp.-infected animals (Knowles et al., 1996; de Echaide et al., 1998; Strik et al., 2007). However, the test cannot differentiate the three *Anaplasma* spp., namely *A. marginale*, *A. centrale* and *A. ovis*, because they all express the MSP5 antigen (Visser et al., 1992), and induce antibodies recognised by the MSP5-specific monoclonal antibody. Other findings suggest that this MSP5 ELISA may also recognise *A. phagocytophilum* antibodies in infected cattle (Dreher et al., 2005). The MSP5 sequence is highly conserved and thus similar among strains of *A. marginale*, as well as between *A. marginale*, *A. centrale* and *A. phagocytophilum*. According to the manufacturer's information, the cELISA has a sensitivity of 95% and a specificity of 98% when used to identify persistently infected cattle at a cut-off point of 30% inhibition (VMRD, Inc., Pullman, WA). However, the cELISA for *A. marginale* may be limited by the low sensitivity for the detection of early infections (Knowles et al., 1996; Coetzee et al., 2007), and insufficient specificity for identifying true negative cattle at the time of chemosterilisation (Reinbold et al., 2010a). If the cELISA is to be used to determine the success of chemosterilisation, enough time has to be allowed between treatment and testing (Aubry and Geale, 2011). Internationally-validated ELISAs for the diagnosis of *B. bovis* infection have been developed (de Echaide et al., 1995; Molloy et al., 1998a) but, despite concerted efforts, there is still no similarly validated ELISA for *B. bigemina* (OIE, 2015b). Typically, ELISAs for antibodies to *B. bigemina* suffer from poor specificity (OIE, 2015b). Competitive ELISAs developed and validated in Australia (Molloy et al., 1998b) and USA (Goff et al., 2008) are apparently the only ELISAs in routine use for *B.*

bigemina (OIE, 2015b). Unlike *B. bovis* where animals are thought to remain carriers for life after infection, *B. bigemina* infection may be cleared and antibody levels may decline below the negative threshold within months after infection (Goff et al., 2008). Inconclusive results may occur around the negative threshold values, and this phenomenon can provide a diagnostic challenge in animals where titres are declining if the animals have cleared the infection (OIE, 2015b). An ELISA test for detection of *E. ruminantium* was developed (Neitz et al., 1986); however, the test suffered from cross-reactions with antibodies against related *Ehrlichia* and *Anaplasma* spp., resulting in the common occurrence of false positive results (Holland et al., 1987; Du Plessis and Malan, 1987a). Several other serological tests were developed over a period of years, detecting antibodies to the immunodominant *E. ruminantium* outer membrane microtubule-associated protein 1 (MAP1), but they all suffered from a high rate of false positives and false negatives. The reason for these problems is the existence of homologous families of immunodominant outer membrane proteins in several *Ehrlichia* and *Anaplasma* spp. (Palmer et al., 1994; Ohashi et al., 2001; Van Heerden et al., 2004). In an attempt to overcome the problem, the cELISA test for *E. ruminantium* was modified by the use of a recombinant fragment of MAP1, designated MAP1B, in an indirect ELISA format (Van Vliet et al., 1995). This test shows higher specificity for *E. ruminantium*, but still detects antibodies to *E. canis*, *E. chaffeensis*, an unidentified *Ehrlichia* sp. (Katz et al., 1996), and *Anaplasma* sp. Omatjenne (Allsopp et al., 1997). The MAP1B ELISA also gave false positive results when used in lambs and kids in Gambia (Faburay et al., 2007a). An additional problem affecting all serological tests for heartwater in cattle is that antibody levels against *E. ruminantium* are often too low to be detected, even in animals that have been vaccinated or are under continuous natural challenge by infected ticks (De Waal et al., 2000; Semu et al., 2001). Therefore, care must be taken in interpreting results of cattle tested with serological tests since such animals may be tick-infective subclinical carriers of heartwater.

2.8.3.3 Card agglutination test

The card agglutination test (CAT) for *A. marginale* is sensitive, may be undertaken either in the laboratory or in the field and gives a result within 30 min (OIE, 2015a). However, non-specific reactions may be a problem, and subjectivity in interpreting assay reactions can result in variability in test results. In addition, the CAT antigen can be difficult to prepare and can

vary from batch to batch and from laboratory to laboratory, and requires the infection of splenectomised calves by intravenous inoculation with blood containing *Anaplasma*-infected erythrocytes (OIE, 2015a).

Other serological tests including capillary tube agglutination, conglutination and indirect haemagglutination assay have been described for *T. parva* antigens or antibodies (Lawrence et al., 2004b). A dot enzyme-linked immunosorbent assay (dot ELISA) has also been described. Compared with the indirect ELISA, the dot ELISA has the potential advantages of being rapid, inexpensive and simple to perform. The dot ELISA has been reported to have a sensitivity of 93% and a specificity of 96%. Cross-reactivity was not observed with *B. bovis* or *B. bigemina* (Montenegro-James et al., 1990).

2.8.4 DNA-based methods

Molecular methods, with a high degree of sensitivity and specificity, have been developed to identify DNA in tick-borne infections (Bekker et al., 2002; Carelli et al., 2007; Liu et al., 2012). The critical factors which allow this are the specificity of DNA-DNA hybridisation probes, the sensitivity which follows on-target amplification by the polymerase chain reaction (PCR), and the availability of public databanks of genetic information (Allsopp et al., 2004). Whereas serological tests detect exposure to infection (and not necessarily current infection), molecular diagnostic techniques detect current/active infection as well as carrier animals with very low parasitaemias (Bekker et al., 2002; Sibeko et al., 2008; Salih et al., 2010; Liu et al., 2012). The commonly used molecular methods in diagnosis of tick-borne haemoparasite infections are: (i) isothermal amplification, (ii) conventional PCR, (iii) reverse line blotting (RLB), and (iv) quantitative real-time PCR (qPCR).

2.8.4.1 Isothermal amplification

The loop-mediated isothermal amplification (LAMP) is expected to amplify 10^9 copies in less than 1h, and provides high specificity because four primers are used for the detection of six distinctive sequences (Notomi et al., 2000). Most importantly, LAMP assays do not require expensive or complicated equipment; an ordinary water bath or heating block is sufficient. Direct visual identification can also be performed using SYBR Green 1 under an ultraviolet

lamp (Liu et al., 2012). A LAMP method based on two species-specific primer sets was used for the rapid identification of *B. bovis* and *B. bigemina* (Liu et al., 2012). In another study, a LAMP assay was developed for specific detection of *E. ruminantium* (Nakao et al., 2010). However, the use of several enzymes and/or several primers in the amplification process makes LAMP assays costlier than PCR tests. As a consequence, isothermal amplification tests have not been widely used (Criado-Fornelio, 2007).

2.8.4.2 Conventional polymerase chain reaction (PCR)

A number of diagnostic tests employed in haemoparasite research are based on final-time PCR and related techniques. One of the genes most commonly employed in PCR assays has been the gene encoding the small ribosomal subunit (made up of several proteins as well as the small subunit ribosomal RNA [SSU rRNA]), although alternative genes (membrane proteins, cytochrome b, etc.) have also been used (Criado-Fornelio, 2007). Ribosomal ribonucleic acid (rRNA) genes are widely used, since amplification primers can be designed in conserved regions of the gene to amplify the gene from all related organisms, while the variable regions can be used to differentiate between different species. The number of copies of the genes employed in amplification methods has special impact on the sensitivity of molecular diagnostics (Criado-Fornelio, 2007). PCR methods can detect low levels of parasite infection in cattle (carrier and early stage of infection), and are therefore more sensitive than microscopy for diagnosis (Radwan et al., 2013). The levels of parasitaemia detected by PCR-related methods usually range from 0.001 to 0.0000001% (the latter figure could only be attained by PCR combined with blotting) (Criado-Fornelio, 2007). PCR assays may also be useful as confirmatory tests, in some cases for regulatory testing, and as markers for vaccine strains (Bock et al., 2000). However, unlike serological tests, PCR assays generally may not be convenient to use for large-scale testing in epidemiological studies (Bock et al., 2000). PCR performance may also be limited by the number of gene sequences available in the databases for some haemoparasites like *Babesia* and *Theileria* (Criado-Fornelio, 2007). Furthermore, conventional PCR-based methods do not provide quantitative information on parasitaemia levels (Bekker et al., 2002; Molad et al., 2006). PCR techniques that use *T. parva*-specific primers based on single copy genes (p104 and PIM antigen genes) (Iams et al., 1990; Toye et al., 1991) or a repetitive gene sequence (TpR) (Bishop et al., 1992) were developed for the

detection of *T. parva* carrier animals. The PCR assay based on the sporozoite 104 kDa rhoptry antigen (p104) gene is sensitive and specific and has been successfully exploited in the detection of *T. parva* infection particularly in carrier animals (Sibeko et al., 2011; Muleya et al., 2012; Kabi et al., 2014; Muhanguzi et al., 2014).

2.8.4.3 Reverse line blot hybridisation

This method of molecular diagnosis combines PCR amplification with hybridisation of amplified products to oligonucleotide probes. Unlike the standard Southern blot, the probe is not in solution but linked to a membrane, hence this method was named reverse line blotting [RLB] (Oura et al., 2004a). A mini blotter apparatus is used to apply the genus-and species-specific oligonucleotide probes to a membrane for the simultaneous detection and differentiation of *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria* species, making specific PCR reactions for each individual species unnecessary (Gubbels et al., 1999; Georges et al., 2001; Oura et al., 2004a; Nijhof et al., 2005). The first step is PCR amplification of a variable region in the 16S rRNA gene (*Ehrlichia* and *Anaplasma*) and/or 18S rRNA gene (*Theileria* and *Babesia*) using two sets of primers located within conserved parts of the rRNA gene (Schouls et al., 1999). The primers are specific to the rRNA gene of the target organisms and are not complementary to the rRNA genes of either the hosts or the ticks, resulting in a high specificity of the PCR reaction. Both PCR primer sets have matching melting temperatures; therefore, the same PCR program is used for both reactions. In the second step, the PCR products are hybridised on a blot with non-radioactive species-specific oligonucleotide probes (Gubbels et al., 1999; Bekker et al., 2002). The assay can identify carrier animals, in which low numbers of erythrocytes remain infected. This is important for the assessment of infection risk, given that carrier animals serve as reservoirs for infection of ticks and, ultimately wider infection of the herd (Calder et al., 1996). The sensitivity of the assay is 1,000 fold higher than that of single conventional PCR. Moreover, detection is based on chemiluminescence instead of radioactivity, making the kit more user-friendly. The membrane that contains the oligonucleotide probes can be re-used 10 to 20 times. Furthermore, only a limited number of PCR amplifications are required, which enables economic use of resources. The RLB kit incorporates cloned plasmid controls that can be used as standardised positive controls (Gubbels et al., 1999; Nijhof et al., 2005; Oosthuizen et al., 2009). Reverse line blot

hybridisation has been used to study tick-borne protozoa and bacteria in some parts of Uganda (Oura et al., 2004a; Muhanguzi et al., 2010a; Muhanguzi et al., 2010b; Oura et al., 2011b; Asiimwe et al., 2013).

2.8.4.4 Real-time polymerase chain reaction

The quantitative real-time PCR (qPCR) assay is based on the detection of the fluorescence produced by a reporter molecule that increases in concentration as the reaction proceeds, due to accumulation of the fluorescently-labelled PCR product (Criado-Fornelio, 2007). The qPCR technology has the advantages of being able to detect and quantify specific DNA in multiple samples against multiple probes at the same time (Sibeko et al., 2008; Steyn et al., 2008). Therefore, qPCR gives a precise estimation of the parasitaemia levels, which makes it suitable for diagnosis and epidemiological studies (Steyn et al., 2008). The assay requires less time to perform, and enables early detection and treatment of infection in animals (Steyn et al., 2008; Aubry and Geale, 2011). Real-time PCR also allows differentiation of species or strains of important pathogenic organisms (Criado-Fornelio, 2007). Amplification and detection can be accomplished in a closed capillary tube, thereby minimising contamination problems (Criado-Fornelio, 2007). A hybridisation probe qPCR assay was designed for the specific detection of *T. parva* by amplifying a 167 bp fragment of the V4 hyper-variable region of the 18S SSU rRNA gene, using a *T. parva*-'specific' forward and a *Theileria* genus-specific reverse primer set (Sibeko et al., 2008). This *T. parva*-specific qPCR assay has been successfully used to amplify and detect *T. parva*, *Theileria* sp. (buffalo) and *Theileria* sp. (bougasvlei) in Cape buffalo (*Syncerus caffer*) in southern Africa (Chaisi et al., 2011; Mans et al., 2011; Pienaar et al., 2011). The assay is highly reproducible and can reliably detect the parasite in carrier animals with a piroplasm parasitaemia as low as 8.79×10^{-4} %, and is therefore more sensitive than conventional PCR and RLB assays (Sibeko et al., 2008). Another qPCR assay based on TaqMan probe chemistry was developed for the detection of *T. parva* in buffalo and cattle (Papli et al., 2011). The assay used a *Theileria* genus-specific PCR primer set and a *T. parva*-specific probe to amplify and hybridise with a species-specific part of the 18S rRNA gene for the parasite (Papli et al., 2011). The assay was shown to detect parasitaemia as low as 2×10^{-6}%, and was equally sensitive to the qPCR hybridisation probe assay. The assay offers certain

advantages in terms of cost, simplicity and interpretation of results as no melting curve analysis is required (Papli et al., 2011).

Real-time PCR assays were developed for detection and quantification of *A*. *marginale msp*5 gene copies and *msp*2 RNA transcripts from amplified DNA in infected ticks, using SYBR Green intercalating dye (Löhr et al., 2002; Futse et al., 2003). However, the methods though sensitive, lacked determination of diagnostic specificity with particular regard to the related species, *A*. *centrale*. Recently, qPCR was successfully applied for the rapid detection and accurate quantification of *A. marginale* DNA (*msp*1b gene) in the blood of naturally infected cattle (Carelli et al., 2007). The test proved to be highly specific. There were no cross-reactions with other *Anaplasma* species of ruminants, including the closely related *A. centrale*, or other haemoparasites of ruminants (*A*. *bovis*, *A*. *ovis*, *A*. *phagocytophilum*, *B*. *bovis*, *B*. *bigemina*, *T*. *annulata* and *T*. *buffeli*), which were present in 70% of bovine samples tested, as determined by RLB hybridisation. The qPCR assay for *A*. *marginale* overcomes the limitations of existing diagnostic methods and could be employed for: (i) detection and quantification of rickettsemia in carrier, pre-symptomatic and symptomatic cattle, (ii) assessment of the precise correlation between levels of rickettsemia and occurrence of clinical signs, (iii) evaluation of the efficacy of vaccines, and (iv) evaluation of the follow-up after treatment with anti-rickettsial drugs (Carelli et al., 2007). Later, a duplex TaqMan qPCR assay with hybridisation probes labeled with different fluorophores was developed for simultaneous detection and quantification of *A*. *marginale* and *A*. *centrale* DNA in bovine blood samples (Decaro et al., 2008). The assay was able to detect as few as 10^1 DNA copies of *A*. *marginale* and 10^2 copies for *A*. *centrale*, with optimal specificity and reproducibility (Decaro et al., 2008). The duplex assay can detect and quantify the two *Anaplasma* spp., even if present simultaneously in the same blood sample, and could be used in pathogenesis studies on acute bovine anaplasmosis (Decaro et al., 2008). In another study, a duplex real-time quantitative reverse transcriptase PCR (qRT-PCR) assay for the detection of *A*. *marginale* and *A*. *phagocytophilum* 16S rRNA in bovine blood samples was developed (Reinbold et al., 2010b). The assay was found to be a highly sensitive and specific diagnostic tool, and was able to detect as few as 100 copies of 16S rRNA of both *A*. *marginale* and *A*. *phagocytophilum* in the

same reaction (Reinbold et al., 2010b). The duplex qRT-PCR assay was found to be more sensitive than cELISA in detecting *Anaplasma* infection in cattle (Reinbold et al., 2010a).

A pCS20 qPCR was developed for specific detection of *E. ruminantium* DNA in cell culture, blood and ticks (Peixoto et al., 2005; Steyn et al., 2008). The assay is more sensitive than the standard pCS20 PCR and pCS20 PCR/^{32P}-probe. The pCS20 qPCR TaqMan probe provides the best sensitivity with a detection limit of one gene copy per reaction, which is 100 times higher than that of conventional pCS20 PCR. However, the qPCR cross-reacts with both *E. chaffeensis* and *E. canis*.

2.9 Control of tick-borne diseases

Control of TBDs can be achieved by tick management, immunisation, and the use of chemotherapeutic and chemoprophylactic drugs (Norval et al., 1992b; Bock et al., 2004; Mugisha et al., 2005), or by a combination of these approaches. The control of ticks and TBDs constitutes a significant proportion of total disease control in cattle production systems, for example, 86% in south-western Uganda (Ocaido et al., 2009).

2.9.1 Tick control

Tick control is a widespread method of controlling TBDs, by using acaricides to limit infestation with the tick vectors. Tick control methods include direct application of acaricides to cattle through dipping, spray races, hand spray, pour-ons and hand dressing (Gachohi et al., 2012). The advantage of acaricides is that they control all tick species, thereby controlling all TBDs, as well as reducing hide damage (GALVmed, 2015a). Tick control can be either intensive or strategic (Bezuidenhout and Bigalke, 1987). The objective of the intensive method is to control all stages of ticks throughout the year. However, intensive acaricide application disrupts endemic stability due to lack of natural challenge, thereby creating a susceptible cattle population, even in endemic areas with indigenous breeds (Norval et al., 1992b; Perry and Young, 1995; Perry et al., 1998; Minjauw and McLeod, 2003). Therefore, any breakdown of the intensive control regimen then results in heavy losses from TBDs (Allsopp et al., 2004). Strategic tick control implies regulating the number of ticks present so that

natural infection of livestock occurs, but also preventing the debilitating effects of severe tick infestations (Allsopp et al., 2004). The aim is to achieve an epidemiologically stable situation with respect to TBDs (Allsopp et al., 2004). Economic studies have demonstrated that strategic tick control is both a more economical and a more practical option for limiting losses from TBDs (Meltzer et al., 1995). Strategic use of acaricides also prevents rapid selection for acaricide resistance, and increases the acaricide use threshold (George et al., 2004). Although the establishment of endemic stability using limited acaricide application can be successful, if herd immunity breaks down then serious disease outbreaks can occur. This can happen if new immunotypes enter an area where immunity to the previously prevalent strains offers no or limited protection. Such a situation is likely to occur if widespread genetic exchange occurs in the field between different strains, as is the case for *E*. *ruminantium* (Allsopp and Allsopp, 2007) and *T*. *parva* (Oura et al., 2007).

The use of acaricides to control ticks has drawbacks: acaricides are expensive for the average livestock keeper (Okello-Onen et al., 1998; Mugisha et al., 2005), there is need for regular application, and there is a possibility of contaminating food supplies and the environment (Norval et al., 1992a; George et al., 2004). Furthermore, the inappropriate use of acaricides by some livestock keepers (Chenyambuga et al., 2010; Mugabi et al., 2010; Kasozi et al., 2014) can result in development of resistant tick and fly populations (George et al., 2004; SNV, 2013b). In the event that resistant tick populations become established, the incidence of TBDs is likely to increase (Kocan et al., 2010). The cost of managing resistant tick populations makes acaricide treatments more expensive and raises the costs of a control programme (George et al., 2004). In addition, the use of acaricides requires large volumes of water, and the presence of alternative hosts, mainly wild ungulates, may compromise effectiveness (Mukhebi, 1992). The use of acaricides is also compromised by stock movement during drought and insufficient knowledge of tick ecology (Cox, 1991). A successful control programme depends on detailed knowledge of the biology and ecology of the targeted tick. Without this information, weak points in the life cycle cannot be determined and exploited (Barre and Garris, 1990). Pastoralists need to have local ecological knowledge of the seasonal activity of ticks if strategic treatment with acaricides is to be successful (Latif and Walker, 2004). For example, adults of *A*. *variegatum* feed during the rainy season and the immature stages feed during the dry

(Prine et al., 2013). The adults are easiest to estimate and are probably the best stage for strategic control because of the impact on reproduction (Latif and Walker, 2004). Acaricide treatment intervals must coincide with the length of the feeding period of the female tick. Each subsequent treatment must be applied before the female mates, completes the engorgement process, and detaches from the host (Barre and Garris, 1990). Therefore treatment of cattle is started when adults are becoming active but before their numbers become high (natural peak). Treatment continues at sufficient frequency to maintain constant tick control during most of the season of adult feeding (Latif and Walker, 2004). One-host ticks in the *Boophilus* sub-genus of the genus *Rhipicephalus* can be easily controlled on cattle and cleared from pastures by a combination of acaricide treatment on animals and management methods that reduce tick numbers on vegetation [e.g. heavy grazing to keep pasture short] (Latif and Walker, 2004).

The other problem concerning acaricide use in the control of ticks is that partial removal of one parasite by chemical control results in invasion of others. For instance, the eradication of *R*. *decoloratus* in south-eastern African countries resulted in the invasion of its competitor, *R*. *microplus* (Sutherst, 2001). Furthermore, in situations where both biological (ticks) and mechanical transmission (haematophagous arthropods and blood-contaminated fomites) are involved, as is the case for *A*. *marginale*, tick control can only partially prevent transmission (Kocan et al., 2003). In these situations, products that are effective against both ticks and flies are necessary (or a combination of products). After nearly a century of acaricides utilisation, it is widely believed that acaricides alone do not provide a sustainable solution to tick and TBD control (Norval et al., 1992a).

In most production systems in eastern Africa, the use of acaricides is the most common method for control of ticks in livestock, mostly through spraying with hand sprayers and spray pumps (Minjauw and McLeod, 2003; Mugisha et al., 2005; Mugisha et al., 2008; Chenyambuga et al., 2010; Mugabi et al., 2010). Some livestock keepers do not apply acaricides at all (Chenyambuga et al., 2010). Hand spraying is less effective: the procedure is time-consuming and its efficacy depends on the degree of care taken to ensure that all body parts are treated. It is therefore only suitable for small numbers of animals (Minjauw and McLeod, 2003). Dip

tanks are an efficient, practical and convenient means of applying acaricide to a herd of livestock. However, government veterinary services in eastern Africa are often constrained by poor or inadequate infrastructure (Perry and Young, 1995), including cattle dip tanks and diagnostic facilities, which constrains the diagnosis and control of TBDs (Musoke et al., 2004; Kasozi et al., 2014). The decentralisation and privatisation of veterinary services in eastern Africa emphasises a private sector demand-driven control of TBDs (Minjauw and McLeod, 2003; SNV, 2013b; Kasozi et al., 2014; Muhanguzi et al., 2015). However, most rural pastoralist communities have very limited financial resources to maintain the cattle dips (George et al., 2004; Mugisha et al., 2005; Mbassa et al., 2009). The main factor that drives the choice of acaricides by cattle keepers is price; for example, in Sembabule and Mbarara Districts in southern-western Uganda (Mugisha et al., 2005; Mugisha et al., 2008) and Nakasongola District of central Uganda (Mugabi et al., 2010), the commonly used acaricides for tick control were amitraz formulations. Although about 10 litres of acaricide wash (at about 0.2% concentration) is recommended for use per animal (irrespective of the breed and size) (Minjauw and McLeod, 2003; Mugisha et al., 2005; Mugabi et al., 2010), cattle keepers often use acaricides of lower strength and less quantity than recommended by manufacturers (Mugisha et al., 2005; Mugisha et al., 2008; Mugabi et al., 2010). Moreover, herders use uncalibrated materials for measuring acaricides (Mugisha et al., 2005). Economic reasons force livestock keepers to use understrength acaricides (Mugisha et al., 2005; Mugisha et al., 2008; Mugabi et al., 2010). Due to the costs associated with the use of conventional methods of control, the use of ethnoveterinary knowledge is also a common practice in the control of ticks and TBDs in rural livestock keeping communities (Gradé et al., 2009; Chenyambuga et al., 2010; Nabukenya et al., 2014). Ethnoveterinary plants have been evaluated and found to have acaricidal activities. A study in South Africa showed that ethanol extracts of the plant species *Calpurnia aurea* (leaves, flowers) and *Cissus quadrangularis* (stems) had a good acaricidal activity (>80% mortality) against larvae of *R. decoloratus* in an *in vitro* experiment (Fouche et al., 2016). In another *in vitro* study in the Democratic Republic of Congo, Kalume et al. (2012) demonstrated a 100% acaricidal effect of ethanol extracts of *Tephrosia vogelii* against adults of *R. appendiculatus*.

2.9.2 Chemotherapy and chemoprophylaxis

Treatment is a secondary strategy in the control of TBDs. Theileriosis in cattle is effectively treated by derivatives of hydroxynaphthoquinone compounds (parvaquone and buparvaquone) and a quinazolinone compound (halofuginone). The drugs are very effective, if administered early, and safe with a wide therapeutic index, and they specifically target the causative parasite (Lawrence et al., 2004b). In field conditions, parvaquone and buparvaquone are active against both schizont and piroplasm stages of *T. parva*, while halofuginone appears to be active against only the schizont stage, which makes the drug most effective against the early stages of ECF (Lawrence et al., 2004b). However, these drugs are expensive and this limits their use in the field (Lawrence et al., 2004b). Moreover, accurate differential diagnosis in the field is difficult and may lead to inappropriate or delayed treatment (GALVmed, 2015a). Cattle may still die if treatment is done in advanced stages with pulmonary oedema (Muraguri et al., 1999). However, treatment of ECF cases with the pulmonary oedema syndrome can be improved by incorporating furosemide, which has a diuretic effect (Musoke et al., 2004). Treatment of ECF also faces the challenges of limited access to anti-theilerial drugs, unreliable quality of available products and the possibility of under-dosing by cattle keepers (Homewood et al., 2006; Lynen et al., 2006). The other problem is the carrier state which follows treatment of naturally infected animals with the curative drugs, thus maintaining the infection in cattle populations (Dolan, 1986; Kabi et al., 2014). In animals affected by environmental stress factors, such as poor nutrition, the ECF drugs can be sufficiently toxic to lead to death of the treated animals (GALVmed, 2015a). Antimicrobial therapy for bovine anaplasmosis employs mainly tetracyclines and imidocarb drugs (Potgieter and Stoltsz, 2004). Antimicrobial therapy is directed towards control of active infection (on apparently healthy animals during the vector season to limit the clinical effects of infection), and treatment of clinical anaplasmosis. Imidocarb reportedly eliminates *Anaplasma marginale* from carrier animals (Roby and Mazzola, 1972; Potgieter and Stoltsz, 2004). Tetracyclines are widely used; however, there are concerns about their capacity to eliminate persistent *A. marginale* infections in cattle, also termed chemosterilisation (Aubry and Geale, 2011). There have been conflicting reports between studies conducted prior to and after the year 2000 regarding the efficacy of tetracyclines for chemosterilisation against *A. marginale*, suggesting the existence of differences in susceptibility between *A. marginale*

isolates (Aubry and Geale, 2011). Two multidrug resistance pumps in the genome of *A. marginale* were identified, although the clinical significance of these pumps has yet to be examined (Brayton et al., 2005). If differences in susceptibility between strains exist, it would be necessary to know which *A. marginale* strain (s) is present in the herd and the efficacy of tetracycline for chemosterilisation of those strains. It is also important to note that animals successfully chemosterilised are still fully susceptible to re-infection with *A. marginale* (Reinbold et al., 2010a). Therefore, in addition to the antimicrobial treatment at the required dose and duration, the exposure needs to be prevented in order for the animal to remain free of *Anaplasma* (Aubry and Geale, 2011). Although a number of drugs have been used in the treatment of babesiosis, only a few are available commercially and none is ideal for the purpose. Currently, diminazene aceturate and imidocarb dipropionate (imidocarb) are the most widely used babesiacides (Bock et al., 2004). Imidocarb and diminazene are also the only drugs with useful prophylactic properties for the short-term control or prevention of babesiosis (De Vos et al., 2004). However, problems of residues in the food chain and limited supply by manufacturers have affected the use of babesiacides in many countries (Zintl et al., 2003; De Vos et al., 2004). Furthermore, since *Babesia bovis* and *Babesia bigemina* vaccine strains might be more sensitive to babesiacides, drug residues remaining in treated animals may interfere with vaccination efforts (Combrink et al., 2002). At high doses, imidocarb also eliminates *B. bovis* and *B. bigemina* from carrier animals (Bock et al., 2004). However, imidocarb-resistant *B. bovis* parasites have been expressed experimentally (Rodriguez and Trees, 1996), and inappropriate use of the babesicides may lead to emergence of drug-resistant *Babesia* strains in the field (Zintl et al., 2003). Tetracyclines, especially oxytetracycline, are the most widely used drugs in the treatment of heartwater (Allsopp et al., 2004). In prophylactic treatment of heartwater, oxytetracycline injections may be used to protect susceptible animals against heartwater when they are introduced into an endemic area (Allsopp et al., 2004).

In general, chemotherapy is expensive, effective only for a short period, and often may be difficult to apply effectively in range cattle. In Uganda, many livestock owners particularly those in rural remote areas face challenges including a lack of knowledge of livestock diseases, and accessibility and affordability of the drugs they require (SNV, 2013a). Due to

government's liberalisation policy, the human resources that are already limited at the district level are under-utilised (Kasozi et al., 2014). There is, therefore, lack of appropriate guidance on proper drug usage. Livestock keepers treat sick animals themselves or do nothing at all, rather than consulting veterinary personnel (Mugisha et al., 2005; SNV, 2013b; Kasozi et al., 2014). Therefore, the use of understrength concentrations of drugs or inappropriate application methods is common, which leads to development of carriers and the proliferation of tick-borne infections within the livestock populations, and consequently increased animal losses (SNV, 2013b; Kasozi et al., 2014). The inadequate supervision of the drug industry has also led to development of drug resistance (SNV, 2013a; SNV, 2013b). Oxytetracycline is the drug most commonly used in the treatment of TBDs in cattle among livestock keeping communities in Uganda, because it is cheaper (Mugisha et al., 2005; Chenyambuga et al., 2010). Tetracycline administration is accompanied by the disadvantages of continuous costs incurred, and meat and milk withholding periods (Kocan et al., 2010).

2.9.3 Vaccination

Cattle develop durable, long-lasting immunity after a single infection with tick-borne pathogens and this feature has been exploited to immunise cattle against TBDs. Vaccination can significantly contribute to the reduction in the use of different veterinary drugs, therefore preventing the emergence of resistance among parasites (Marcelino et al., 2012). Immunisation of cattle by the infection and treatment method (ITM) offers a valuable option for control of ECF (Lynen et al., 2006; Oura et al., 2007; Lynen et al., 2012). The method involves inoculation of a known strain (s) of *T. parva* live sporozoites with the concurrent administration of long-acting formulations of oxytetracycline antibiotic. This procedure results in a mild and controlled reaction to the parasite infection and leads to development of immunity to subsequent infections (Radley et al., 1975a; Radley et al., 1975b; Di Giulio et al., 2009; McKeever, 2009). The immunity lasts up to three years in the absence of further tick infestations, but it is life-long if infected ticks continue to challenge the immunised animal regularly (Di Giulio et al., 2009). There is strong evidence that protection is mediated by parasite-specific major histocompatibility complex (MHC) class I-restricted $CD8^+$ cytotoxic T lymphocytes (CTLs) that target the schizont-infected lymphoblasts, although $CD4^+$ T cells may be involved (Morrison et al., 1987; McKeever et al., 1994; Jenkins, 2001; McKeever, 2007).

However, the CTL response does not prevent infection because it does not take effect until the schizont parasitosis is established (Morrison et al., 1987). Moreover, immunisation generally results in a persistent 'carrier' state which is potentially tick-transmissible (Young et al., 1986; Bishop et al., 1992; Kariuki et al., 1995; Oura et al., 2004b). The transmission of vaccine components from vaccinated ECF carriers to non-immunised co-grazing animals (De Deken et al., 2007; Oura et al., 2007; Geysen, 2008) may also result in a heavier ECF challenge in non-immunised calves (Babo Martins et al., 2010). The vaccine parasite strains or specific alleles that are introduced into the field could also undergo recombination in the vector and thereby create opportunities for immune evasion by the pathogen (McKeever, 2007; Oura et al., 2007). The other problem is that high diversity of *T. parva* parasite populations in the field, both antigenic (Irvin et al., 1983; Minami et al., 1983) and molecular (Bishop et al., 1993; Bishop et al., 1996; Oura et al., 2004b; McKeever, 2009), results in a lack of cross-protection between isolates of the parasite (Cunningham et al., 1974; Irvin et al., 1983; McKeever, 2009). However, broad protection against most field isolates can be accomplished by combining different strains, suggesting that antigenic variation might be limited (Radley et al., 1975b; Uilenberg, 1999; McKeever, 2009). The most widely used vaccine is the original trivalent Muguga cocktail (Radley et al., 1975b) which comprises three stocks of the parasite - Muguga, Kiambu 5 and Serengeti-transformed isolate from a Mara buffalo, and protects against a range of geographically disparate isolates (McKeever, 2007). However, the control of ECF by vaccination also faces complexities because of variations in challenge intensity associated with vector dynamics (McKeever, 2007). There is also the risk of live parasites in the vaccine causing morbidity and mortality in vaccinates. Furthermore, vaccine production requires extensive infectivity testing and titration in cattle, to determine the safety and efficacy of the immunising dose (Di Giulio et al., 2009). Production of the ECF vaccine is complicated, time-consuming and expensive. To produce one million doses of vaccine requires 130 cattle that have not previously been exposed to the disease, 500 rabbits and at least 600,000 ticks. The entire process of making the batch takes up to 18 months (Macmillan, 2014). Nevertheless, the vaccine has been used in eastern, central and southern Africa to protect cattle against ECF (Perry et al., 1998; Minjauw and McLeod, 2003; Homewood et al., 2006; Lynen et al., 2006; Di Giulio et al., 2009). The main targets of immunisation are the taurine and taurine-cross cattle, due to high susceptibility. However, indigenous Zebu cattle may also be targets for

immunisation where endemic instability to *T. parva* exists (Perry et al., 1998). In Uganda, ITM has been used in 43 districts, but mostly on exotic breeds and their crosses, achieving immunity in about 85% of vaccinated cattle (SNV, 2013b). Widespread use in Africa has been restricted due to high costs of the vaccine and liquid nitrogen, and limited shelf-life of the vaccine (Di Giulio et al., 2009; SNV, 2013b). Furthermore, inadequate expertise to monitor the use of chemotherapy to moderate any severe effects of immunisation is a challenge in rural livestock-based communities (Di Giulio et al., 2009; Oura et al., 2011b). However, a study in northern Tanzania showed that ITM was adapted by cross-border pastoral communities, which led to reduced ECF incidence, decreased annual calf mortality (by 80-95%), and improved rate of weight gain and quality of animals (Homewood et al., 2006; Lynen et al., 2006). There was also a reduction in the use of acaricides to control ticks and increased herd size (Homewood et al., 2006; Lynen et al., 2006). Farmers reported higher market price for vaccinated animals (GALVmed, 2015b). Other studies in Tanzania showed that the vaccine is a cost-effective control option against ECF with a vaccine efficacy of 97% (Babo Martins et al., 2010), and ITM prevented ECF cases in crossbred dairy cattle which resulted in reduced acaricide use, without compromising survival in the face of other TBDs (Lynen et al., 2006). The ECF-ITM approach, which typically provides life-long immunity, comprises a single dose vaccine ideally given to calves, by injection close to the parotid lymph node, behind the ear, which is the natural attachment site for the tick *R. appendiculatus*. Controlled spraying or dipping against ticks is necessary to maintain immunity and control other TBDs (GALVmed, 2015a). Currently, the Centre for Ticks and Tick Borne Diseases (CTTBD) in Malawi is producing the ECF vaccine, and the Global Alliance for Livestock Veterinary Medicines (GALVmed) is providing support to ensure vaccine availability fron CTTBD, process improvements to ECF-ITM vaccine (e.g. reduce the time it takes to manufacture the vaccine), finding an approach to conserve sporozoite viability without the need for liquid nitrogen storage, and promote collaboration between governments, scientific think-tanks and private business innovators. GALVmed is also building delivery capabilities (vaccinator training) and market awareness, and facilitating official product registration in different countries (GALVmed, 2015a; GALVmed, 2015b). Research in Tanzania has been undertaken with the objectives of making the ECF vaccine safer, more effective, more easily managed, and more acceptable to farmers. This research has shown that replacement of the traditional 20% oxytetracycline formulation

with a longer-acting 30% formulation (Alamycin LA 300®) reduced the percentage of ECF reactors from an average of 5-6% to less than 0.2%. It also allowed those conducting the treatment to abandon the intensive monitoring after ECF immunisation, and made the technology much more acceptable to both private veterinarians and livestock owners (Minjauw and McLeod, 2003). Research has also moved towards developing subunit vaccines from native antigens of *T. parva* parasites or as recombinant proteins from cloned DNA. Subunit vaccines offer an attractive alternative to virulent or attenuated parasites because of the use of bacteria or lower eukaryotes to produce recombinant proteins in batch culture, they are less costly to produce, are relatively stable, and do not require a cold chain, making the logistics of access and delivery easier. Moreover, there is flexibility to incorporate only those antigens that elicit 'protective' immune responses (Jenkins, 2001). Milestones have included field evaluation of the p67 sporozoite recombinant vaccine, which has shown a degree of efficacy (Musoke et al., 2005). There has also been identification of vaccine candidate antigens from the *T. parva* schizont stage (Graham et al., 2006) that represent targets of $CD8^+$ T-cell responses induced by live immunisation.

Despite the far-reaching economic impacts of anaplasmosis, there is no vaccine universally accepted as safe and efficacious (Aubry and Geale, 2011; Ducken et al., 2015). Both killed and live vaccines, which rely on erythrocyte-derived antigen sources, have been used worldwide to induce protective immunity that mutes or prevents clinical disease; however, the vaccines neither prevent cattle from becoming persistently infected with *A. marginale* nor from becoming reservoirs of infections (Kocan et al., 2003). Moreover, the development of an effective bovine anaplasmosis vaccine is complicated by the increasing numbers of *A. marginale* field strains that occur in a given geographical area (de la Fuente et al., 2005; Aubry and Geale, 2011). Therefore, premunising isolates of *A. marginale* may not be cross-protective within and across geographical areas. Live vaccines involve inoculating cattle with erythrocytes infected with attenuated strains of *A. marginale* or the less pathogenic *A. centrale* (Kocan et al., 2003). During production of live vaccines, splenectomised calves that are maintained under quarantine conditions are experimentally inoculated with stabilates of defined strains of *A. marginale* or *A. centrale* and serve as a source of infective blood (Kocan et al., 2003; OIE, 2015a). Vaccinated cattle develop persistent infections, which induce lifelong

protective immunity in cattle (Kocan et al., 2003). Advantages offered by a live vaccine include a full complement of surface antigens in their native conformations, and presentation of new surface protein variants over time (Hammac et al., 2013). However, live blood vaccines have disadvantages that include the risk of co-transmission of other pathogens, risk of haemolytic disease in calves born to vaccinated dams, and a requirement for a stringent cold chain (Bell-Sakyi et al., 2015). Moreover, attenuated *A. marginale* vaccines could become virulent after successive passage through cattle or by ticks (Aubry and Geale, 2011), and the vaccine is not recommended in cattle older than 12 months (Kocan et al., 2003). *Anaplasma centrale* (Theiler, 1911) is the most widely used live vaccine for control of bovine anaplasmosis, being used in Africa, Australia, Israel and Latin American (Aubry and Geale, 2011). Cross-protection by the *A. centrale* live vaccine is attributed to similar antigenic variation of major surface protein (MSP) 2 that occurs during persistent infections of *A. centrale* and *A. marginale* (Shkap et al., 2002), and conserved $CD4^+$ T-cell epitopes between the two species (Shkap et al., 2002; Molad et al., 2004; Agnes et al., 2011). However, *A. centrale* vaccine success also varies with *A. marginale* strains to which vaccinated cattle are exposed (Hammac et al., 2013). There is a much lower degree of conservation between the deduced amino acid sequences of surface proteins of *A. centrale* and sequenced *A. marginale* strains than between any two *A. marginale* strains examined to date (Herndon et al., 2010; Agnes et al., 2011). Killed (inactivated) vaccines have some advantages over live vaccines: the risk of contamination with undesirable infectious agents is low, storage is inexpensive, and post-inoculation reactions are of minimal clinical relevance. However, the disadvantage of killed *A. marginale* vaccines is the high cost of producing and using the vaccines due to the need for annual boosters, extensive purification of *A. marginale* from erythrocytes, dependence upon live animals as antigen source, and difficulty of standardisation (Kocan et al., 2003). There is also lack of cross-protection among isolates from different geographical areas, and the protective immunity afforded by killed vaccines is usually lower than that of live vaccines (Kocan et al., 2003). Therefore, killed vaccines are not used worldwide as frequently as live vaccines (Kocan et al., 2003). As a prospect for *Anaplasma* vaccines, a cell culture system has been developed for propagation of *A. marginale* in a continuous tick cell line derived from embryonic *Ixodes scapularis* (Kocan et al., 2003). *Anaplasma marginale* derived from the cultured tick cells provides a potential source of antigen for the development of improved killed and live

vaccines, and this would eliminate the use of cattle in vaccine production (Kocan et al., 2003). Recently, a transformed and cell-culture derived *A*. *marginale* St. Maries strain showed better clinical protection than *A*. *centrale* vaccine following challenge with *A*. *marginale* (Hammac et al., 2013). In another study, *A*. *centrale* Israel vaccine strain was successively propagated in tick cell lines, which opens up the possibility of generating a safer and ethical vaccine for bovine anaplasmosis (Bell-Sakyi et al., 2015). The ideal vaccine for bovine anaplasmosis would be one that is able to mimic or redirect the host response during natural infections and blocks infection of cattle and ticks that are potential vectors. Additionally, the possibility of blocking the biological transmission of *A. marginale* is an important goal of vaccines for bovine anaplasmosis (Kocan et al., 2003). Recent proteomic and genomic research approaches have identified 21 new proteins within the outer membrane immunogen in addition to the well-characterised major surface proteins (MSP1-MSP5). The identification of these novel antigenic proteins expands current knowledge of the composition of the protective immunogens and provides hope for the development of new vaccines (Lopez et al., 2005; Brayton et al., 2006). A recent study evaluated the protective capacity of sub-dominant outer membrane protein antigens but with less success (Ducken et al., 2015). Therefore, progressing from the protective capacity of outer membrane formulations to recombinant vaccines requires testing of additional antigens, optimisation of the vaccine formulation and a better understanding of the protective immune response (Aubry and Geale, 2011; Ducken et al., 2015).

Attenuated *Babesia* parasites are used in vaccination against babesiosis (Suarez and Noh, 2011). The attenuated *B*. *bovis* used in vaccines was derived by serial and rapid blood passages of a virulent strain among 20-30 splenectomised steers (Callow et al., 1979). Since the spleen is important in the trapping and destruction of infected erythrocytes, the use of splenectomised bovines yields adequately high parasitaemias (Bock et al., 2004). In contrast, *B. bigemina* attenuation is usually performed by slow successive passages of virulent strains among spleen-intact steers (Shkap and Pipano, 2000). Live *Babesia* vaccines are recommended for use in 4 - to 10 - month-old calves that generally show good tolerance, though a transient clinical response to vaccination can sometimes take place (Fish et al., 2008). Adult animals, on the other hand, can develop acute babesiosis upon vaccination, for

which daily monitoring for up to 21 days is suggested and babesiacide treatment is often needed (Shkap et al., 2005; de Waal and Combrink, 2006; Suarez and Noh, 2011). Live vaccines against *B*. *bovis* and *B*. *bigemina* are currently commercialised around the world (Florin-Christensen et al., 2014), and have a desirable level of efficacy (over 95% from a single vaccination) (Bock et al., 2004). Several vaccines are based on the Australian vaccinal strains, while some countries, such as Argentina, South Africa, Mexico and Uzbekistan, use their own locally-generated attenuated strains (Florin-Christensen et al., 2014). Protective immunity develops 3-4 weeks after vaccination and normally lasts at least four years (Bock and de Vos, 2001; OIE, 2015b). It was observed in South Africa that, after chemosterilisation of infections, sterile immunity to *B*. *bigemina* lasted for only 16 months, without further boosting of immunity from tick-acquired infections, while immunity to *B*. *bovis* lasted for over three years (Florin-Christensen et al., 2014). Thus, complete tick control after vaccination is discouraged, so that natural infections through tick bites can aid the acquisition of a long-term protective status (Bock et al., 2004; de Waal and Combrink, 2006; Florin-Christensen et al., 2014). In contrast with the live vaccine of *B*. *divergens*, which produces sterile immunity, *B*. *bovis* and *B*. *bigemina* live vaccines do not eliminate the parasites but rather produce disease-resistant carrier animals that may act as reservoirs for tick-transmission (Zintl et al., 2003; Bock et al., 2004). Additionally, live vaccine production can be cumbersome, expensive and unattractive for private industry undertakings; therefore, current vaccines around the world are mostly produced with government support (Florin-Christensen et al., 2014). Donor bovines should be kept free of other pathogens, such as bovine leukaemia virus, and vaccines need to undergo strict quality controls to avoid pathogen dissemination at vaccination (Bock et al., 2004; Shkap et al., 2007; Florin-Christensen et al., 2014). Donors of blood for vaccines should also be free of *Babesia* spp., which can be particularly challenging when vaccines are produced in tick-endemic regions (Florin-Christensen et al., 2014). In order to ensure parasite viability, a strict cold chain needs to be sustained during preparation, maintenance and transportation to the end users (Florin-Christensen et al., 2014). Refrigerated vaccines have a short shelf life (4 to 7 days at 4°C), and in the case of frozen vaccines, the timing of application is critical, since parasites rapidly die after thawing (Shkap et al., 2007). Adverse reactions to vaccination with live parasites, including abortions in pregnant cattle have been reported in highly susceptible cattle, such as high-yielding cows (Florin-Christensen et al., 2014).

Additionally, vaccine failures can take place due to incorrect handling or storage, administration of chemotherapeutics immediately before or during vaccination, stress or concomitant infections, pathogenicity reversion of the attenuated vaccine strains, and/or changes in the parasite population that can lead to lack of protection (Bock and de Vos, 2001; de Waal and Combrink, 2006). Live vaccines also require sensitisation against blood groups and tick transmissibility (Shkap and Pipano, 2000; Bock et al., 2004; Fish et al., 2008). Thus, in spite of the general efficiency of current vaccines in preventing clinical cases of bovine babesiosis, there is need to develop improved subunit vaccines that are safer, provide protection against disease, and are easier to handle and produce (Florin-Christensen et al., 2014). It is possible to develop alternative subunit vaccines (Suarez and Noh, 2011) based on the following facts: (i) cattle persistently infected with *Babesia* are generally resistant to re-infection with related strains, termed concomitant immunity (Brown et al., 2006), (ii) immunity following immunisation with live, attenuated *B*. *bovis* vaccine is known to last after parasites have been eliminated by drug treatment (Brown et al., 2006), and (iii) immunisation with killed parasites or parasite extracts can confer some level of protective immunity following homologous or heterologous strain challenge (Bock et al., 1992; Patarroyo et al., 1995). Therefore, development of subunit vaccines requires a more refined understanding of the nature of the protective immune responses against *Babesia* parasites. Since relatively few blood stage antigens have been tested as vaccines, new vaccine candidate antigens need to be identified, and many other new avenues of subunit vaccine development using the already available vaccine candidates remain yet to be explored (Suarez and Noh, 2011). Recent research development in: (a) the complete genome sequence of *Babesia* parasites, and (b) the availability of novel functional genomics tools, including a transfection system for *B. bovis*, in conjunction with the availability of *Babesia* culture systems and novel genomic, transcriptomic and proteomic techniques, has great potential to impact vaccine development. In addition, other important recent advances are the characterisation of the mechanisms of antigenic variation, the adhesion properties of infected erythrocytes and the mechanisms of cell invasion (Allred and Al-Khedery, 2006; Hutchings et al., 2007; Barbet et al., 2009; Gohil et al., 2010). *In vitro* cultivation of bovine *Babesia* spp. allows vaccine preparations, cheap maintenance of field strains for antigen characterisation, drug testing,

sero-neutralisation assays, production of transgenic variants, morphological studies, and invasion assays (Müller and Hemphill, 2013).

At the moment, the only commercially available vaccine against heartwater is a cryopreserved preparation of blood from sheep infected with virulent *E. ruminantium* organisms of the Ball 3 isolate (which is less virulent than other strains), used in an infection and treatment strategy (Bezuidenhout and Wright, 1989; Nakao et al., 2012). The blood is injected intravenously in animals to be immunised, the rectal temperature is monitored daily, and antibiotic treatment is applied to prevent a more serious course of the disease or animal death (Allsopp, 2009; Marcelino et al., 2012). However, there are several practical disadvantages to this infection and treatment procedure: (i) the duration and effectiveness of the immunity is uncertain, (ii) because live organisms are involved, the procedure cannot be used in non-endemic areas (Allsopp, 2009), (iii) *E. ruminantium* organisms rapidly lose infectivity even at 4°C, so the infective blood must be preserved on dry ice and thawed immediately before inoculation which makes the procedure inappropriate for use in rural areas (Allsopp, 2009), and (iv) the procedure must be supervised by trained staff because of the need for intravenous injection and subsequent careful monitoring of the animals' condition (Allsopp, 2009). The procedure is, however, successfully used to protect susceptible animals against the disease, especially when they are first introduced into endemic areas, or if they are particularly valuable (Allsopp et al., 2004). The Ball 3 stock was chosen as the 'vaccine' stock because it produces an early temperature rise several days before any other serious clinical signs appear. This makes it relatively easy to decide when to treat (Marcelino et al., 2012). However, antigenic strain differences and high diversity limits the protection offered by Ball 3 genotype against some common virulent genotypes, because cross protection between immunotypes of *E. ruminantium* may be minimal (Collins et al., 2003; Allsopp and Allsopp, 2007; Barbet et al., 2009). Some stocks, such as the highly virulent Welgevonden isolate, can cause death very shortly after a rapid temperature rise, and are therefore not suitable for the infection and treatment method of immunisation (Allsopp, 2009). The Senegal isolate of *E. ruminantium* was the first isolate to be attenuated; however, it reduced mortality by a small percentage (from 70% to 43%) (Allsopp, 2010). The Welgevonden stock was attenuated in a canine macrophage-monocyte cell line culture, and then re-adapted to grow in bovine endothelial

cells (Zweygarth et al., 2005). The Welgevonden isolate offers a wider spectrum of cross-protection than other stocks (Zweygarth et al., 2005), so the attenuated form may provide better protection than the attenuated Senegal isolate. Other forms of heartwater vaccines are in an inactivated form, consisting of organisms derived from culture which have been rendered non-viable by chemical treatment (Allsopp, 2010). Under field conditions, the inactivated vaccine reduces mortality levels, but protection levels have been disappointing (Mahan et al., 2001; Faburay et al., 2007a). Furthermore, the high cost of batch culture systems for *E. ruminantium* is a factor which has long been an obstacle to its development for large scale use in the field (Esteves et al., 2004). However, a recent study on inactivated *E. ruminantium* vaccine with ISA70M and ISA70 adjuvants demonstrated the ability to rapidly produce several hundred vaccine doses with minimal effort and ensure consistency in vaccine batches (Marcelino et al., 2015). Inactivation also enables the preparation of the vaccines long before vaccination due to the increased stability at 4°C and minimise the adverse effects of cold chain rupture up to 4 days at 37°C, as could happen under field conditions (Marcelino et al., 2015). A recombinant vaccine could in principle be cheap and effective, and unlike the attenuated vaccine it could be used to stop an outbreak in a non-endemic area. DNA vaccination with different *E. ruminantium* genes has also been attempted. Experiments showed that the naked DNA-induced immunity was boosted with microtubule-associated protein (MAP) 1, and as a result protection levels were increased from a range of 13–27%, without boosting, to a range of 53–67% (Nyika et al., 2002). The genome sequence of *E. ruminantium* shows that there are 888 genes from which to choose vaccine candidates (Collins et al., 2005), but the problem is that there are no reliable strategies for identifying the genes which code for antigens which stimulate the protective T-cell response (Esteves et al., 2004). In order to develop *E. ruminantium* vaccines, it is important to find genotypes which can confer cross-immunity to as wide a range of *E. ruminantium* field strains as possible (Allsopp, 2010).

With regard to vaccination against TBDs, the development of new-generation vaccines will require improved knowledge of the complex host-vector-pathogen interactions involved in the pathology, immunopathology and protective immune mechanisms of TBDs, in order to block critical host-pathogen or vector-pathogen interactions (Marcelino et al., 2012). Perhaps

the ultimate vaccine against TBDs will incorporate a cocktail of several antigens derived from different parasite developmental stages, so contributing to lifelong protective and robust immunity against a spectrum of economically devastating diseases (Kivaria, 2010). It is also critically important that the new generation of recombinant antigen vaccines permit the artificial establishment and maintenance of endemic stability (Kivaria, 2010).

Approaches for the control of TBDs also involve long-term research into the development of anti-tick vaccines (de la Fuente et al., 2007a; SNV, 2013b). Control of ticks by vaccination has the advantages of being cost-effective, reducing environmental contamination, and preventing the selection of drug-resistant ticks that result from repeated acaricide application (Marcelino et al., 2012). The saliva of ticks has been shown to contain an array of biologically active proteins with functions essential to feeding success. Repeated host exposure to tick feeding has been shown to result in the development of resistance against future infestations, most likely through development of an adaptive immune response targeting the saliva proteins (Bowman and Sauer, 2004; Valenzuela, 2004). This natural ability of animals to develop resistance to ticks forms the conceptual basis for development of vaccines targeting tick feeding. Vaccinating with tick saliva antigens can induce production of antibodies that bind to and interfere with the function of the proteins in tick saliva (Olds et al., 2016). Tick vaccines reduce the number of engorging female ticks, their weight and reproductive capacity, meaning that the greatest vaccination effect is seen as a reduced larval infestation in the subsequent generation (Willadsen, 2006). The Bm86 antigen, as originally identified in *R. microplus*, is the basis of commercial tick vaccines against this tick species (de Vos et al., 2001). De Vos et al. (2001) demonstrated a reduction in the number of engorged female adult ticks, their weight and egg-laying capacity, leading to a reduction in reproductive capacity of 74% for *R. microplus* and 70% for *R. decoloratus*. There was also an overall 50% reduction in the total weight of nymphs of *Hyalomma anatolicum anatolicum* engorging on vaccinated calves, and a suggestion of a subsequent effect on feeding adults (de Vos et al., 2001). In other studies, the feasibility of controlling tick infestations through immunisation of hosts using recombinant antigens such as Bm86 (Tick-GARD) (Pipano et al., 2003), ferritin 2 (*FER2*) (Hajdusek et al., 2010), and recombinant fractions containing the chimeric proteins BM95-MSP1a and subolesin (SUB-MSP1a) (Almazán et al., 2012) has been demonstrated. It was also

shown that using the tick protective antigen, subolesin (SUB), reduced *A*. *marginale* and *A*. *phagocytophilum* infection levels in ticks (de la Fuente et al., 2006). Furthermore, Merino et al. (2011) demonstrated a reduction in *R*. *microplus* infestation in cattle and a reduction in *A*. *marginale* and *B*. *bigemina* infection levels in ticks by vaccination with subolesin. However, a recent anti-tick vaccination study showed that a multivalent cocktail containing three *R*. *appendiculatus* histamine binding proteins, two different *R*. *appendiculatus* cement cone protein antigens TRP64 and TRP truncate variants, the *R*. *appendiculatus* subolesin homologue, and *T*. *parva* sporozoite antigen p67C showed that the vaccine did not appear to affect tick feeding success or reduce transmission of *T*. *parva* (Olds et al., 2016). A major disadvantage of some of the tick vaccines in current use is that they may not offer protection against multiple tick species. However, controlled immunisation trials conducted by de Vos et al. (2001) indicated that the *R*. *microplus* BM86-containing vaccines protect also against other tick species. Since tick infestation is rarely a one-species issue, tick vaccines should aim at a broader protection against the main species of economical and epidemiological interest. Another limitation in the use of anti-tick vaccines is the delay in the 'knock down' effect of the vaccines. Therefore vaccine use is often coupled with limited acaricide application for short-term control of unacceptable tick burdens (Mapholi et al., 2014). At the moment, two vaccines based on the Bm86 antigen are commercially available, namely Tick-GARD (in Australia) and GAVAC (in Cuba and parts of South America), but they are not fully efficacious (Marcelino et al., 2012).

2.9.4 Animal genetics

Purebred *Bos indicus* cattle are more resistant to ticks, and can withstand higher levels of challenge by TBDs than *Bos taurus* and crossbred cattle (Bock et al., 1999; Minjauw and McLeod, 2003). Therefore, *Bos indicus* breeds (e.g., N'Dama, Sanga and Zebu) can be exploited in areas where ticks and TBDs are big constraints to animal production (Bock et al., 1999a; Mattioli et al., 2000; De Vos et al., 2004). This is of benefit especially for the low-input traditional farming systems where the use of chemicals to counter the negative effects of pathogens is limited by their relatively high cost (Mattioli et al., 2000). The genotype of cattle also plays a role in the development of protective immunity following immunisation, with *Bos taurus* breeds more likely to show a deficient immunity than *Bos indicus* (Bock et al., 2004).

2.9.5 Integrated control

Given the shortcomings of individual control methods against TBDs, integrated control approaches that take into consideration the epidemiology of TBDs in an area are required for control. An effective control of ticks and TBDs can be achieved by integrating the strategic use of acaricides, exploitation of endemic stability, the application of vaccines, and the use of tick-resistant breeds of cattle (Norval et al., 1992a; Perry et al., 1998; Minjauw and McLeod, 2003; George et al., 2004). The use of immunisation and tick-resistant breeds of cattle considerably reduces the dependence on acaricides (Minjauw and McLeod, 2003). Integrated control approaches also include reducing the host-finding rate of ticks by changing the host density and controlling cattle movements (George et al., 2004). Increased exploitation of livestock genetic resistance is likely to remain the most cost-effective strategy in livestock-dependent and certain forms of traditional crop-livestock production systems that are characterised by low levels of acaricide use and therapeutic resources, leading to 'natural' disease control (Gachohi et al., 2012). However, because of the high mortality in young non-immune calves (Homewood et al., 2006; Gachohi et al., 2012), integration of the relative genetic resistance of livestock with immunisation and seasonal tick control could be more beneficial (Gachohi et al., 2012). In a population where a large proportion of cattle are susceptible, and vector ticks are absent or of low density, and therefore not a reliable indicator of occurrence of infection, it is logically and financially difficult to sustain immunisation; moreover, there would be little or no challenge from natural infected ticks which are required to sustain the immunity (Gachohi et al., 2012; Gachohi et al., 2013). In such areas, the most cost-effective control methods seem to be strategic tick control during periods when the vector is found and early and effective treatment in clinical cases of successful transmission (Gachohi et al., 2012). In endemically unstable areas, which are characterised by cattle populations with different levels of antibodies at the herd level, control measures, for instance immunisation, depend on the proportion of the susceptible population (Deem et al., 1993; Gachohi et al., 2012). The goal of the immunisation approach is to increase the proportion of immune animals to endemic stability status and minimise tick control to allow the infected ticks to sustain endemic stability naturally through continuous challenge (Gachohi et al., 2012). *Bos taurus* cattle breeds which are more susceptible than *Bos indicus* to TBDs warrant the use of preventive measures such as vaccination in areas with a high incidence of ticks and TBDs

(Perry et al., 1998; Minjauw and McLeod, 2003; Bock et al., 2004). However, indigenous zebu cattle may also be targets for immunisation where endemic instability to tick-borne pathogens exists (Perry et al., 1998).

Veterinary services and appropriate information dissemination methods should be used to advise on the choice of control (Minjauw and McLeod, 2003). Therefore, a more creative and 'farmer-friendly' use of information networks is needed (Minjauw and McLeod, 2003). There is need to increase farmer awareness about the biology and ecology of tick species on their land and the clinical picture of TBDs, so as to optimise both the use of acaricides, by applying the most appropriate frequency, and early management of infection (Minjauw and McLeod, 2003; Gachohi et al., 2013; SNV, 2013b). A rational strategy for the control of ticks must also feature appropriate policies on acaricide usage and management (George et al., 2004). Government promotion of immunisation technology and assistance in remote areas is necessary to allow for fast adoption once vaccine delivery becomes available (Lynen et al., 2006). Due to constrained resources, there is need to consider production systems with their unique needs in terms of TBD control. Therefore, attention should focus on identifying the significance of tick-borne infections in the specific production systems and then integrate strategic control measures based on the epidemiology and economics of TBDs. Given the impact of TBDs in cattle, control would be a major step to increase productivity of livestock production systems and contribute to food security.

2.10 References

ADL, S.M., SIMPSON, A.G.B., LANE, C.E., LUKĚS, J., BASS, D., BOWSER, S.S., BROWN, M.W., BURKI, F., DUNTHORN, M., HAMPL, V., HEISS, A., HOPPENRATH, M., LARA, E., GALL, L.L., LYNN, D.H., MCMANUS, H., MITCHELL, E.A.D., MOZLEY-STANRIDGE, S.E., PARFREY, L.W., PAWLOWSKI, J., RUECKERT, S., SHADWICK, L., SCHOCH, C.L., SMIRNOV, A., SPIEGEL, F.W., 2012. The revised classification of eukaryotes. The Journal of Eukaryotic Microbiology 59, 429-493.

AGNES, J.T., BRAYTON, K.A., LAFOLLETT, M., NORIMINE, J., BROWN, W.C., PALMER, G.H., 2011. Identification of *Anaplasma marginale* outer membrane protein antigens conserved between *A*. *marginale* sensu stricto strains and the live *A*. *marginale* subsp. *centrale* vaccine. Infection and Immunity 79, 1311-1318.

AHMED, J.S., 2002. The role of cytokines in immunity and immunopathogenesis of piroplasmosis. Parasitology Research 88, S48-S50.

AHMED, J.S., LUO, J., SCHNITTGER, L., SEITZER, U., JONGEJAN, F., YIN, H., 2006. Phylogenetic position of small-ruminant infecting piroplasms. Annals of the New York Academy of Sciences 1081, 498-504.

AKABWAI, D., ATEYO, P.E., 2007. The Scramble for Cattle, Power and Guns in Karamoja. Feinstein International Center, Tufts University, 200 Boston Avenue, Suite 4800, Medford, MA 02155, USA, 49 pp. http://fic.tufts.edu/assets/The+Scramble+for+Cattle1.pdf. Accessed October 12, 2015.

ALLRED, D.R., 2001. Antigenic variation in babesiosis: is there more than one ˈwhyˈ? Microbes and Infection 3, 481-491.

ALLRED, D.R., AL-KHEDERY, B., 2006. Antigenic variation as an exploitable weakness of babesial parasites. Veterinary Parasitology 138, 50-60.

ALLRED, D.R., MCGUIRE, T.C., PALMER, G.H., LEIB, S.R., HARKINS, T.M., MCELWAIN, T.F., BARBET, A.F., 1990. Molecular basis for surface antigen size polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. Proceedings of the National Academy of Sciences of the United States of America 87, 3220-3224.

ALLSOPP, B.A., 2009. Trends in the control of heartwater. Onderstepoort Journal of Veterinary Research 76, 81-88.

ALLSOPP, B.A., 2010. Natural history of *Ehrlichia ruminantium*. Veterinary Parasitology 167, 123-135.

ALLSOPP, B.A., BEZUIDENHOUT, J.D., PROZESKY, L., 2004. Heartwater. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 507-535.

ALLSOPP, M.T.E.P., ALLSOPP, B.A., 2007. Extensive genetic recombination occurs in the field between different genotypes of *Ehrlichia ruminantium*. Veterinary Microbiology 124, 58-65.

ALLSOPP, M.T.E.P., CAVALIER-SMITH, T., WAAL, D.T.D., ALLSOPP, B.A., 1994. Phylogeny and evolution of the piroplasms. Parasitology 108, 147-152.

ALLSOPP, M.T.E.P., LOUW, M., MEYER, E.C., 2005. *Ehrlichia ruminantium*: an emerging human pathogen? Annals of the New York Academy of Sciences 1063, 358-360.

ALLSOPP, M.T.E.P., VISSER, E.S., DU PLESSIS, J.L., VOGEL, S.W., ALLSOPP, B.A., 1997. Different organisms associated with heartwater as shown by analysis of 16S ribosomal RNA gene sequences. Veterinary Parasitology 71, 283-300.

ALMAZÁN, C., MORENO-CANTÚ, O., MORENO-CID, J.A., GALINDO, R.C., CANALES, M., VILLAR, M., DE LA FUENTE, J., 2012. Control of tick infestations in cattle vaccinated with bacterial membranes containing surface-exposed tick protective antigens. Vaccine 30, 265-272.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

ANDREW, H.R., NORVAL, R.A.I., 1989a. The carrier status of sheep, cattle and African buffalo recovered from heartwater. Veterinary Parasitology 34, 261-266.

ANDREW, H.R., NORVAL, R.A.I., 1989b. The role of males of the bont tick (*Amblyomma hebraeum*) in the transmission of *Cowdriaruminantium* (heartwater). Veterinary Parasitology 34, 15-23.

ASIIMWE, B.B., WEIR, W., TAIT, A., LUBEGA, G.W., OURA, C.A.L., 2013. Haemoparasite infection kinetics and the population structure of *Theileria parva* on a single farm in Uganda. Veterinary Parasitology 193, 8-14.

ATIF, F.A., 2015. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. Parasitology Research 114, 3941-3957.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

AYANTUNDE, A.A., ASSE, R., SAID, M.Y., FALL, A., 2014. Transhumant pastoralism, sustainable management of natural resources and endemic ruminant livestock in the sub-humid zone of West Africa. Environment, Development and Sustainability 16, 1097-1117.

BABO MARTINS, S., DI GIULIO, G., LYNEN, G., PETERS, A., RUSHTON, J., 2010. Assessing the impact of East Coast fever immunisation by the infection and treatment method in Tanzanian pastoralist systems. Preventive Veterinary Medicine 97, 175-182.

BALDWIN, C.L., BLACK, S.J., BROWN, W.C., CONRAD, P.A., GODDEERIS, B.M., KINUTHIA, S.W., LALOR, P.A., MACHUGH, N.D., MORRISON, W.I., MORZARIA, S.P., 1988. Bovine T cells, B cells, and null cells are transformed by the protozoan parasite *Theileria parva*. Infection and Immunity 56, 462-467.

BARBET, A.F., BYROM, B., MAHAN, S.M., 2009. Diversity of *Ehrlichia ruminantium* major antigenic protein 1-2 in field isolates and infected sheep. Infection and Immunity 77, 2304-2310.

BARNARD, B.J.H., VAN DER LUGT, J.J., MUSHI, E.Z., 1994. Malignant catarrhal fever. In: Infectious Diseases of Livestock with special reference to Southern Africa, COETZER, J.A.W., THOMSON, G.R., TUSTIN, R.C. (Eds.), Oxford University Press Southern Africa, Cape Town, South Africa, pp. 946-957.

BARRE, N., GARRIS, G.I., 1990. Biology and ecology of *Amblyomma variegatum* (Acari: Ixodidae) in the Caribbean: Implications for a regional eradication program. Journal of Agricultural Entomology 7, 1-9.

BAZARUSANGA, T., GEYSEN, D., VERCRUYSSE, J., MARCOTTY, T., 2008. The sensitivity of PCR and serology in different *Theileria parva* epidemiological situations in Rwanda. Veterinary Parasitology 154, 21-31.

BEKKER, C.P.J., DE VOS, S., TAOUFIK, A., SPARAGANO, O.A.E., JONGEJAN, F., 2002. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Veterinary Microbiology 89, 223-238.

BELL-SAKYI, L., PALOMAR, A.M., BRADFORD, E.L., SHKAP, V., 2015. Propagation of the Israeli vaccine strain of *Anaplasma centrale* in tick cell lines. Veterinary Microbiology 179, 270-276.

BENSON, T., MUGARURA, S., 2013. Livestock development planning in Uganda: Identification of areas of opportunity and challenge. Land Use Policy 35, 131-139.

BESSENGER, R., SCHOEMAN, J.H., 1983. Serological response of cattle to infection with *Babesia bigemina* and *Babesia bovis* in southern Africa. The Onderstepoort Journal of Veterinary Research 50, 115-117.

BEZUIDENHOUT, J.D., WRIGHT, I.G., 1989. Cowdria vaccines. Veterinary protozoan and hemoparasite vaccines 31-42.

BEZUIDENHOUT, J.D., 1987. Natural transmission of heartwater. The Onderstepoort Journal of Veterinary Research 54, 349-351.

BEZUIDENHOUT, J.D., 1988. Certain aspects of the transmission of heartwater, the occurrence of the organism in ticks and *in vitro* culture. DVSc Thesis, University of Pretoria, South Africa.

BEZUIDENHOUT, J.D., BIGALKE, R.D., 1987. The control of heartwater by means of tick control. Onderstepoort Journal of Veterinary Research 54, 525-528.

BILGIÇ, H.B., KARAGENÇ, T., SIMUUNZA, M., SHIELS, B., TAIT, A., EREN, H., WEIR, W., 2013. Development of a multiplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis* and *Anaplasma marginale* in cattle. Experimental Parasitology 133, 222-229.

BILLIOUW, M., BERKVENS, D., 1999. The current epidemiological status of bovine theileriosis in eastern Zambia. Tropical Medicine and International Health 4, A28-A33.

BILLIOUW, M., BRANDT, J., VERCRUYSSE, J., SPEYBROECK, N., MARCOTTY, T., MULUMBA, M., BERKVENS, D., 2005. Evaluation of the indirect fluorescent antibody test as a diagnostic tool for East Coast fever in eastern Zambia. Veterinary Parasitology 127, 189-198.

BISHOP, R., SOHANPAL, B., MORZARIA, S., 1993. *Theileria parva*: detection of genomic polymorphisms by polymerase chain reaction amplification of DNA using arbitrary primers. Experimental Parasitology 77, 53-61.

BISHOP, R.P., SPOONER, P.R., SOHANPAL, B.K., 1996. Genetic fingerprinting of *Theileria parva* using a telomeric DNA probe. Parasitology Research 82, 264-266.

BISHOP, R., MUSOKE, A., MORZARIA, S., GARDNER, M., NENE, V., 2004. *Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology 129, S271-S283.

BISHOP, R., SOHANPAL, B., KARIUKI, D.P., YOUNG, A.S., NENE, V., BAYLIS, H., ALLSOPP, B.A., SPOONER, P.R., DOLAN, T.T., MORZARIA, S.P., 1992. Detection of a carrier state in *Theileria parva*-infected cattle by the polymerase chain reaction. Parasitology 104, 215-232.

BOCK, R.E., DE VOS, A.J., 2001. Immunity following use of Australian tick fever vaccine: a review of the evidence. Australian Veterinary Journal 79, 832-839.

BOCK, R., JACKSON, L., DE VOS, A., JORGENSEN, W., 2004. Babesiosis of cattle. Parasitology 129, S247-S269.

BOCK, R.E., KINGSTON, T.G., DE VOS, A.J., 1999. Effect of breed of cattle on transmission rate and innate resistance to infection with *Babesia bovis* and *B bigemina* transmitted by *Boophilus microplus* . Australian Veterinary Journal 77, 461-464.

BOCK, R.E., LEW, A.E., MINCHIN, C.M., JESTON, P.J., JORGENSEN, W.K., 2000. Application of PCR assays to determine the genotype of *Babesia bovis* parasites isolated from cattle with clinical babesiosis soon after vaccination against tick fever. Australian Veterinary Journal 78, 179-181.

BOCK, R., DE VOS, A., KINGSTON, T., SHIELS, I., DALGLIESH, R., 1992. Investigations of breakdowns in protection provided by living *Babesia bovis* vaccine. Veterinary Parasitology 43, 45-56.

BOWMAN, A.S., SAUER, J.R., 2004. Tick salivary glands: function, physiology and future. Parasitology 129, S67-S81.

BRAYTON, K.A., KAPPMEYER, L.S., HERNDON, D.R., DARK, M.J., TIBBALS, D.L., PALMER, G.H., MCGUIRE, T.C., KNOWLES, D.P., 2005. Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins. Proceedings of the National Academy of Sciences of the United States of America 102, 844-849.

BRAYTON, K.A., PALMER, G.H., BROWN, W.C., 2006. Genomic and proteomic approaches to vaccine candidate identification for *Anaplasma marginale*. Expert Review of Vaccines 5, 95-101.

BRAYTON, K.A., MEEUS, P.F., BARBET, A.F., PALMER, G.H., 2003. Simultaneous variation of the immunodominant outer membrane proteins, MSP2 and MSP3, during *Anaplasma marginale* persistence *in vivo*. Infection and Immunity 71, 6627-6632.

BROWN, W.C., MCGUIRE, T.C., MWANGI, W., KEGERREIS, K.A., MACMILLAN, H., LEWIN, H.A., PALMER, G.H., 2002. Major histocompatibility complex class II DR-restricted memory $CD4^+$ T lymphocytes recognize conserved immunodominant epitopes of *Anaplasma marginale* major surface protein 1a. Infection and Immunity 70, 5521-5532.

BROWN, W.C., MCGUIRE, T.C., ZHU, D., LEWIN, H.A., SOSNOW, J., PALMER, G.H., 2001. Highly conserved regions of the immunodominant major surface protein 2 of the genogroup II ehrlichial pathogen *Anaplasma marginale* are rich in naturally derived $CD4^+$ T lymphocyte epitopes that elicit strong recall responses. The Journal of Immunology 166, 1114-1124.

BROWN, W.C., NORIMINE, J., GOFF, W.L., SUAREZ, C.E., MCELWAIN, T.F., 2006. Prospects for recombinant vaccines against *Babesia bovis* and related parasites. Parasite Immunology 28, 315-327.

BURRIDGE, M.J., BROWN, C.G.D., KIMBER, C.D., 1974. *Theileria annulata*: cross-reactions between a cell culture schizont antigen and antigens of East African species in the indirect fluorescent antibody test. Experimental Parasitology 35, 374-380.

BURRIDGE, M.J., KIMBER, C.D., 1972. The indirect fluorescent antibody test for experimental East Coast fever (*Theileria parva* infection of cattle). Evaluation of a cell culture schizont antigen. Research in Veterinary Science 13, 451-455.

BURRIDGE, M.J., SIMMONS, L., PETER, T.F., MAHAN, S.M., 2002. Increasing risks of introduction of heartwater onto the American mainland associated with animal movements. Annals of the New York Academy of Sciences 969, 269-274.

BÜSCHER, G., 1988. The infection of various tick species with *Babesia bigemina*, its transmission and identification. Parasitology Research 74, 324-330.

CABEZAS-CRUZ, A., DE LA FUENTE, J., 2015. *Anaplasma marginale* major surface protein 1a: A marker of strain diversity with implications for control of bovine anaplasmosis. Ticks and Tick-borne Diseases 6, 205-210.

CABEZAS-CRUZ, A., PASSOS, L.M.F., LIS, K., KENNEIL, R., VALDÉS, J.J., FERROLHO, J., TONK, M., POHL, A.E., GRUBHOFFER, L., ZWEYGARTH, E., SHKAP, V., RIBEIRO, M.F.B., ESTRADA-PEÑA, A., KOCAN, K.M., DE LA FUENTE, J., 2013. Functional and immunological relevance of *Anaplasma marginale* major surface protein 1a sequence and structural analysis. PLoS ONE 8 (6), e65243. doi: 10.1371/jounal.pone.0065243.

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, 2748-2755.

CALLOW, L.L., 1984. Animal health in Australia, Volume 5. In: Protozoal and rickettsial diseases, Australian Government Publishing Service, 264 pp.

CALLOW, L.L., DALGLIESH, R.J., 1980. The development of effective, safe vaccination against babesiosis and anaplasmosis in Australia. In: Proceedings of the symposium of the 56th Annual Conference of the Australian Veterinary Association 1979, Ticks and Tick-borne Diseases, 4-8.

CALLOW, L.L., MELLORS, L.T., MCGREGOR, W., 1979. Reduction in virulence of *Babesia bovis* due to rapid passage in splenectomized cattle. International Journal for Parasitology 9, 333-338.

CALLOW, L.L., ROGERS, R.J., DE VOS, A.J., 1993. Tick-borne diseases. In: Australian Standard Diagnostic Techniques for Animal Diseases, CORNER, L.A., BAGUST, T.J. (Eds.), CSIRO Information Services, East Melbourne, pp. 1-16.

CAMUS, E., BARRÉ, N., 1992. The role of *Amblyomma variegatum* in the transmission of heartwater with special reference to Guadeloupe. Annals of the New York Academy of Sciences 653, 33-41.

CANTU, A., ORTEGA-S, J.A., MOSQUEDA, J., GARCIA-VAZQUEZ, Z., HENKE, S.E., GEORGE, J.E., 2007. Immunologic and molecular identification of *Babesia bovis* and *Babesia bigemina* in free-ranging white-tailed deer in northern Mexico. Journal of Wildlife Diseases 43, 504-507.

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, 107-114.

CASTAÑEDA-ORTIZ, E.J., UETI, M.W., CAMACHO-NUEZ, M., MOSQUEDA, J.J., MOUSEL, M.R., JOHNSON, W.C., PALMER, G.H., 2015. Association of *Anaplasma marginale* strain superinfection with infection prevalence within tropical regions. PLoS One 10 (3), e0120748. doi: 10.1371/journal.pone.0120748.

CATLEY, A., 2016. Use of participatory epidemiology to compare the clinical veterinary knowledge of pastoralists and veterinarians in East Africa. Tropical Animal Health and Production 38, 171-184.

CATLEY, A., ADMASSU, B., BEKELE, G., ABEBE, D., 2014. Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. Disasters 38, 500-516.

CATLEY, A., ALDERS, R.G., WOOD, J.L.N., 2012. Participatory epidemiology: Approaches, methods, experiences. The Veterinary Journal 191, 151-160.

CATLEY, A., CHIBUNDA, R.T., RANGA, E., MAKUNGU, S., MAGAYANE, F.T., MAGOMA, G., MADEGE, M.J., VOSLOO, W., 2004. Participatory diagnosis of a heat-intolerance syndrome in cattle in Tanzania and association with foot-and-mouth disease. Preventive Veterinary Medicine 65, 17-30.

CATLEY, A., OKOTH, S., OSMAN, J., FISON, T., NJIRU, Z., MWANGI, J., JONES, B.A., LEYLAND, T.J., 2001. Participatory diagnosis of a chronic wasting disease in cattle in southern Sudan. Preventive Veterinary Medicine 51, 161-181.

CHAISI, M.E., SIBEKO, K.P., COLLINS, N.E., POTGIETER, F.T., OOSTHUIZEN, M.C., 2011. Identification of *Theileria parva* and *Theileria* sp. (buffalo) 18S rRNA gene sequence variants in the African Buffalo (*Syncerus caffer*) in southern Africa. Veterinary Parasitology 182, 150-162.

CHAMBERS, R., 1994. The origins and practice of participatory rural appraisal. World Development 22, 953-969.

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, 37.

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. Livestock Research for Rural Development Volume 22 (7), Article # 135. Retrieved May 2, 2013, from http://www.lrrd.org/lrrd22/7/chen22135.htm.

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, 872-878.

COLLINS, N.E., LIEBENBERG, J., DE VILLIERS, E.P., BRAYTON, K.A., LOUW, E., PRETORIUS, A., FABER, F.E., VAN HEERDEN, H., JOSEMANS, A., VAN KLEEF, M., STEYN, H.C., VAN STRIJP, M.F., ZWEYGARTH, E., JONGEJAN, F., MAILLARD, J.C., BERTHIER, D., BOTHA, M., JOUBERT, F., CORTON, C.H., THOMSON, N.R., ALLSOPP, M.T., ALLSOPP, B.A., 2005. The genome of the heartwater agent *Ehrlichia ruminantium* contains multiple tandem repeats of actively variable copy number. Proceedings of the National Academy of Sciences of the United States of America 102, 838-843.

COLLINS, N.E., PRETORIUS, A., VAN KLEEF, M., BRAYTON, K.A., ALLSOPP, M.T., ZWEYGARTH, E., ALLSOPP, B.A., 2003. Development of improved attenuated and nucleic acid vaccines for heartwater. Developments in Biologicals 114, 121-136.

COMBRINK, M.P., TROSKIE, P.C., DE WAAL, D.T., 2002. Residual effect of antibabesial drugs on the live redwater blood vaccines. Annals of the New York Academy of Sciences 969, 169-173.

CONRAD, P.A., DENHAM, D., BROWN, C.G.D., 1986. Intraerythrocytic multiplication of *Theileria parva in vitro*: An ultrastructural study. International Journal for Parasitology 16, 223-229.

COX, F.E.G., 1991. Vector control: making predictions. Parasitology Today 7, 223 pp.

CRIADO-FORNELIO, A., 2007. A review of nucleic acid-based diagnostic tests for *Babesia* and *Theileria*, with emphasis on bovine piroplasms. Parassitologia 49, 39-44.

CUNNINGHAM, M.P., BROWN, C.G.D., BURRIDGE, M.J., IRVIN, A.D., KIRIMI, I.M., PURNELL, R.E., RADLEY, D.E., WAGNER, G.G., 1974. Theileriosis: The exposure of immunised cattle in a *Theileria lawrencei* enzootic area. Tropical Animal Health and Production 6, 39-43.

DAGRIS, 2007. Domestic Animal Genetic Resources Information System. International Livestock Research Institute (ILRI). Accessed October 23, 2016, from dagris.ilri.cgiar.org/display.asp?ID=37.

DA SILVEIRA, J.A.G., RABELO, E.M.L., RIBEIRO, M.F.B., 2011. Detection of *Theileria* and *Babesia* in brown brocket deer (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*) in the State of Minas Gerais, Brazil. Veterinary Parasitology 177, 61-66.

DALRYMPLE, B.P., JORGENSEN, W.K., DE VOS, A.J., WRIGHT, I.G., 1992. Analysis of the composition of samples of *Babesia bovis* and the influence of different environmental conditions on genetically distinct subpopulations. International Journal for Parasitology 22, 731-737.

DE DEKEN, R., MARTIN, V., SAIDO, A., MADDER, M., BRANDT, J., GEYSEN, D., 2007. An outbreak of East Coast fever on the Comoros: A consequence of the import of immunised cattle from Tanzania? Veterinary Parasitology 143, 245-253.

DE ECHAIDE, S.T., ECHAIDE, I.E., GAIDO, A.B., MANGOLD, A.J., LUGARESI, C.I., VANZINI, V.R., GUGLIELMONE, A.A., 1995. Evaluation of an enzyme-linked immunosorbent assay kit to detect *Babesia bovis* antibodies in cattle. Preventive Veterinary Medicine 24, 277-283.

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, 777-782.

DE LA FUENTE, J., ALMAZÁN, C., BLOUIN, E.F., NARANJO, V., KOCAN, K.M., 2006. Reduction of tick infections with *Anaplasma marginale* and *A. phagocytophilum* by targeting the tick protective antigen subolesin. Parasitology Research 100, 85-91.

DE LA FUENTE, J., KOCAN, K.M., BLOUIN, E.F., 2007a. Tick vaccines and the transmission of tick-borne pathogens. Veterinary Research Communications 31, 85-90.

DE LA FUENTE, J., GARCIA-GARCIA, J.C., BARBET, A.F., BLOUIN, E.F., KOCAN, K.M., 2004a. Adhesion of outer membrane proteins containing tandem repeats of *Anaplasma* and *Ehrlichia* species (Rickettsiales: Anaplasmataceae) to tick cells. Veterinary Microbiology 98, 313-322.

DE LA FUENTE, J., GARCIA-GARCIA, J.C., BLOUIN, E.F., MCEWEN, B.R., CLAWSON, D., KOCAN, K.M., 2001. Major surface protein 1a effects tick infection and transmission of *Anaplasma marginale*. International Journal for Parasitology 31, 1705-1714.

DE LA FUENTE, J., LEW, A., LUTZ, H., MELI, M.L., HOFMANN-LEHMANN, R., SHKAP, V., MOLAD, T., MANGOLD, A.J., ALMAZÁN, C., NARANJO, V., GORTÁZAR, C., TORINA, A., CARACAPPA, S., GARCÍA-PÉREZ, A.L., BARRAL, M., OPORTO, B., CECI, L., CARELLI, G., BLOUIN, E.F., KOCAN, K.M., 2005. Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. Animal Health Research Reviews 6, 75-89.

DE LA FUENTE, J., RUYBAL, P., MTSHALI, M.S., NARANJO, V., SHUQING, L., MANGOLD, A.J., RODRÍGUEZ, S.D., JIMÉNEZ, R., VICENTE, J., MORETTA, R., TORINA, A., ALMAZÁN, C., MBATI, P.M., DE ECHAIDE, S.T., FARBER, M., ROSARIO-CRUZ, R., GORTAZAR, C., KOCAN, K.M., 2007b. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. Veterinary Microbiology 119, 382-390.

DE LA FUENTE, J., THOMAS, E.J.G., VAN DEN BUSSCHE, R.A., HAMILTON, R.G., TANAKA, E.E., DRUHAN, S.E., KOCAN, K.M., 2003. Characterization of *Anaplasma marginale* isolated from North American bison. Applied and Environmental Microbiology 69, 5001-5005.

DE LA FUENTE, J., VICENTE, J., HÖFLE, U., RUIZ-FONS, F., FERNÁNDEZ DE MERA, I.G., VAN DEN BUSSCHE, R.A., KOCAN, K.M., GORTAZAR, C., 2004b. *Anaplasma* infection in free-ranging Iberian red deer in the region of Castilla-La Mancha, Spain. Veterinary Microbiology 100, 163-173.

DE VOS, A.J., BESSENGER, R., FOURIE, C.G., 1982. Virulence and heterologous strain immunity of South African and Australian *Babesia bovis* strains with reduced pathogenicity. The Onderstepoort Journal of Veterinary Research 49, 133-136.

DE VOS, A.J., DE WAAL, D.T., JACKSON, L.A., 2004. Bovine babesiosis. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), Oxford University Press Southern Africa, Cape Town, South Africa, pp. 406-424.

DE VOS, S., ZEINSTRA, L., TAOUFIK, A., WILLADSEN, P., JONGEJAN, F., 2001. Evidence for the utility of the Bm86 antigen from *Boophilus microplus* in vaccination against other tick species. Experimental & Applied Acarology 25, 245-261.

DE WAAL, D.T., COMBRINK, M.P., 2006. Live vaccines against bovine babesiosis. Veterinary Parasitology 138, 88-96.

DE WAAL, D.T., MATTHEE, O., JONGEJAN, F., 2000. Evaluation of the MAP1b ELISA for the diagnosis of heartwater in South Africa. Annals of the New York Academy of Sciences 916, 622-627.

DECARO, N., CARELLI, G., LORUSSO, E., LUCENTE, M.S., GRECO, G., LORUSSO, A., RADOGNA, A., CECI, L., BUONAVOGLIA, C., 2008. Duplex real-time polymerase chain reaction for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale*. Journal of Veterinary Diagnostic Investigation 20, 606-611.

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, 171-187.

DEEM, S.L., DONACHIE, P.L., NORVAL, R.A.I., 1996a. Colostrum from dams living in a heartwater-endemic area influences calfhood immunity to *Cowdria ruminantium*. Veterinary Parasitology 61, 133-144.

DEEM, S.L., NORVAL, R.A.I., DONACHIE, P.L., MAHAN, S.M., 1996b. Demonstration of vertical transmission of *Cowdria ruminantium*, the causative agent of heartwater, from cows to their calves. Veterinary Parasitology 61, 119-132.

DI GIULIO, G., LYNEN, G., MORZARIA, S., OURA, C., BISHOP, R., 2009. Live immunization against East Coast fever - current status. Trends in Parasitology 25, 85-92.

DOLAN, T.T., 1986. Chemotherapy of East Coast fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. Journal of Comparative Pathology 96, 137-146.

DREHER, U.M., DE LA FUENTE, J., HOFMANN-LEHMANN, R., MELI, M.L., PUSTERLA, N., KOCAN, K.M., WOLDEHIWET, Z., BRAUN, U., REGULA, G., STAERK, K.D.C., LUTZ, H., 2005. Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. Clinical and Vaccine Immunology 12, 1177-1183.

DU PLESSIS, J.L., MALAN, L., 1987a. Problems with the interpretation of epidemiological data in heartwater: a study on 23 farms. Onderstepoort Journal of Veterinary Research 54, 427-433.

DU PLESSIS, J.L., MALAN, L., 1987b. The application of the indirect fluorescent antibody test in research on heartwater. Onderstepoort Journal of Veterinary Research 54, 319-325.

DU PLESSIS, J.L., MALAN, L., 1987c. The non-specific resistance of cattle to heartwater. The Onderstepoort Journal of Veterinary Research 54, 333-336.

DUCKEN, D.R., BROWN, W.C., ALPERIN, D.C., BRAYTON, K.A., REIF, K.E., TURSE, J.E., PALMER, G.H., NOH, S.M., 2015. Subdominant outer membrane antigens in *Anaplasma marginale*: conservation, antigenicity, and protective capacity using recombinant protein. PLoS ONE 10 (6), e0129309.

DUMLER, J.S., BARBET, A.F., BEKKER, C.P.J., 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 combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. International Journal of Systematic and Evolutionary Microbiology 51, 2145-2165.

EGERU, A., WASONGA, O., KYAGULANYI, J., MAJALIWA, G.J.M., MACOPIYO, L., MBURU, J., 2014. Spatio-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda. Pastoralism: Research, Policy and Practice 4, 6.

EMERY, D.L., EUGUI, E.M., NELSON, R.T., TENYWA, T., 1981. Cell-mediated immune responses to *Theileria parva* (East Coast fever) during immunization and lethal infections in cattle. Immunology 43, 323-336.

ESTEVES, I., VACHIÉRY, N., MARTINEZ, D., TOTTE, P., 2004. Analysis of *Ehrlichia ruminantium*-specific T1/T2 responses during vaccination with a protective killed vaccine and challenge of goats. Parasite Immunology 26, 95-103.

FABURAY, B., GEYSEN, D., CEESAY, A., MARCELINO, I., ALVES, P.M., TAOUFIK, A., POSTIGO, M., BELL-SAKYI, L., JONGEJAN, F., 2007a. Immunisation of sheep against heartwater in The Gambia using inactivated and attenuated *Ehrlichia ruminantium* vaccines. Vaccine 25, 7939-7947.

FABURAY, B., GEYSEN, D., MUNSTERMANN, S., BELL-SAKYI, L., JONGEJAN, F., 2007b. Longitudinal monitoring of *Ehrlichia ruminantium* infection in Gambian lambs and kids by pCS20 PCR and MAP1-B ELISA. BMC Infectious Diseases 7, 85.

FANDAMU, P., DUCHATEAU, L., SPEYBROECK, N., MARCOTTY, T., MBAO, V., MTAMBO, J., MULUMBA, M., BERKVENS, D., 2005. *Theileria parva* seroprevalence in traditionally kept cattle in southern Zambia and El Niño. International Journal for Parasitology 35, 391-396.

FAO, 2013. FAO at work in Karamoja. Food and Agriculture Organization of the United nations (FAO) Representation in Uganda. http://www.fao.org/emergencies/resources/documents/resources-detail/en/c/212585/. Accessed October 23, 2015.

FERRERI, L., BENITEZ, D., DOMINGUEZ, M., RODRIGUEZ, A., ASENZO, G., MESPLET, M., FLORIN-CHRISTENSEN, M., SCHNITTGER, L., 2008. Water buffalos as carriers of *Babesia bovis* in Argentina. Annals of the New York Academy of Sciences 1149, 149-151.

FISH, L., LEIBOVICH, B., KRIGEL, Y., MCELWAIN, T., SHKAP, V., 2008. Vaccination of cattle against *B. bovis* infection with live attenuated parasites and non-viable immunogens. Vaccine 26, G29-G33.

FOUCHE, G., RAMAFUTHULA, M., MASELELA, V., MOKOENA, M., SENABE, J., LEBOHO, T., SAKONG, B.M., ADENUBI, O.T., ELOFF, J.N., WELLINGTON, K.W., 2016. Acaricidal activity of the organic extracts of thirteen South African plants against *Rhipicephalus* (*Boophilus*) *decoloratus* (Acari: Ixodidae). Veterinary Parasitology 224, 39-43.

FLORIN-CHRISTENSEN, M., SUAREZ, C.E., RODRIGUEZ, A.E., FLORES, D.A., SCHNITTGER, L., 2014. Vaccines against bovine babesiosis: where we are now and possible roads ahead. Parasitology 141, 1563-1592.

FRENCH, D.M., BROWN, W.C., PALMER, G.H., 1999. Emergence of *Anaplasma marginale* antigenic variants during persistent rickettsemia. Infection and Immunity 67, 5834-5840.

FRIEDHOFF, K.T., 1988. Transmission of *Babesia*. In: Babesiosis of domestic animals and man, RISTIC, M. (Ed.), CRC Press Inc., Boca Raton, Florida, pp. 23-52.

FUJISAKI, K., 1992. A review of the taxonomy of *Theileria sergenti*/*buffeli*/*orientalis* group parasites in cattle. The Journal of Protozoology Research 3, 87-96.

FUTSE, J.E., UETI, M.W., KNOWLES, 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, 3829-3834.

FYUMAGWA, R.D., RUNYORO, V., HORAK, I.G., HOARE, R., 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. South African Journal of Wildlife Research 37, 79-90.

FYUMAGWA, R.D., MDAKI, M.L., SOOMBE, C., NYAKI, A., 2013. African buffalo is an important reservior of *Anaplasma bovis* in the Ngorongoro Crater, Tanzania. Research Opinions in Animal and Veterinary Sciences 3, 105-110.

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, 194.

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, 103-113.

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, 208-223.

GALVmed, 2015a. East Coast Fever Infection and Treatment Method. GALVmed Position Paper 1. The Global Alliance for Livestock Veterinary Medicines. Accessed on November 26, 2016, from www.galvmed.org/wp-content/uploads/2015/02/GALVmed-Position-Paper-on-ECF-Feb-2015.pdf.

GALVMED, 2015b. The CTTBD ECF Vaccine: Greater opportunity to control East Coast fever and other tick-borne diseases in Africa. Global Alliance for Livestock Veterinary Medicines, Pentlands SciencePark, Bush Loan, Edinburgh EH26 OPZ, UK.

GARCIA-GARCIA, J.C., DE LA FUENTE, J., KOCAN, K.M., BLOUIN, E.F., HALBUR, T., ONET, V.C., SALIKI, J.T., 2004. Mapping of B-cell epitopes in the N-terminal repeated peptides of *Anaplasma marginale* major surface protein 1a and characterization of the humoral immune response of cattle immunized with recombinant and whole organism antigens. Veterinary Immunology and Immunopathology 98, 137-151.

GE, N., KOCAN, K.M., BLOUIN, E.F., MURPHY, G.L., 1996. Developmental studies of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari: Ixodidae) infected as adults by using nonradioactive *in situ* hybridization and microscopy. Journal of Medical Entomology 33, 911-920.

GEORGE, J.E., POUND, J.M., DAVEY, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129, S353-S366.

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, 273-286.

GEYSEN, D., 2008. Live immunisation against *Theileria parva*: spreading the disease? Trends in Parasitology 24, 245-246.

GITAU, G.K., MCDERMOTT, J.J., KATENDE, J.M., O'CALLAGHAN, C.J., BROWN, R.N., PERRY, B.D., 2000. Differences in the epidemiology of theileriosis on smallholder dairy farms in contrasting agro-ecological and grazing strata of highland Kenya. Epidemiology and Infection 124, 325-335.

GODDEERIS, B.M., KATENDE, J.M., IRVIN, A.D., CHUMO, R.S., 1982. Indirect fluorescent antibody test for experimental and epizoological studies on East Coast fever (*Theileria parva* infection in cattle). Evaluation of a cell culture schizont antigen fixed and stored in suspension. Research in Veterinary Science 33, 360-365.

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., MOSQUEDA, J., PALMER, G.H., SUAREZ, C.E., KNOWLES, D.P., MCELWAIN, T.F., 2008. Validation of a competitive enzyme-linked immunosorbent assay for detection of *Babesia bigemina* antibodies in cattle. Clinical and Vaccine Immunology 15, 1316-1321.

GOHIL, S., KATS, L.M., STURM, A., COOKE, B.M., 2010. Recent insights into alteration of red blood cells by *Babesia bovis*: moovin' forward. Trends in Parasitology 26, 591-599.

GOLDMAN, M.J., RIOSMENA, F., 2013. Adaptive capacity in Tanzanian Maasailand: changing strategies to cope with drought in fragmented landscapes. Global Environmental Change 23, 588-597.

GRADÉ, J.T., TABUTI, J.R.S., VAN DAMME, P., 2009. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. Journal of Ethnopharmacology 122, 273-293.

GRAHAM, S.P., PELLÉ, R., HONDA, Y., MWANGI, D.M., TONUKARI, N.J., YAMAGE, M., GLEW, E.J., DE VILLIERS, E.P., SHAH, T., BISHOP, R., ABUYA, E., AWINO, E., GACHANJA, J., LUYAI, A.E., MBWIKA, F., MUTHIANI, A.M., NDEGWA, D.M., NJAHIRA, M., NYANJUI, J.K., ONONO, F.O., OSASO, J., SAYA, R.M., WILDMANN, C., FRASER, C.M., MAUDLIN, I., GARDNER, M.J., MORZARIA, S.P., LOOSMORE, S., GILBERT, S.C., AUDONNET, J., VAN DER BRUGGEN, P., NENE, V., TARACHA, E.L.N., 2006. *Theileria parva* candidate vaccine antigens recognized by immune bovine cytotoxic T lymphocytes. Proceedings of the National Academy of Sciences of the United States of America 103, 3286-3291.

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, 1782-1789.

HAJDUSEK, O., ALMAZÁN, C., LOOSOVA, G., VILLAR, M., CANALES, M., GRUBHOFFER, L., KOPACEK, P., DE LA FUENTE, J., 2010. Characterization of ferritin 2 for the control of tick infestations. Vaccine 28, 2993-2998.

HAMMAC, G.K., KU, P., GALLETTI, M.F., NOH, S.M., SCOLES, G.A., PALMER, G.H., BRAYTON, K.A., 2013. Protective immunity induced by immunization with a live, cultured *Anaplasma marginale* strain. Vaccine 31, 3617-3622.

HASSAN, S.M., SALIH, D.A., 2013. An overview of factors responsible for geographic distribution pattern of ixodid ticks in the Sudan. Sokoto Journal of Veterinary Sciences 11, 1-9.

HERNDON, D.R., PALMER, G.H., SHKAP, V., KNOWLES, D.P., BRAYTON, K.A., 2010. Complete genome sequence of *Anaplasma marginale* subsp. *centrale*. Journal of Bacteriology 192, 379-380.

HOLLAND, C.J., LOGAN, L.L., MEBUS, C.A., RISTIC, M., 1987. The serological relationship between *Cowdria ruminantium* and certain members of the genus *Ehrlichia*. Onderstepoort Journal of Veterinary Research 54, 331.

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, 248-271.

HUGHES, V.L., RANDOLPH, S.E., 2001. Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. Journal of Parasitology 87, 49-54.

HUNFELD, K.P., HILDEBRANDT, A., GRAY, J.S., 2008. Babesiosis: Recent insights into an ancient disease. International Journal for Parasitology 38, 1219-1237.

HUTCHINGS, C.L., LI, A., FERNANDEZ, K.M., FLETCHER, T., JACKSON, L.A., MOLLOY, J.B., JORGENSEN, W.K., LIM, C.T., COOKE, B.M., 2007. New insights into the altered adhesive and mechanical properties of red blood cells parasitized by *Babesia bovis*. Molecular Microbiology 65, 1092-1105.

IAMS, K.P., YOUNG, J.R., NENE, V., DESAI, J., WEBSTER, P., OLE-MOIYOI, O.K., MUSOKE, A.J., 1990. Characterisation of the gene encoding a 104-kilodalton micronemerhoptry protein of *Theileria parva*. Molecular and Biochemical Parasitology 39, 47-60.

IRVIN, A.D., DOBBELAERE, D.A., MWAMACHI, D.M., MINAMI, T., SPOONER, P.R., OCAMA, J.G., 1983. Immunisation against East Coast fever: correlation between monoclonal antibody profiles of *Theileria parva* stocks and cross immunity *in vivo*. Research in Veterinary Science 35, 341-346.

IRVIN, A.D., MWAMACHI, D.M., 1983. Clinical and diagnostic features of East Coast fever (*Theileria parva*) infection of cattle. The Veterinary Record 113, 192-198.

JARRETT, W.F.H., CRIGHTON, G.W., PIRIE, H.M., 1969. *Theileria parva*: kinetics of replication. Experimental Parasitology 24, 9-25.

JENKINS, M.C., 2001. Advances and prospects for subunit vaccines against protozoa of veterinary importance. Veterinary Parasitology 101, 291-310.

JONGEJAN, F., MUSISI, F.L., MOORHOUSE, P.D.S., SNACKEN, M., UILENBERG, G., 1986. *Theileria taurotragi* in Zambia. Veterinary Quarterly 8, 261-263.

JONGEJAN, F., ZANDBERGEN, T.A., VAN DE WIEL, P.A., DE GROOT, M., UILENBERG, G., 1991. The tick-borne rickettsia *Cowdria ruminantium* has a *Chlamydia*-like developmental cycle. The Onderstepoort Journal of Veterinary Research 58, 227-237.

JONSSON, N.N., BOCK, R.E., JORGENSEN, W.K., 2008. Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses, and the effects of differing intensity of tick control in Australia. Veterinary Parasitology 155, 1-9.

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, 85-89.

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. Journal of Protozoology Research 18, 61-70.

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, 414.

KABUUSU, R.M., ALEXANDER, R., KABUUSU, A.M., MUWANGA, S.N., ATIMNEDI, P., MACPHERSON, C., 2013. Effect of wildlife-livestock interface on the prevalence of intra-erythrocytic haemoparasites in cattle. Open Journal of Veterinary Medicine 3, 315-318.

KALUME, M.K., LOSSON, B., ANGENOT, L., TITS, M., WAUTERS, J.N., FRÉDÉRICH, M., SAEGERMAN, C., 2012. Rotenoid content and *in vitro* acaricidal activity of *Tephrosia vogelii* leaf extract on the tick *Rhipicephalus appendiculatus*. Veterinary Parasitology 190, 204-209.

KALUME, M.K., SAEGERMAN, C., MBAHIKYAVOLO, D.K., MAKUMYAVIRI, A.M., MARCOTTY, T., MADDER, M., CARON, Y., LEMPEREUR, L., LOSSON, B., 2013. Identification of hard ticks (Acari: *Ixodidae*) and seroprevalence to *Theileria parva* in cattle raised in North Kivu Province, Democratic Republic of Congo. Parasitology Research 112, 789-797.

KAMAU, J., DE VOS, A.J., PLAYFORD, M., SALIM, B., KINYANJUI, P., SUGIMOTO, C., 2011. Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. Parasites & Vectors 4, 22.

KANYARI, P.W.N., KAGIRA, J., 2000. The role of parasitic diseases as causes of mortality in cattle in a high potential area of central Kenya: a quantitative analysis. Onderstepoort Journal of Veterinary Research 67, 157-161.

KARIUKI, D.P., YOUNG, A.S., MORZARIA, S.P., LESAN, A.C., MINING, S.K., OMWOYO, P., WAFULA, J.L.M., MOLYNEUX, D.H., 1995. *Theileria parva* carrier state in naturally infected and artificially immunised cattle. Tropical Animal Health and Production 27, 15-25.

KASOZI, K.I., MATOVU, E., TAYEBWA, D.S., NATUHWERA, J., MUGEZI, I., MAHERO, M., 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. Open Journal of Veterinary Medicine 4, 220-231.

KATENDE, J., MORZARIA, S., TOYE, P., SKILTON, R., NENE, V., NKONGE, C., MUSOKE, A., 1998. An enzyme-linked immunosorbent assay for detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. Parasitology Research 84, 408-416.

KATZ, J.B., BARBET, A.F., MAHAN, S.M., KUMBULA, D., LOCKHART, J.M., KEEL, M.K., DAWSON, J.E., OLSON, J.G., EWING, S.A., 1996. A recombinant antigen from the heartwater agent (*Cowdria ruminatium*) reactive with antibodies in some southeastern United States white-tailed deer (*Odocoileus virginianus*), but not cattle, sera. Journal of Wildlife Diseases 32, 424-430.

KATZER, F., NGUGI, D., WALKER, A.R., MCKEEVER, D.J., 2010. Genotypic diversity, a survival strategy for the apicomplexan parasite *Theileria parva*. Veterinary parasitology 167, 236-243.

KAWAHARA, M., RIKIHISA, Y., LIN, Q., ISOGAI, E., TAHARA, K., ITAGAKI, A., HIRAMITSU, Y., TAJIMA, T., 2006. Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. Applied and Environmental Microbiology 72, 1102-1109.

KAWAZU, S., SUGIMOTO, C., KAMIO, T., FUJISAKI, K., 1992. Antigenic differences between Japanese *Theileria sergenti* and other benign *Theileria* species of cattle from Australia (*T. buffeli*) and Britain (*T. orientalis*). Parasitology Research 78, 130-135.

KEHRLI, M.E., NONNECKE, B.J., ROTH, J.A., 1989. Alterations in bovine neutrophil function during the peripartureint period. American Journal of Veterinary Research 50, 207-214.

KIM, C.M., KIM, M.S., PARK, M.S., PARK, J.H., CHAE, J.S., 2003. Identification of *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, and *A. bovis* in *Haemaphysalis longicornis* and *Ixodes persulcatus* ticks from Korea. Vector-Borne and Zoonotic Diseases 3, 17-26.

KIVARIA, F.M., 2010. Climate change and the epidemiology of tick-borne diseases of cattle in Africa. The Veterinary Journal 184, 7-8.

KIVARIA, F.M., HEUER, C., JONGEJAN, F., OKELLO-ONEN, J., RUTAGWENDA, T., UNGER, F., BOEHLE, W., 2004. Endemic stability for *Theileria parva* infections in Ankole calves of the Ankole ranching scheme, Uganda. Onderstepoort Journal of Veterinary Research 71, 189-195.

KNOWLES, D., TORIONI DE ECHAIDE, S., 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, 2225-2230.

KOCAN, K.M., DE LA FUENTE, J., 2003. Co-feeding studies of ticks infected with *Anaplasma marginale*. Veterinary Parasitology 112, 295-305.

KOCAN, K.M., BEZUIDENHOUT, J.D., HART, A., 1987. Ultrastructural features of *Cowdria ruminantium* in midgut epithelial cells and salivary glands of nymphal *Amblyomma hebraeum*. The Onderstepoort Journal of Veterinary Research 54, 87-92.

KOCAN, K.M., DE LA FUENTE, J., BLOUIN, E.F., GARCIA-GARCIA, J.C., 2004a. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology 129, S285-S300.

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, 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, 698-712.

KOCAN, K.M., DE LA FUENTE, J., THOMAS, E.J.G., VAN DEN BUSSCHE, R.A., HAMILTON, R.G., TANAKA, E.E., DRUHAN, S.E., 2004b. Recent studies on the characterization of *Anaplasma marginale* isolated from North American bison. Annals of the New York Academy of Sciences 1026, 114-117.

KOCAN, K.M., GOFF, W.L., STILLER, D., CLAYPOOL, P.L., EDWARDS, W., EWING, S.A., HAIR, J.A., BARRON, S.J., 1992. Persistence of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari: Ixodidae) transferred successively from infected to susceptible calves. Journal of Medical Entomology 29, 657-668.

KUTTLER, K.L., 1984. *Anaplasma* infections in wild and domestic ruminants: a review. Journal of Wildlife Diseases 20, 12-20.

LAISSER, E.L.K., CHENYAMBUGA, S.W., MSALYA, G., KIPANYULA, M.J., MDEGELA, R.H., KARIMURIBO, E.D., MWILAWA, A.J., KUSILUKA, L.J.M., 2015. Knowledge and perception of ticks, tick-borne diseases and indigenous cattle tolerance to East Coast fever in agro-pastoral communities of Lake Zone in Tanzania. Livestock Research for Rural Development Volume 27, Article #64. Retrieved October 15, 2016, from http://www.lrrd.org/lrrd27/4/lais27064.htm.

LAMY, E., VAN HARTEN, S., SALES-BAPTISTA, E., GUERRA, M.M.M., DE ALMEIDA, A.M., 2012. Factors influencing livestock productivity. In: Environmental Stress and Amelioration in Livestock Production, SEJIAN, V., NAQVI, S.M.K., EZEJI, T., LAKRITZ, J., LAI, R. (Eds.), pp. 19-51.

LATIF, A.A., HOVE, T., 2011. History and critical review of *Theileria parva* (Boleni), the vaccine stock against Zimbabwean cattle theileriosis. Ticks and Tick borne Diseases 2, 163-167.

LATIF, A.A., WALKER, A.R., 2004. An introduction to the biology and control of ticks in Africa, 29 pp. Accessed October 19, 2016, from www.alanrwalker.com/assets/PDF/tick-biology-africa.pdf.

LAWRENCE, J.A., PERRY, B.D., WILLIAMSON, S.M., 2004a. Corridor disease. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 468-471.

LAWRENCE, J.A., PERRY, B.D., WILLIAMSON, S.M., 2004b. East Coast fever. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 448-467.

LAWRENCE, J.A., PERRY, B.D., WILLIAMSON, S.M., 2004c. Zimbabwe theileriosis. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 472-474.

LEEMANS, I., HOOSHMAND-RAD, P., UGGLA, A., 1997. The indirect fluorescent antibody test based on schizont antigen for study of the sheep parasite *Theileria lestoquardi*. Veterinary Parasitology 69, 9-18.

LÉGER, E., VOURC'H, G., VIAL, L., CHEVILLON, C., MCCOY, K.D., 2013. Changing distributions of ticks: causes and consequences. Experimental and Applied Acarology 59, 219-244.

LETA, S., DE CLERCQ, E.M., MADDER, M., 2013. High-resolution predictive mapping for *Rhipicephalus appendiculatus* (Acari: Ixodidae) in the Horn of Africa. Experimental and Applied Acarology 60, 531-542.

LIU, A., GUAN, G., DU, P., GOU, H., LIU, Z., LIU, J., MA, M., YANG, J., LI, Y., NIU, Q., REN, Q., BAI, Q., YIN, H., LUO, J., 2012. Loop-mediated isothermal amplification (LAMP) method based on two species-specific primer sets for the rapid identification of Chinese *Babesia bovis* and *B. bigemina*. Parasitology International 61, 658-663.

LIU, Z., ZHAO, J., MA, L., YAO, B., 1997. *Babesia orientalis* sp. nov. parasitized in buffalo *Bubalus bubalis* in China (Piroplasmida: Babesiidae). Acta Veterinaria et Zootechnica Sinica 28, 84-89.

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, 114-120.

LOPEZ, J.E., SIEMS, W.F., PALMER, G.H., BRAYTON, K.A., MCGUIRE, T.C., NORIMINE, J., BROWN, W.C., 2005. Identification of novel antigenic proteins in a complex *Anaplasma marginale* outer membrane immunogen by mass spectrometry and genomic mapping. Infection and Immunity 73, 8109-8118.

LYNEN, G., DI GIULIO, G., HOMEWOOD, K., REID, R., MWILAWA, A., 2006. Deployment of a live ECF vaccine in pastoral areas: lessons learned from Tanzania. In: The role of biotechnology in animal agriculture to address poverty in Africa: opportunities and challenges. Proceedings of the 4th All African Conference on Animal Agriculture and the 31st Annual Meeting of the Tanzania Society for Animal Production (TSAP), 20-24 September, 2005, REGE, J.E.O., NYAMU, A.M., SENDALO, D. (Eds.), Arusha, Tanzania, All Africa Society for Animal Production (AASAP), Nairobi, Kenya, 193-201.

LYNEN, G., YRJÖ-KOSKINEN, A.E., BAKUNAME, C., DI GIULIO, G., MLINGA, N., KHAMA, I., HANKS, J., TAYLOR, N.M., JAMES, A.D., MCKEEVER, D., PETERS, A.R., RUSHTON, J., 2012. East Coast fever immunisation field trail in crossbred dairy cattle in Hanang and Handeni districts in northern Tanzania. Tropical Animal Health and Production 44, 567-572.

MAAIF, 2010. Agriculture for Food and Income Security. Agriculture Sector Development Strategy and Investment Plan: 2010/11-2014/15. Ministry of Agriculture, Animal Industry & Fisheries, Uganda, 160 pp. Retrieved June 21, 2015, from agriculture.go.ug/userfiles/Agricultural Sector Development Strategy and Investment Plan (2).pdf.

MAAIF, 2011. Statistical Abstract, Agricultural Planning Department, Ministry of Agriculture, Animal Industry and Fisheries. www.agriculture.go.ug/userfiles/Statistical Abstract 2011.pdf. Accessed June 7, 2013.

MACMILLAN, S., 2014. ILVAC: A short history of the 'live' vaccine protecting Africa's cattle against East Coast fever. International Livestock Research Institute. Accessed on November 7, 2016 from https://ilvac.net/2014/08/19/a-short-history-of-the-live-vaccine-protecting-africas-cattle-against-east-coast-fever/.

MCCORKLE, C.M., MATHIAS-MUNDY, E., 1992. Ethnoveterinary Medicine in Africa. Africa: Journal of the International African Institute 62, 59-93.

MADDER, M., SPEYBROECK, N., BRANDT, J., TIRRY, L., HODEK, I., BERKVENS, D., 2002. Geographic variation in diapause response of adult *Rhipicephalus appendiculatus* ticks. Experimental and Applied Acarology 27, 209-221.

MAGONA, J.W., WALUBENGO, J., KABI, F., 2011a. Response of Nkedi Zebu and Ankole cattle to tick infestation and natural tick-borne, helminth and trypanosome infections in Uganda. Tropical Animal Health and Production 43, 1019-1033.

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, 273-280.

MAGONA, J.W., WALUBENGO, J., OLAHO-MUKANI, W., JONSSON, N.N., WELBURN, S.W., EISLER, M.C., 2011b. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55, 203-213.

MAHAN, S.M., PETER, T.F., SEMU, S.M., SIMBI, B.H., NORVAL, R.A.I., BARBET, A.F., 1995. Laboratory reared *Amblyomma hebraeum* and *Amblyomma variegatum* ticks differ in their susceptibility to infection with *Cowdria ruminantium*. Epidemiology and Infection 115, 345-353.

MAHAN, S.M., PETER, T.F., SIMBI, B.H., KOCAN, K., CAMUS, E., BARBET, A.F., BURRIDGE, M.J., 2000. Comparison of efficacy of American and African *Amblyomma* ticks as vectors of heartwater (*Cowdria ruminantium*) infection by molecular analyses and transmission trials. The Journal of Parasitology 86, 44-49.

MAHAN, S.M., SMITH, G.E., KUMBULA, D., BURRIDGE, M.J., BARBET, A.F., 2001. Reduction in mortality from heartwater in cattle, sheep and goats exposed to field challenge using an inactivated vaccine. Veterinary Parasitology 97, 295-308.

MAHONEY, D.F., MIRRE, G.B., 1979. A note on the transmission of *Babesia bovis* (syn *B argentina*) by the one-host tick, *Boophilus miroplus*. Research in Veterinary Science 26, 253-254.

MAHONEY, D.F., ROSS, D.R., 1972. Epizootiological factors in the control of bovine babesiosis . Australian Veterinary Journal 48, 292-298.

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, 216-223.

MANS, B.J., PIENAAR, R., LATIF, A.A., 2015. A review of *Theileria* diagnostics and epidemiology International Journal for Parasitology: Parasites and Wildlife 4, 104-118.

MANS, B.J., PIENAAR, R., LATIF, A.A., POTGIETER, F.T., 2011. Diversity in the 18S SSU rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitology 138, 766-779.

MAPHOLI, N.O., MARUFU, M.C., MAIWASHE, A., BANGA, C.B., MUCHENJE, V., MACNEIL, M.D., CHIMONYO, M., DZAMA, K., 2014. Towards a genomics approach to tick (Acari: Ixodidae) control in cattle: A review. Ticks and Tick-borne Diseases 5, 475-483.

MARCELINO, I., DE ALMEIDA, A.M., VENTOSA, M., PRUNEAU, L., MEYER, D.F., MARTINEZ, D., LEFRANÇOIS, T., VACHIÉRY, N., COELHO, A.V., 2012. Tick-borne diseases in cattle: Applications of proteomics to develop new generation vaccines. Journal of Proteomics 75, 4232-4250.

MARCELINO, I., LEFRANÇOIS, T., MARTINEZ, D., GIRAUD-GIRARD, K., APRELON, R., MANDONNET, N., GAUCHERON, J., BERTRAND, F., VACHIÉRY, N., 2015. A user-friendly and scalable process to prepare a ready-to-use inactivated vaccine: The example of heartwater in ruminants under tropical conditions. Vaccine 33, 678-685.

MARINER, J.C., HENDRICKX, S., PFEIFFER, D.U., COSTARD, S., KNOPF, L., OKUTHE, S., CHIBEU, D., PARMLEY, J., MUSENERO, M., PISANG, C., ZINGESER, J., JONES, B.A., ALI, S.N., BETT, B., MCLAWS, M., UNGER, F., ARABA, A., MEHTA, P., JOST, C.C., 2011. Integration of participatory approaches into surveillance systems. Scientific and Technical Review of the Office International des Epizooties (Paris) 30, 653-659.

MARINER, J.C., PASKIN, R., 2000. Manual on participatory epidemiology: methods for the collection of action-oriented epidemiological intelligence. FAO Animal Health Manual 10. Food and Agriculture Organisation of the United Nations, Rome 2000, 81 pp. Accessed October 17, 2016, from www.fao.org/docrep/003/x8833e/x8833e00.htm.

MARUFU, M.C., CHIMONYO, M., DZAMA, K., MAPIYE, C., 2010. Seroprevalence of tick-borne diseases in communal cattle reared on sweet and sour rangelands in a semi-arid area of South Africa. The Veterinary Journal 184, 71-76.

MASON, C.A., NORVAL, R.A.I., 1981. The transfer of *Boophilus microplus* (Acarina: Ixodidae) from infested to uninfested cattle under field conditions. Veterinary Parasitology 8, 185-188.

MATHEW, S., BOYD, R., 2014. The cost of cowardice: punitive sentiments towards free riders in Turkana raids. Evolution and Human behaviour 35, 58-64.

MATSON, B.A., 1967. Theileriosis in Rhodesia (Zimbabwe): a study of diagnostic specimens over two seasons. Journal of South African Veterinary Medical Association 38, 93-97.

MATTHYSSE, J.G., COLBO, M.H., 1987. The Ixodid Ticks of Uganda. Entomological Society of America, College Park, Maryland, pp. xii + 426 pp.

MATTIOLI, R.C., PANDEY, V.S., MURRAY, M., FITZPATRICK, J.L., 2000. Immunogenetic influences on tick resistance in African cattle with particular reference to trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Gobra zebu (*Bos indicus*) cattle. Acta Tropica 75, 263-277.

MBASSA, G.K., MGONGO, F.O.K., MELAU, L.S.B., MLANGWA, J.E.D., SILAYO, R.S., BIMBITA, E.N., HAYGHAIMO, A.A., MBIHA, E.R., 2009. A financing system for the control of tick-borne diseases in pastoral herds: The Kambala (Tanzania) model. Livestock Research for Rural Development 21 (3), Article #44. http://www.lrrd.org/lrrd21/3/mbas21044.htm. Accessed May 20, 2015.

MBIZENI, S., POTGIETER, F.T., TROSKIE, C., MANS, B.J., PENZHORN, B.L., LATIF, A.A., 2013. Field and laboratory studies on Corridor disease (*Theileria parva* infection) in cattle population at the livestock/game interface of uPhongolo-Mkuze area, South Africa. Ticks and Tick-borne Diseases 4, 227-234.

MCCORKLE, C.M., MATHIAS-MUNDY, E., 1992. Ethnoveterinary medicine in Africa. Africa: Journal of the International African Institute 62, 59-93.

MCGAREY, D.J., BARBET, A.F., PALMER, G.H., MCGUIRE, T.C., ALLRED, D.R., 1994. Putative adhesins of *Anaplasma marginale*: major surface polypeptides 1a and 1b. Infection and Immunity 62, 4594-4601.

MCKEEVER, D.J., 2007. Live immunisation against *Theileria parva*: containing or spreading the disease? Trends in Parasitology 23, 565-568.

MCKEEVER, D.J., 2009. Bovine immunity - a driver for diversity in *Theileria* parasites? Trends in Parasitology 25, 269-276.

MCKEEVER, D.J., TARACHA, E.L.N., INNES, E.L., MACHUGH, N.D., AWINO, E., GODDEERIS, B.M., MORRISON, W.I., 1994. Adoptive transfer of immunity to *Theileria parva* in the CD8+ fraction of responding efferent lymph. Proceedings of the National Academy of Sciences of the United States of America 91, 1959-1963.

MEHLHORN, H., SCHEIN, E., 1985. The piroplasms: life cycle and sexual stages. Advances in Parasitology 23, 37-103.

MELTZER, M.I., 1996. A possible explanation of the apparent breed-related resistance in cattle to bont tick (*Amblyomma hebraeum*) infestations. Veterinary Parasitology 67, 275-279.

MELTZER, M.I., NORVAL, R.A.I., DONACHIE, P.L., 1995. Effects of tick infestation and tick-borne disease infections (heartwater, anaplasmosis and babesiosis) on the lactation and weight gain of mashona cattle in South-Eastern Zimbabwe. Tropical Animal Health and Production 27, 129-144.

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, 8575-8579.

MINAMI, T., SPOONER, P.R., IRVIN, A.D., OCAMA, J.G., DOBBELAERE, D.A., FUJINAGA, T., 1983. Characterisation of stocks of *Theileria parva* by monoclonal antibody profiles. Research in Veterinary Science 35, 334-340.

MINJAUW, B., MCLEOD, A., 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, 116 pp.

MOLAD, T., BRAYTON, K.A., PALMER, G.H., MICHAELI, S., SHKAP, V., 2004. Molecular conservation of MSP4 and MSP5 in *Anaplasma marginale* and *A*. *centrale* vaccine strain. Veterinary Microbiology 100, 55-64.

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, 55-62.

MOLL, G., LOHDING, A., YOUNG, A.S., LEITCH, B.L., 1986. Epidemiology of theileriosis in calves in an endemic area of Kenya. Veterinary Parasitology 19, 255-273.

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., 1998a. Evaluation of an ELISA for detection of antibodies to *Babesia bovis* in cattle in Australia and Zimbabwe. Preventive Veterinary Medicine 33, 59-67.

MOLLOY, J.B., BOWLES, P.M., JESTON, P.J., BRUYERES, A.G., BOWDEN, J.M., BOCK, R.E., JORGENSEN, W.K., BLIGHT, G.W., DALGLIESH, R.J., 1998b. Development of an enzyme-linked immunosorbent assay for detection of antibodies to *Babesia bigemina* in cattle. Parasitology Research 84, 651-656.

MONTENEGRO-JAMES, S., GUILLEN, A.T., MA, S.J., TAPANG, P., ABDEL-GAWAD, A., TORO, M., RISTIC, M., 1990. Use of the dot enzyme-linked immunosorbent assay with isolated *Anaplasma marginale* initial bodies for serodiagnosis of anaplasmosis in cattle. American Journal of Veterinary Research 51, 1518-1521.

MOREL, P.C., UILENBERG, G., 1981. The nomenclature of some *Theileria* species (Sporozoa, Babesioidea) of domestic ruminants. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux 34, 139-143.

MORRISON, W.I., BUSCHER, G., MURRAY, M., EMERY, D.L., MASAKE, R.A., COOK, R.H., WELLS, P.W., 1981. *Theileria parva*: Kinetics of infection in the lymphoid system of cattle. Experimental Parasitology 52, 248-260.

MORRISON, W.I., GODDEERIS, B.M., TEALE, A.J., GROOCOCK, C.M., KEMP, S.J., STAGG, D.A., 1987. Cytotoxic T-cells elicited in cattle challenged with *Theileria parva* (Muguga): evidence for restriction by class I MHC determinants and parasite strain specificity. Parasite Immunology 9, 563-578.

MORRISON, W.I., MACHUGH, N.D., LALOR, P.A., 1996. Pathogenicity of *Theileria parva* is influenced by the host cell type infected by the parasite. Infection and Immunity 64, 557-562.

MUBIRU, D.N., 2010. Climate change and adaptation options in Karamoja. European Commission and Food and Agriculture Organisation of the United Nations, 62 pp. Retrieved May 9, 2014, from www.fao.org/fileadmin/user_upload/drought/docs/Karamoja_Climate_Change_and_Adaptation_Options.pdf.

MUGABI, K.N., MUGISHA, A., OCAIDO, M., 2010. Socio-economic factors influencing the use of acaricides on livestock: a case study of the pastoralist communities of Nakasongola District, Central Uganda. Tropical Animal Health and Production 42, 131-136.

MUGISHA, A., MCLEOD, A., PERCY, R., KYEWALABYE, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

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.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010a. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

MUHANGUZI, D., MATOVU, E., WAISWA, C., 2010b. Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. International Journal of Animal and Veterinary Advances 2, 51-58.

MUHANGUZI, D., OKELLO, W.O., KABASA, J.D., WAISWA, C., WELBURN, S.C., SHAW, A.P.M., 2015. Cost analysis of options for management of African Animal Trypanosomiasis using interventions targeted at cattle in Tororo District; south-eastern Uganda. Parasites & Vectors 8, 387.

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, 91.

MUKHEBI, A.W., 1992. Economic importance of theileriosis and its control in Africa. In: Epidemiology of theileriosis in Africa, NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S. (Eds.), Academic Press, London, pp. 379 – 403.

MUKHEBI, A.W., CHAMBOKO, T., O'CALLAGHAN, C.J., PETER, T.F., KRUSKA, R.L., MEDLEY, G.F., MAHAN, S.M., PERRY, B.D., 1999. An assessment of the economic impact of heartwater (*Cowdria ruminantium* infection) and its control in Zimbabwe. Preventive Veterinary Medicine 39, 173-189.

MULEYA, W., NAMANGALA, B., SIMUUNZA, M., NAKAO, R., INOUE, N., KIMURA, T., ITO, K., SUGIMOTO, C., SAWA, H., 2012. Population genetic analysis and sub-structuring of *Theileria parva* in the northern and eastern parts of Zambia. Parasites & Vectors 5, 255.

MÜLLER, J., HEMPHILL, A., 2013. *In vitro* culture systems for the study of apicomplexan parasites in farm animals. International Journal for Parasitology 43, 115-124.

MURAGURI, G.R., KIARA, H.K., MCHARDY, N., 1999. Treatment of East Coast fever: a comparison of parvaquone and buparvaquone. Veterinary Parasitology 87, 25-37.

MURAGURI, G.R., MCLEOD, A., MCDERMOTT, J.J., TAYLOR, N., 2005. The incidence of calf morbidity and mortality due to vector-borne infections in smallholder dairy farms in Kwale District, Kenya. Veterinary Parasitology 130, 305-315.

MUSISI, F.L., JONGEJAN, F., PEGRAM, R.G., 1984. Isolation and transmission of *Theileria mutans* (Chisamba) in Zambia. Research in Veterinary Science 36, 129-131.

MUSOKE, A., ROWLANDS, J., NENE, V., NYANJUI, J., KATENDE, J., SPOONER, P., MWAURA, S., ODONGO, D., NKONGE, C., MBOGO, S., BISHOP, R., MORZARIA, S., 2005. Subunit vaccine based on the p67 major surface protein of *Theileria parva* sporozoites reduces severity of infection derived from field tick challenge. Vaccine 23, 3084-3095.

MUSOKE, R.A., TWEYONGYERE, R., BIZIMENYERA, E., WAISWA, C., MUGISHA, A., BIRYOMUMAISHO, S., MCHARDY, N., 2004. Treatment of East Coast fever of cattle with a combination of parvaquone and frusemide. Tropical Animal Health and Production 36, 233-245.

MUTSHEMBELE, A.M., CABEZAS-CRUZ, A., MTSHALI, M.S., THEKISOE, O.M.M., GALINDO, R.C., DE LA FUENTE, J., 2014. Epidemiology and evolution of the genetic variability of *Anaplasma marginale* in South Africa. Ticks and Tick-borne Diseases 5, 624-631.

MUWANIKA, V., KABI, F., MASEMBE, C., 2016. Population genetic structure of *Theileria parva* field isolates from indigenous cattle populations of Uganda. Ticks and Tick-borne Diseases 7, 291-297.

MWAI, O., HANOTTE, O., KWON, Y., CHO, S., 2015. African indigenous cattle: unique genetic resources in rapidly changing world. Asian-Australasian Journal of Animal Sciences 28, 911-921.

MWEBAZE, S.M.N., 2006. Country pasture/forage resource profiles, Uganda. Food and Agriculture Organization of the United Nations (FAO), 17 pp. Retrieved June 21, 2015, from www.fao.org/ag/agp/AGPC/doc/Counprof/PDF files/Uganda_English.pdf.

MWEGA, E., HASAN, S.D., MOSES, N., ELPIDIUS, R., SKILTON, R., GWAKISA, P., 2015. Genetic and antigenic diversity of *Theileria parva* in cattle in eastern and southern zones of Tanzania. A study to support control of East Coast fever. Parasitology 142, 698-705.

NABUKENYA, I., RUBAIRE-AKIIKI, C., OLILA, D., IKWAP, K., HÖGLUND, J., 2014. Ethnopharmacological practices by livestock farmers in Uganda: Survey experiences from Mpigi and Gulu districts. Journal of Ethnobiology and Ethnomedicine 10, 9.

NAKAO, R., MORRISON, L.J., ZHOU, L., MAGONA, J.W., JONGEJAN, F., SUGIMOTO, C., 2012. Development of multiple-locus variable-number tandem-repeat analysis for rapid genotyping of *Ehrlichia ruminantium* and its application to infected *Amblyomma variegatum* collected in heartwater endemic areas in Uganda. Parasitology 139, 69-82.

NAKAO, R., STROMDAHL, E., MAGONA, J.W., FABURAY, B., NAMANGALA, B., MALELE, I., INOUE, N., GEYSEN, D., KAJINO, K., JONGEJAN, F., 2010. Development of loop-mediated isothermal amplification (LAMP) assays for rapid detection of *Ehrlichia ruminantium*. BMC Microbiology 10, 296.

NALUBWAMA, S.M., MUGISHA, A., VAARST, M., 2011. Organic livestock production in Uganda: potentials, challenges and prospects. Tropical Animal Health and Production 43, 749-757.

NEITZ, A.W., VILJOEN, G.J., BEZUIDENHOUT, J.D., OBEREM, P.T., VISSER, L., VERMEULEN, N.M., 1986. Detection of *Cowdria ruminantium* antigen and antibody during the course of heartwater disease in sheep by means of an enzyme-linked immunosorbent assay. The Onderstepoort Journal of Veterinary Research 53, 205-207.

NEITZ, W.O., 1955. Corridor disease: a fatal form of bovine theileriosis encountered in Zululand. Bulletin of Epizootic Diseases of Africa 3, 121-123.

NEVILS, M.A., FIGUEROA, J.V., TURK, J.R., CANTO, G.J., LE, V., ELLERSIECK, M.R., CARSON, C.A., 2000. Cloned lines of *Babesia bovis* differ in their ability to induce cerebral babesiosis in cattle. Parasitology Research 86, 437-443.

NIJHOF, A.M., PILLAY, V., STEYL, J., PROZESKY, L., STOLTSZ, W.H., LAWRENCE, J.A., PENZHORN, B.L., JONGEJAN, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. Journal of Clinical Microbiology 43, 5907-5911.

NORVAL, R.A., PERRY, B.D., HARGREAVES, S.K., 1992a. Tick and tick-borne disease control in Zimbabwe: what might the future hold? Zimbabwe Veterinary Journal 23, 1-15.

NORVAL, R.A.I., 1994. Vectors: ticks. In: Infectious diseases of livestock with special reference to Southern Africa, COETZER, J.A.W., THOMSON, G.R., TUSTIN, R.C. (Eds.), Volume 1, Oxford University Press Southern Africa, Cape Town, South Africa, 3-24.

NORVAL, R.A.I., DONACHIE, P.L., MELTZER, M.I., DEEM, S.L., MAHAN, S.M., 1995. The relationship between tick (*Amblyomma hebraeum*) infestation and immunity to heartwater (*Cowdria ruminantium* infection) in calves in Zimbabwe . Veterinary Parasitology 58, 335-352.

NORVAL, R.A.I., PERRY, B.D., GEBREAB, F., LESSARD, P., 1991. East Coast fever: a problem of the future for the horn of Africa? Preventive Veterinary Medicine 10, 163-172.

NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S., LAWRENCE, J.A., MUKHEBI, A.W., BISHOP, R.P., MCKEEVER, D.J., SHAW, M.K., 1992b. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

NOTOMI, T., OKAYAMA, H., MASUBUCHI, H., YONEKAWA, T., WATANABE, K., AMINO, N., HASE, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 28, e63.

NYIKA, A., BARBET, A.F., BURRIDGE, M.J., MAHAN, S.M., 2002. DNA vaccination with *map1* gene followed by protein boost augments protection against challenge with *Cowdria ruminantium*, the agent of heartwater. Vaccine 20, 1215-1225.

NYSSEN, J., DESCHEEMAEKER, K., ZENEBE, A., POESEN, J., DECKERS, J., HAILE, M., 2009. Transhumance in the Tigray highlands (Ethiopia). Mountain Research and Development 29, 255-264.

OCAIDO, M., MUWAZI, R.T., OPUDA, J.A., 2009. Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. Tropical Animal Health and Production 41, 731-739.

OCAIDO, M., OTIM, C.P., OKUNA, N.M., ERUME, J., SSEKITTO, C., WAFULA, R.Z.O., KAKAIRE, D., WALUBENGO, J., MONRAD, J., 2005. Socio-economic and livestock disease survey of agro-pastoral communities in Serere County, Soroti District, Uganda. Livestock Research for Rural Development Volume 17 (8), Article #93. Retrieved March 4, 2013, from http://www.lrrd.org/lrrd17/8/ocai17093.htm.

OCHANDA, H., 2006. Comparison of the survival of *Theileria parva*-infected adult *Rhipicephalus appendiculatus* (Acari: Ixodidae) and their infection under simulated climate conditions in the laboratory and in the field. International Journal of Tropical Insect Science 26, 101-107.

ODONGO, D.O., OURA, C.A.L., SPOONER, P.R., KIARA, H., MBURU, D., HANOTTE, O.H., BISHOP, R.P., 2006. Linkage disequilibrium between alleles at highly polymorphic mini-and micro-satellite loci of *Theileria parva* isolated from cattle in three regions of Kenya. International Journal for Parasitology 36, 937-946.

OHASHI, N., RIKIHISA, Y., UNVER, A., 2001. Analysis of transcriptionally active gene clusters of major outer membrane protein multigene family in *Ehrlichia canis* and *E. chaffeensis*. Infection and Immunity 69, 2083-2091.

OIE, 2015a. Bovine anaplasmosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015. OIE World Organisation for Animal Health, Paris. Accessed from http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ on September 9, 2015,.

OIE, 2015b. Bovine babesiosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010. OIE World Organisation for Animal Health, Paris. Accessed from http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ on September 9, 2015,.

OKELLO-ONEN, J., MUKHEBI, A.W., TUKAHIRWA, E.M., MUSISI, G., BODE, E., HEINONEN, R., PERRY, B.D., OPUDA-ASIBO, J., 1998. Financial analysis of dipping strategies for indigenous cattle under ranch conditions in Uganda. Preventive Veterinary Medicine 33, 241-250.

OKELLO-ONEN, J., TUKAHIRWA, E.M., SSENYONGA, G.S.Z., PERRY, B.D., KATENDE, J.M., MUSISI, G., MWAYI, W.T., 1994. Epidemiology of *Theileria parva* under ranch conditions in Uganda. The Kenya Veterinarian 18, 362-365.

OLDS, C.L., MWAURA, S., ODONGO, D.O., SCOLES, G.A., BISHOP, R., DAUBENBERGER, C., 2016. Induction of humoral immune response to multiple recombinant *Rhipicephalus appendiculatus* antigens and their effect on tick feeding success and pathogen transmission. Parasites & Vectors 9, 484.

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, 108-120.

ONONO, J.O., WIELAND, B., RUSHTON, J., 2013. Constraints to cattle production in a semiarid pastoral system in Kenya. Tropical Animal Health and Production 45, 1415-1422.

OOSHIRO, M., ZAKIMI, S., MATSUKAWA, Y., KATAGIRI, Y., INOKUMA, H., 2008. Detection of *Anaplasma bovis* and *Anaplasma phagocytophilum* from cattle on Yonaguni Island, Okinawa, Japan. Veterinary Parasitology 154, 360-364.

OOSTHUIZEN, M.C., ALLSOPP, B.A., TROSKIE, M., COLLINS, N.E., PENZHORN, B.L., 2009. Identification of novel *Babesia* and *Theileria* species in South African giraffe (*Giraffa camelopardalis*, Linnaeus, 1758) and roan antelope (*Hippotragus equinus*, Desmarest 1804). Veterinary Parasitology 163, 39-46.

OTIM, C.P., OCAIDO, M., OKUNA, N.M., ERUME, J., SSEKITTO, C., WAFULA, R.Z.O., KAKAIRE, D., WALUBENGO, J., OKELLO, A., MUGISHA, A., MONRAD, J., 2004. Disease and vector constraints affecting cattle production in pastoral communities of Ssembabule district, Uganda. Livestock Research for Rural Development Volume 16 (5), Article #35. Retrieved September 12, 2013, from http://www.lrrd.org/lrrd16/5/otim16035.htm.

OURA, C.A., BISHOP, R., WAMPANDE, E.M., LUBEGA, G.W., TAIT, A., 2004b. The persistence of component *Theileria parva* stocks in cattle immunized with the 'Muguga cocktail' live vaccine against East Coast fever in Uganda. Parasitology 129, 27-42.

OURA, C.A.L., ASIIMWE, B.B., WEIR, W., LUBEGA, G.W., TAIT, A., 2005. Population genetic analysis and sub-structuring of *Theileria parva* in Uganda. Molecular and Biochemical Parasitology 140, 229-239.

OURA, C.A.L., BISHOP, R., ASIIMWE, B.B., SPOONER, P., LUBEGA, G.W., TAIT, A., 2007. *Theileria parva* live vaccination: parasite transmission, persistence and heterologous challenge in the field. Parasitology 134, 1205-1213.

OURA, C.A.L., BISHOP, R.P., WAMPANDE, E.M., LUBEGA, G.W., TAIT, A., 2004a. Application of a reverse line blot assay to the study of haemoparasites in cattle in Uganda. International Journal for Parasitology 34, 603-613.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011a. Haemoparasite prevalence and *Theileria parva* strain diversity in Cape buffalo (*Syncerus caffer*) in Uganda. Veterinary Parasitology 175, 212-219.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011b. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

PALMER, G.H., BARBET, A.F., CANTOR, G.H., MCGUIRE, T.C., 1989. Immunization of cattle with the MSP-1 surface protein complex induces protection against a structurally variant *Anaplasma marginale* isolate. Infection and Immunity 57, 3666-3669.

PALMER, G.H., BROWN, W.C., RURANGIRWA, F.R., 2000. Antigenic variation in the persistence and transmission of the ehrlichia *Anaplasma marginale*. Microbes and Infection 2, 167-176.

PALMER, G.H., RURANGIRWA, F.R., KOCAN, K.M., BROWN, W.C., 1999. Molecular basis for vaccine development against the erhrlichial pathogen *Anaplasma marginale*. Parasitology Today 15, 281-286.

PALMER, G.H., BRAYTON, K.A., 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15, 1969-1975.

PALMER, G.H., RURANGIRWA, F.R., MCELWAIN, T.F., 2001. Strain composition of the ehrlichia *Anaplasma marginale* within persistently infected cattle, a mammalian reservoir for tick transmission. Journal of Clinical Microbiology 39, 631-635.

PALMER, G.H., WAGHELA, S.D., BARBET, A.F., DAVIS, W.C., MCGUIRE, T.C., 1987. Characterization of a neutralization-sensitive epitope on the Am 105 surface protein of *Anaplasma marginale*. International Journal for Parasitology 17, 1279-1285.

PALMER, G.H., EID, G., BARBET, A.F., MCGUIRE, T.C., MCELWAIN, T.F., 1994. The immunoprotective *Anaplasma marginale* major surface protein 2 is encoded by a polymorphic multigene family. Infection and Immunity 62, 3808-3816.

PAPLI, N., LANDT, O., FLEISCHER, C., KOEKEMOER, J.O., MANS, B.J., PIENAAR, R., JOSEMANS, A., ZWEYGARTH, E., POTGIETER, F., LATIF, A.A., 2011. Evaluation of a TaqMan real-time PCR for the detection of *Theileria parva* in buffalo and cattle. Veterinary Parasitology 175, 356-359.

PATARROYO, J.H., PRATES, A.A., TAVARES, C.A.P., MAFRA, C.L., VARGAS, M.I., 1995. Exoantigens of an attenuated strain of *Babesia bovis* used as a vaccine against bovine babesiosis. Veterinary Parasitology 59, 189-199.

PEGRAM, R.G., BANDA, D.S., 1990. Ecology and phenology of cattle ticks in Zambia: Development and survival of free-living stages. Experimental & Applied Acarology 8, 291-301.

PEIXOTO, C.C., MARCELINO, I., VACHIÉRY, N., BENSAID, A., MARTINEZ, D., CARRONDO, M.J.T., ALVES, P.M., 2005. Quantification of *Ehrlichia ruminantium* by real time PCR. Veterinary Microbiology 107, 273-278.

PERRY, B.D., CHAMBOKO, T., MAHAN, S.M., MEDLEY, G.F., MINJAUW, B., O'CALLAGHAN, C.J., PETER, T.F., 1998. The economics of integrated tick and tick-borne disease control on commercial farms in Zimbabwe. Zimbabwe Veterinary Journal 29, 21-29.

PERRY, B.D., RANDOLPH, T.F., 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Veterinary Parasitology 84, 145-168.

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, 107-120.

PESQUERA, C., PORTILLO, A., PALOMAR, A.M., OTEO, J.A., 2015. Investigation of tick-borne bacteria (*Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador. Parasites & Vectors 8, 46.

PETER, T.F., BURRIDGE, M.J., MAHAN, S.M., 2000. Competence of the African tortoise tick, *Amblyomma marmoreum* (Acari: Ixodidae), as a vector of the agent of heartwater (*Cowdria ruminantium*). Journal of Parasitology 86, 438-441.

PETERSON, P.H., NDUMU, D.B., KIWUWA, G.H., KYOMO, M.L., SEMAMBO, D.K.N., ROWLANDS, G.J., NAGDA, S.N., NAKIMBUGWE, H., 2004. Characteristics of Ankole Longhorn cattle and their production environments in south western Uganda: milk offtake and body measurements. Animal Genetic Resources Information, Food and Agriculture Organisation of the United Nations, Volume 34.

PIENAAR, R., POTGIETER, F.T., LATIF, A.A., THEKISOE, O.M.M., MANS, B.J., 2011. Mixed *Theileria* infections in free-ranging buffalo herds: implications for diagnosing *Theileria parva* infections in Cape buffalo (*Syncerus caffer*). Parasitology 138, 884-895.

PIENAAR, R., POTGIETER, F.T., LATIF, A.A., THEKISOE, O.M.M., MANS, B.J., 2014. Geographic distribution of *Theileria* sp. (buffalo) and *Theileria* sp. (bougasvlei) in Cape buffalo (*Syncerus caffer*) in southern Africa: implications for speciation. Parasitology 141, 411-424.

PIPANO, E., ALEKCEEV, E., GALKER, F., FISH, L., SAMISH, M., SHKAP, V., 2003. Immunity against *Boophilus annulatus* induced by the Bm86 (Tick-GARD) vaccine Experimental and Applied Acarology 29, 141-149.

PLOWRIGHT, W., 1998. Professional commitment to the success of the global rinderpest eradication campaign. Technical consultation on the global rinderpest eradication programme, FAO Rome, 28-30 September 1998.

POLIN, H., HOFNAGL, P., HAUNSCHMID, R., GRUBER, F., LADURNER, G., 2004. Molecular evidence of *Anaplasma phagocytophiulm* in *Ixodes ricinus* ticks and wild animals in Austria. Journal of Clinical Microbiology 42, 2285-2286.

POTGIETER, F.T., VAN RENSBURG, L., 1987b. Tick transmission of *Anaplasma centrale*. Onderstepoort Journal of Veterinary Research 54, 5-7.

POTGIETER, F.T., 1977. The life cycle of *Babesia bovis* and *Babesia bigemina* in ticks and cattle in South Africa. Thesis, Randse Afrikaanse Universiteit.

POTGIETER, F.T., STOLTSZ, W.H., 2004. Bovine anaplasmosis. In: Infectious diseases of livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 594-616.

POTGIETER, F.T., STOLTSZ, W.H., BLOUIN, E.F., ROOS, J.A., 1988. Corridor disease in South Africa: a review of the current status. Journal of the South African Veterinary Association 59, 155-160.

POTGIETER, F.T., VAN RENSBURG, L., 1987a. The persistence of colostral *Anaplasma* antibodies and incidence of *in utero* transmission of *Anaplasma* infections in calves under laboratory conditions. The Onderstepoort Journal of Veterinary Research 54, 557-560.

PRETORIUS, A., VAN KLEEF, M., COLLINS, N.E., TSHIKUDO, N., LOUW, E., FABER, F.E., VAN STRIJP, M.F., ALLSOPP, B.A., 2008. A heterologous prime/boost immunisation strategy protects against virulent *E. ruminantium* Welgevonden needle challenge but not against tick challenge. Vaccine 26, 4363-4371.

PRETTY, J.N., 1995. Participatory learning for sustainable agriculture. World Development 23, 1247-1263.

PRINE, K.C., HODGES, A.C., 2013. Entomology and Nematology: Featured Creatures. University of Florida. Accessed October 19, 2016, from www.entnemdept.ufl.edu/creatures/livestock/ticks/tropical_bont_tick.htm.

PROVOST, A., BEZUIDENHOUT, J.D., 1987. The historical background and global importance of heartwater. The Onderstepoort Journal of Veterinary Research 54, 165-169.

PROZESKY, L., BEZUIDENHOUT, J.D., PATERSON, C.L., 1986. Heartwater: an *in vitro* study of the ultrastructure of *Cowdria ruminantium*. The Onderstepoort Journal of Veterinary Research 53, 153-159.

PROZESKY, L., DU PLESSIS, J.L., 1985. The pathology of heartwater. I. A study of mice infected with the Welgevonden strain of *Cowdria ruminantium*. The Onderstepoort Journal of Veterinary Research 52, 71-79.

RADLEY, D.E., BROWN, C.G.D., BURRIDGE, M.J., CUNNINGHAM, M.P., KIRIMI, I.M., PURNELL, R.E., YOUNG, A.S., 1975a. East coast fever: 1. Chemoprophylactic immunization of cattle against *Theileria parva* (Muguga) and five theilerial strains. Veterinary Parasitology 1, 35-41.

RADLEY, D.E., BROWN, C.G.D., CUNNINGHAM, M.P., KIMBER, C.D., MUSISI, F.L., PAYNE, R.C., PURNELL, R.E., STAGG, S.M., YOUNG, A.S., 1975b. East coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of theilerial strains. Veterinary Parasitology 1, 51-60.

RADWAN, M.E.I., ALI, A.F., HAMIED, O.A., 2013. Epidemiological studies, molecular diagnosis of *Anaplasma marginale* in cattle and biochemical changes associated with it in Kaliobia Governorate. American Journal of Infectious Diseases and Microbiology 1, 46-49.

REEVES, J.D.I., SWIFT, B.L., 1977. Iatrogenic transmission of *Anaplasma marginale* in beef cattle (Protozoan diseases). Veterinary Medicine and Small Animal Clinician 72, 911-914.

REGITANO, L.C.A., PRAYAGA, K., 2010. Ticks and tick-borne diseases in cattle. In: Breeding for Disease Resistance in Farm Animals, BISHOP, S.C., AXFORD, R.F.E., NICHOLAS, F.W., OWENS, J.B. (Eds.), third edn, CAB International, pp. 295-313.

REINBOLD, J.B., COETZEE, J.F., HOLLIS, L.C., NICKELL, J.S., RIEGEL, C., OLSON, K.C., GANTA, R.R., 2010a. The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. Veterinary Microbiology 145, 69-75.

REINBOLD, J.B., COETZEE, J.F., SIRIGIREDDY, K.R., GANTA, R.R., 2010b. 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, 2424-2432.

RICHEY, E.J., PALMER, G.H., 1990. Bovine anaplasmosis. Compendium on Continuing Education for the Practicing Veterinarian 12, 1661-1668.

RIKIHISA, Y., ZHANG, C., CHRISTENSEN, B.M., 2003. Molecular characterization of *Aegyptianella pullorum* (Rickettsiales, Anaplasmataceae). Journal of Clinical Microbiology 41, 5294-5297.

ROBY, T.O., MAZZOLA, V., 1972. Elimination of the carrier state of bovine anaplasmosis with imidocarb. American Journal of Veterinary Research 33, 1931-1933.

RODRIGUEZ, R.I., TREES, A.J., 1996. *In vitro* responsiveness of *Babesia bovis* to imidocarb dipropionate and the selection of a drug-adapted line. Veterinary Parasitology 62, 35-41.

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, 8.

RUBAIRE-AKIIKI, C.M., OKELLO-ONEN, J., MUSUNGA, D., KABAGAMBE, E.K., VAARST, M., OKELLO, D., OPOLOT, C., BISAGAYA, A., OKORI, C., BISAGATI, C., ONGYERA, S., MWAYI, M.T., 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, 251-266.

RYMASZEWSKA, A., GRENDA, S., 2008. Bacteria of the genus *Anaplasma*-characteristics of *Anaplasma* and their vectors: a review. Veterinarni Medicina 53, 573-584.

SALIH, D.A., EL HUSSEIN, A.R.M., AHMED, J., SEITZER, U., 2010. Comparison between reverse line blot and enzyme-linked immunosorbent assay in diagnosis of major tick-borne diseases of cattle in Southern Sudan. Transboundary and Emerging diseases 57, 61-62.

SAMISH, M., PIPANO, E., HADANI, A., 1993. Intrastadial and interstadial transmission of *Anaplasma marginale* by *Boophilus annulatus* ticks in cattle. American Journal of Veterinary Research 54, 411-414.

SANTOS, P.S., SENA, A.A.S., NASCIMENTO, R., ARAÚJO, T.G., MENDES, M.M., MARTINS, J.R.S., MINEO, T.W.P., MINEO, J.R., GOULART, L.R., 2013. Epitope-based vaccines with the *Anaplasma marginale* MSP1a functional motif induce a balanced humoral and cellular immune response in mice. PLOS ONE 8 (4). e60311. doi:10.1371/journal.pone.0060311.

SCHNITTGER, L., RODRIGUEZ, A.E., FLORIN-CHRISTENSEN, M., MORRISON, D.A., 2012. *Babesia*: A world emerging. Infection, Genetics and Evolution 12, 1788-1809.

SCHOULS, L.M., VAN DE POL, I., RIJPKEMA, S.G.T., SCHOT, C.S., 1999. Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. Journal of Clinical Microbiology 37, 2215-2222.

SCOLES, G.A., UETI, M.W., PALMER, G.H., 2005b. Variation among geographically separated populations of *Dermacentor andersoni* (Acari: Ixodidae) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). Journal of Medical Entomology 42, 153-162.

SCOLES, G.A., BROCE, A.B., LYSYK, T.J., PALMER, G.H., 2005a. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* (Acari: Ixodidae) compared with mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology 42, 668-675.

SEMU, S.M., PETER, T.F., MUKWEDEYA, D., BARBET, A.F., JONGEJAN, F., MAHAN, S.M., 2001. Antibody responses to MAP 1B and other *Cowdria ruminantium* antigens are down regulated in cattle challenged with tick-transmitted heartwater. Clinical and Vaccine Immunology 8, 388-396.

SHITAKHA, V.M., NATULYA, V.M., MUSOKE, A.J., RAMASAMY, R., BUSCHER, G., 1983. Complement activation and fibrinolysis during infection with *Theileria parva* (East Coast fever) in cattle. Veterinary Immunology and Immunopathology 4, 361-373.

SHKAP, V., KOCAN, K., MOLAD, T., MAZUZ, M., LEIBOVICH, B., KRIGEL, Y., MICHOYTCHENKO, A., BLOUIN, E., DE LA FUENTE, J., SAMISH, M., MTSHALI, M., ZWEYGARTH, E., FLEIDEROVICH, E.L., FISH, L., 2009. Experimental transmission of field *Anaplasma marginale* and the *A. centrale* vaccine strain by *Hyalomma excavatum*, *Rhipicephalus saguineus* and *Rhipicephalus* (*Boophilus*) *annulatus* ticks. Veterinary Microbiology 134, 254-260.

SHKAP, V., DE VOS, A.J., ZWEYGARTH, E., JONGEJAN, F., 2007. Attenuated vaccines for tropical theileriosis, babesiosis and heartwater: the continuing necessity. Trends in Parasitology 23, 420-426.

SHKAP, V., LEIBOVITZ, B., KRIGEL, Y., HAMMERSCHLAG, J., MARCOVICS, A., FISH, L., MOLAD, T., SAVITSKY, I., MAZUZ, M., 2005. Vaccination of older *Bos taurus* bulls against bovine babesiosis. Veterinary Parasitology 129, 235-242.

SHKAP, V., MOLAD, T., BRAYTON, K.A., BROWN, W.C., PALMER, G.H., 2002. Expression of major surface protein 2 variants with conserved T-cell epitopes in *Anaplasma centrale* vaccinates. Infection and Immunity 70, 642-648.

SHKAP, V., PIPANO, E., 2000. Culture-derived parasites in vaccination of cattle against tick-borne diseases. Annals of the New York Academy of Sciences 916, 154-171.

SIBEKO, K.P., COLLINS, N.E., OOSTHUIZEN, M.C., TROSKIE, M., POTGIETER, F.T., COETZER, J.A.W., GEYSEN, D., 2011. Analyses of genes encoding *Theileria parva* p104 and polymorphic immunodominant molecule (PIM) reveal evidence of the presence of cattle-type alleles in the South African *T. parva* population. Veterinary Parasitology 181, 120-130.

SIBEKO, K.P., OOSTHUIZEN, M.C., COLLINS, N.E., GEYSEN, D., RAMBRITCH, N.E., LATIF, A.A., GROENEVELD, H.T., POTGIETER, F.T., COETZER, J.A.W., 2008. Development and evaluation of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. Veterinary Parasitology 155, 37-48.

SILVA, J.B., GONÇALVES, L.R., VARANI, A.M., ANDRÉ, M.R., MACHADO, R.Z., 2015. Genetic diversity and molecular phylogeny of *Anaplasma marginale* studied longitudinally under natural transmission conditions in Rio de Janeiro, Brazil. Ticks and Tick-borne diseases 6, 499-507.

SILVESTRE, B.T., RABELO, É.M.L., VERSIANI, A.F., DA FONSECA, F.G., SILVEIRA, J.A.G., BUENO, L.L., FUJIWARA, R.T., RIBEIRO, M.F.B., 2014. Evaluation of humoral and cellular immune response of BALB/c mice immunized with a recombinant fragment of MSP1a from *Anaplasma marginale* using carbon nanotubes as a carrier molecule. Vaccine 32, 2160-2166.

SIMUUNZA, M., WEIR, W., COURCIER, E., TAIT, A., SHIELS, B., 2011. Epidemiological analysis of tick-borne diseases in Zambia. Veterinary Parasitology 175, 331-342.

SIVAKUMAR, T., HAYASHIDA, K., SUGIMOTO, C., YOKOYAMA, N., 2014. Evolution and genetic diversity of *Theileria*. Infection, Genetics and Evolution 27, 250-263.

SKILTON, R.A., BISHOP, R.P., KATENDE, J.M., MWAURA, S., MORZARIA, S.P., 2002. The persistence of *Theileria parva* infection in cattle immunized using two stocks which differ in their ability to induce a carrier state: analysis using a novel blood spot PCR assay. Parasitology 124, 265-276.

SMITH, E.R., PARKER, D.M., 2010. Tick communities at the expanding wildlife/cattle interface in the Eastern Cape Province, South Africa: implications for Corridor disease. Journal of the South African Veterinary Association 81, 237-240.

SMITH, R.D., EVANS, D.E., MARTINS, J.R., CERESÉR, V.H., CORREA, B.L., PETRACCIA, C., CARDOZO, H., SOLARI, M.A., NARI, A., 2000. Babesiosis (*Babesia bovis*) stability in unstable environments. Annals of the New York Academy of Sciences 916, 510-520.

SNV, 2013a. Management of Livestock Drug Systems. Dairy Platform. Policy Brief 03. SNV Netherlands Development Organisation, 4 pp. Retrieved August 31, 2015, from http://www.snvworld.org/download/publications/ecf_brief_3_management_pdf.

SNV, 2013b. Policy Brief on Management of Ticks and East Coast fever. SNV Netherlands Development Organisation, 6 pp. Retrieved December 20, 2015, from http://www.m.snvworld.org/en/countries/Uganda/publications/policy-brief-on-management-of-ticks-and-east-coast-fever.

SPARAGANO, O.A.E., 2005. Impact of ticks and tick-borne diseases on agriculture and human populations in Europe. Journal of Agricultural Science 143, 463-468.

SPERANZA, C.I., 2010. Drought coping and adaptation strategies: understanding adaptations to climate change in agro-pastoral livestock production in Makueni District, Kenya. European Journal of Development Research 22, 623-642.

SREEKUMAR, C., ANANDAN, R., BALASUNDARAM, S., RAJAVELU, G., 1996. Morphology and staining characteristics of *Ehrlichia bovis*. Comparative Immunology, Microbiology and Infectious Diseases 19, 79-83.

STEYN, H.C., PRETORIUS, A., MCCRINDLE, C.M.E., STEINMANN, C.M.L., VAN KLEEF, M., 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Veterinary Microbiology 131, 258-265.

STICH, R.W., KOCAN, K.M., PALMER, G.H., EWING, S.A., HAIR, J.A., BARRON, S.J., 1989. Transstadial and attempted transovarial transmission of *Anaplasma marginale* by *Dermacentor variabilis*. American Journal of Veterinary Research 50, 1377-1380.

STITES, E., MITCHARD, E., 2011. Milk Matters in Karamoja: Milk in Children's Diets and Household Livelihoods. Feinstein International Center, Tufts University, 200 Boston Ave., Suite 4800, Medford, MA 02155, USA, 35 pp. (http://www.fao.org/fileadmin/user_upload/drought/docs/MM-Karamoja.pdf. Accessed October 10, 2015.

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., BJOERSDORFF, A., STUEN, S., KNOWLES, D.P., 2007. Characterization of *Anaplasma phagocytophilum* major surface protein 5 and the extent of its cross-reactivity with *A. marginale*. Clinical and Vaccine Immunology 14, 262-268.

SUAREZ, C.E., NOH, S., 2011. Emerging perspectives in the research of bovine babesiosis and anaplasmosis. Veterinary Parasitology 180, 109-125.

SUTHERST, R.W., 2001. The vulnerability of animal and human health to parasites under global change. International Journal for Parasitology 31, 933-948.

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, 34-42.

SWAI, E.S., MBISE, A.N., KESSY, V., KAAYA, E., SANKA, P., LOOMU, P.M., 2005. Farm constraints, cattle disease perception and tick management practices in pastoral Maasai community-Ngorongoro, Tanzania. Livestock Research for Rural Development Volume 17 (2), Article #17. Retrieved June 16, 2015, from http://www.lrrd.org/lrrd17/2/swai17017.htm.

TATTIYAPONG, M., SIVAKUMAR, T., YBANEZ, A.P., YBANEZ, R.H.D., PEREZ, Z.O., GUSWANTO, A., IGARASHI, I., YOKOYAMA, N., 2014. Diversity of *Babesia bovis* merozoite surface antigen genes in the Philippines. Parasitology International 63, 57-63.

TAY, S.T., KOH, F.X., KHO, K.L., ONG, B.L., 2014. Molecular survey and sequence analysis of *Anaplasma* spp. in cattle and ticks in a Malaysian farm. Tropical Biomedicine 31, 769-776.

THEILER, A., 1906. *Piroplasma mutans* (n. spec) of South African cattle. Journal of Comparative Pathology and Therapeutics 19, 292-300.

THEILER, A., 1910. *Anaplasma marginale* (gen.and spec. nov.): the marginal points in the blood of cattle suffering from a specific disease. Report of the Government Veterinary Bacteriologist for the year 1908-1909. Department of Agriculture, South Africa, 7-64.

THEILER, A., 1911. Further investigations into anaplasmosis of South African cattle. First report of the Director of Veterinary Research. Department of Agriculture of the Union of South Africa, 7-46.

TORINA, A., MORENO-CID, J.A., BLANDA, V., DE MERA, I.G.F., DE LA LASTRA, J.M.P., SCIMECA, S., BLANDA, M., SCARIANO, M.E., BRIGANÒ, S., DISCLAFANI, R., PIAZZA, A., VICENTE, J., GORTÁZAR, C., CARACAPPA, S., LELLI, R.C., DE LA FUENTE, J., 2014. Control of tick infestations and pathogen prevalence in cattle and sheep farms vaccinated with the recombinant Subolesin-Major Surface Protein 1a chimeric antigen. Parasites & Vectors 7, 10.

TOYE, P.G., GODDEERIS, B.M., IAMS, K., MUSOKE, A.J., MORRISON, W.I., 1991. Characterization of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva*. Parasite Immunology 13, 49-62.

TRUEMAN, K.F., BLIGHT, G.W., 1978. The effect of age on resistance of cattle to *Babesia bovis*. Australian Veterinary Journal 54, 301-305.

UBOS, 2006. Uganda National Household Survey 2005/2006. Report on the Socio-economic module. Uganda Bureau of Statistics, Retrieved May 4, 2013, from www.ubos.org/onlinefiles/uploads/ubos/pdf documents/UNHSReport20052006.pdf.

UBOS, 2007. Statistical Abstract. Uganda Bureau of Statistics. Retrieved March 6, 2013, from www.ubos.org/onlinefiles/uploads/ubos/pdf documents/abstracts/Statistical Abstract 2007.pdf.

UBOS, 2014. Statistical Abstract. Uganda Bureau of Statistics. www.ubos.org/onlinefiles/uploads/ubos/statistical_abstracts/Statistical_Abstract_2014.pdf. Accessed June 21, 2015.

UETI, M.W., REAGAN, J.O., KNOWLES, D.P., SCOLES, G.A., SHKAP, V., PALMER, G.H., 2007. Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of *Anaplasma marginale*. Infection and Immunity 75, 2959-2964.

UETI, M.W., TAN, Y., BROSCHAT, S.L., CASTAÑEDA-ORTIZ, E.J., CAMACHO-NUEZ, M., MOSQUEDA, J.J., SCOLES, G.A., GRIMES, M., BRAYTON, K.A., PALMER, G.H., 2012. Expansion of variant diversity associated with a high prevalence of pathogen strain superinfection under conditions of natural transmission. Infection and Immunity 80, 2354-2360.

UILENBERG, G., 1983. Heartwater (*Cowdria ruminantium* infection): current status. Advances in Veterinary Science and Comparative Medicine 27, 427-480.

UILENBERG, G., 1999. Immunization against diseases caused by *Theileria parva*: a review. Tropical Medicine and International Health 4, A12-A20.

UILENBERG, G., 2006. *Babesia* - A historical overview. Veterinary Parasitology 138, 3-10.

UILENBERG, G., PERIÉ, N.M., SPANJER, A.A.M., FRANSSEN, F., 1985. *Theileria orientalis*, a cosmopolitan blood parasite of cattle: Demonstration of the schizont stage. Research in Veterinary Science 38, 352-360.

VALENZUELA, J.G., 2004. Exploring tick saliva: from biochemistry to 'sialomes' and functional genomics. Parasitology 129, S83-S94.

VAN DE PYPEKAMP, H.E., PROZESKY, L., 1987. Heartwater. An overview of the clinical signs, susceptibility and differential diagnoses of the disease in domestic ruminants. The Onderstepoort Journal of Veterinary Research 54, 263-266.

VAN HEERDEN, H., COLLINS, N.E., BRAYTON, K.A., RADEMEYER, C., ALLSOPP, B.A., 2004. Characterization of a major outer membrane protein multigene family in *Ehrlichia ruminantium*. Gene 330, 159-168.

VAN VLIET, A.H., VAN DER ZEIJST, B.A., CAMUS, E., MAHAN, S.M., MARTINEZ, D., JONGEJAN, F., 1995. Use of a specific immunogenic region on the *Cowdria ruminantium* MAP1 protein in a serological assay. Journal of Clinical Microbiology 33, 2405-2410.

VISSER, E.S., MCGUIRE, T.C., PALMER, G.H., DAVIS, W.C., SHKAP, V., PIPANO, E., KNOWLES, D.P., 1992. The *Anaplasma marginale* msp5 gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infection and Immunity 60, 5139-5144.

WALKER, A.R., BOUATTOUR, A., CAMICAS, J.-L., ESTRADA-PEÑA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G., PRESTON, P.M., 2013. Ticks of domestic animals in Africa: a guide to identification of species. International Consortium on Ticks and Tick Borne Diseases (ICTTD-2), 221 pp.

WALKER, J.B., OLWAGE, A., 1987. The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. The Onderstepoort Journal of Veterinary Research 54, 353-379.

WALKER, J.G., KLEIN, E.Y., LEVIN, S.A., 2014. Disease at the wildlife-livestock interface: Acaricide use on domestic cattle does not prevent transmission of a tick-borne pathogen with multiple hosts. Veterinary Parasitology 199, 206-214.

WATTS, J.G., PLAYFORD, M.C., HICKEY, K.L., 2016. *Theileria orientalis*. New Zealand Veterinary Journal 64, 3-9.

WILLADSEN, P., 2006. Vaccination against ectoparasites. Parasitology 133, S9-S25.

WOOLHOUSE, M.E.J., THUMBI, S.M., JENNINGS, A., CHASE-TOPPING, M., CALLABY, R., KIARA, H., OOSTHUIZEN, M.C., MBOLE-KARIUKI, M.N., CONRADIE, I., HANDEL, I.G., POOLE, E.J., NJIIRI, E., COLLINS, N.E., MURRAY, G., TAPIO, M., AUGUET, O.T., WEIR, W., MORRISON, W.I., KRUUK, L.E.B., BRONSVOORT, B.M.C., HANOTTE, O., COETZER, K., TOYE, P.G., 2015. Co-infections determine patterns of mortality in a population exposed to parasite infection. Science Advances 1 (2), e1400026. DOI: 10.1126/sciadv.1400026.

WRIGHT, I.G., GOODGER, B.V., LEATCH, G., AYLWARD, J.H., RODE-BRAMANIS, K., WALTISBUHL, D.J., 1987. Protection of *Babesia bigemina*-immune animals against subsequent challenge with virulent *Babesia bovis*. Infection and Immunity 55, 364-368.

YOUNG, A.S., LEITCH, B.L., NEWSON, R.M., CUNNINGHAM, M.P., 1986. Maintenance of *Theileria parva parva* infection in an endemic area of Kenya. Parasitology 93, 9-16.

ZAUGG, J.L., KUTTLER, K.L., 1985. *Anaplasma marginale* infections in American bison: experimental infection and serologic study. American Journal of Veterinary Research 46, 438-441.

ZINTL, A., GRAY, J.S., SKERRETT, H.E., MULCAHY, G., 2005. Possible mechanisms underlying age-related resistance to bovine babesiosis. Parasite Immunology 27, 115-120.

ZINTL, A., MULCAHY, G., SKERRETT, H.E., TAYLOR, S.M., GRAY, J.S., 2003. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clinical Microbiology Reviews 16, 622-636.

ZWANE, A.A., MAIWASHE, A., MAKGAHLELA, M.L., CHOUNDHURY, A., TAYLOR, J.F., VAN MARLE-KÖSTER, E., 2016. Genome-wide identification of breed-informative single-nucleotide polymorphisms in three South African indigenous cattle breeds. South African Journal of Animal Science 46, 302-312.

ZWEYGARTH, E., JOSEMANS, A.I., VAN STRIJP, M.F., LOPEZ-REBOLLAR, L., VAN KLEEF, M., ALLSOPP, B.A., 2005. An attenuated *Ehrlichia ruminantium* (Welgevonden stock) vaccine protects small ruminants against virulent heartwater challenge. Vaccine 23, 1695-1702.

CHAPTER 3

3. Using participatory epidemiology to investigate management options and relative importance of tick-borne diseases among transhumant Zebu cattle in Karamoja Region, Uganda

3.1 Abstract

A participatory epidemiological (PE) study was conducted with livestock keepers in Moroto and Kotido Districts, Karamoja Region, Uganda, between October and December 2013 to determine the management options and relative importance of tick-borne diseases (TBDs) among transhumant zebu cattle. Data collection involved 24 focus group discussions (each comprising 8 to 12 people) in 24 settlement areas (*manyattas*), key informant interviews (30), direct observations, a review of surveillance data, clinical examinations, and laboratory confirmation of cases of TBDs. Methods used in group discussions included semi-structured interviews, simple ranking, pairwise ranking, matrix scoring, proportional piling and participatory mapping. The results of pairwise comparison showed the Ngakarimojong-named diseases, *lokit* (East Coast fever, ECF), *lopid* (anaplasmosis), *loukoi* (contagious bovine pleuropneumonia, CBPP), *lokou* (heartwater) and *lokulam* (babesiosis), were considered the most important cattle diseases in Moroto in that order, while ECF, anaplasmosis, trypanosomosis (*ediit*), CBPP and nonspecific diarrhoea (*loleo*) were most important in Kotido. Strong agreement between informant groups (Kendall's coefficient of concordance W = 0.568 and 0.682; $p < 0.001$) in pairwise ranking indicated that the diseases were a common problem in selected districts. East Coast fever had the highest median score for incidence (18% [range: 2, 33]) in Moroto, followed by anaplasmosis (17.5% [8, 32]) and CBPP (9% [1, 21]). Most animals that suffered from ECF, anaplasmosis, heartwater and babesiosis died, as the respective median scores for case fatality rates (CFR) were 89.5% (42, 100), 82.8% (63, 100), 66.7% (20, 100) and 85.7% (0, 100). In Kotido, diseases with high incidence scores were ECF (21% [6, 32]), anaplasmosis (17% [10, 33]) and trypanosomosis (8% [2, 18]). The CFRs for ECF and anaplasmosis were 81.7% (44, 100) and 70.7% (48, 100), respectively. Matrix scoring revealed that disease indicators showed strong agreement (W = 0.382 - 0.659, $p < 0.05$ - $p <$

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0.001) between informant groups. Inadequate knowledge, poor veterinary services and limited availability of drugs were the main constraints that hindered the control of TBDs. Hand picking of ticks was done by all pastoralists while hand spraying with acaricides was irregular, often determined by availability of drug supplies and money. It was concluded that TBDs, particularly ECF and anaplasmosis were important diseases in this pastoral region. Results from this study may assist in the design of feasible control strategies.

3.2 Introduction

Tick-borne diseases (TBDs) are reported to be the major limitation to cattle production systems in Africa (Malak et al., 2012; Onono et al., 2013). They cause economic losses to farmers through cattle mortality, loss of body weight, milk loss, and control costs through chemotherapy, vaccination, and control of ticks by use of acaricides (Kivaria, 2006; Ocaido et al., 2009a). In Tanzania, the total annual national loss due to TBDs was estimated to be US $364 million (Kivaria, 2006). In Uganda, ticks and TBDs accounted for 75.4% of losses in cattle (Ocaido et al., 2009b), and the costs for control constituted 86% of the total disease control costs in cattle (Ocaido et al., 2009a). Although the effects of TBDs are more pronounced in exotic cattle, they are also a problem in indigenous cattle, especially in situations of endemic instability (Norval et al., 1992).

Pastoralist communities in Africa live in some of the least developed and harshest environments in the world. In these communities, livestock contribute significantly to the social and economic well-being of people (Ocaido et al., 2009a; Onono et al., 2013). In Karamoja Region, the livelihoods of the communities are highly dependent on livestock (IICD, 2010). Cattle in the region are of the short-horned East African zebu type (*Bos indicus*) and make up a relatively large percentage (19.8%) of Uganda's cattle population (MAAIF, 2011). Grazing is extensive on communal land and there is no supplementary feeding. Due to cycles of drought, transhumance is the livestock management system practiced. In the wet season, cattle enclosures ("kraals") are closer to the permanent settlement zone, taking advantage of temporary pools for watering. In the dry season, pastoralists move their animals to neighbouring districts and countries in search of pastures and water (Anderson and Robinson, 2009). Despite the importance of livestock in the region, there is little information from

systematic studies regarding cattle diseases. Local knowledge of pastoralists about the relative importance of cattle diseases, husbandry, and control practices is not fully exploited.

The use of conventional veterinary research and surveillance in pastoral areas is constrained because pastoralists often live in trans-boundary ecosystems and routinely cross national borders to access grazing areas. Furthermore, government veterinary services are poorly funded and the areas are not attractive to veterinarians (Shiferaw et al., 2010). In these areas, insecurity due to cattle raids is common (Chenyambuga et al., 2010), as exemplified by conflicts in South Sudan, Somalia, northern Kenya, eastern Ethiopia and the Karamoja Region of Uganda. As a result, veterinarians have to make best use of local knowledge and experience from the pastoralists, who are valuable sources of disease information (Catley et al., 2002; Catley et al., 2012). Participatory epidemiology (PE) is the systematic use of participatory approaches and methods, with the involvement of communities in defining and prioritising veterinary-related problems, and in the development of solutions to service delivery, disease control or surveillance (Catley et al., 2012). In recent years, PE methods have been adapted by epidemiologists to improve understanding of livestock diseases and impact on pastoralists' livelihoods, for example in Ethiopia and South Sudan (Malak et al., 2012; Catley et al., 2014). This approach is a relatively cheap and rapid way to generate information in marginalised resource-poor areas (Malak et al., 2012; Catley et al., 2014). Compared with conventional questionnaires, PE methods create an open and dynamic interaction between the pastoralists and the researchers, who act as facilitators. Within groups, different people offer ideas that are discussed and refined until the group reaches a collective decision (Catley et al., 2002). Moreover, PE methods such as informal interviewing, visualisation and ranking or scoring reduce non-sampling errors (e.g. badly-phrased or insensitive questions), because there is no need for the pastoralists to state the number of animals owned (Catley et al., 2014). Standardisation and repetition of the method allows for some quantification and statistical analysis (Catley et al., 2014). Furthermore, PE uses triangulation to improve the validity of findings by cross-checking information collected through different methods and sources (Catley et al., 2012).

This study was undertaken to determine the relative importance of TBDs as constraints to cattle production among livestock keepers in the transhumant system of Karamoja Region, Uganda, using PE methods. Further objectives were to establish current control measures used by livestock owners for ticks and TBDs, and to determine the constraints to the control of ticks and TBDs in the region. This information may assist the design of appropriate animal health programmes, to enhance livestock productivity and improve livelihoods of pastoral communities.

3.3 Materials and Methods

3.3.1 Study area

This study was conducted in Moroto and Kotido Districts of Karamoja Region, north-eastern Uganda. The region covers 27,511 km^2 (about 10% of Uganda) and lies between longitudes 33° 30’ E to 35°E and latitudes 1° 30’N to 4°N. It is bordered by South Sudan to the north and Kenya to the east. Karamoja Region is divided into seven administrative districts that are, in turn, divided into sub-counties containing a number of parishes and smaller settlement areas (*manyattas*). The region has a human population of approximately 1,455,200 (UBOS, 2014). The climate is mostly semi-arid with cycles of drought. Rainfall is low (average 500-600 mm per year) with peak rainfall in April-May and July-September (Anderson and Robinson, 2009). Temperatures range from an average minimum of between 15°C and 18°C to an average maximum of between 28°C and 33°C (Anderson and Robinson, 2009). The geographical features include wide savannah plains (1,500 m above sea level) covered with seasonal grasses, thorny plants, and occasional small trees (IICD, 2010).

3.3.2 Selection of study locations

Two districts, Moroto and Kotido, were purposively selected to represent pastoral and agro-pastoral livelihood zones respectively as previously described (Anderson and Robinson, 2009). In each district, two sub-counties were randomly selected, using tabulated random numbers, from a sampling frame drawn up with the help of local leaders. The sampling units were *manyattas*, which are settlement areas occupied by clusters of families. Six *manyattas* were selected from each sub-county. From each *manyatta*, one informant group was mobilised for the interviews. Therefore, a total of 24 *manyattas*, comprising 24 informant groups (12 from

each district) were selected. The *manyattas* and informants were purposively identified in a participatory manner together with community leaders and community-based animal health workers (CAHWs). The informant groups comprised herd owners and cattle herders.

The grid location of each *manyatta* was geo-referenced using a GARMIN global positioning system (GPSMAP® 78s, Garmin International, Inc., USA). The GPS records were mapped using ArcGIS Version 10.2 (Environmental Systems Research Institute, 380 New York Street, Redlands, US). The mapped locations were overlaid onto the administrative map of Karamoja Region (Figure 3.1).

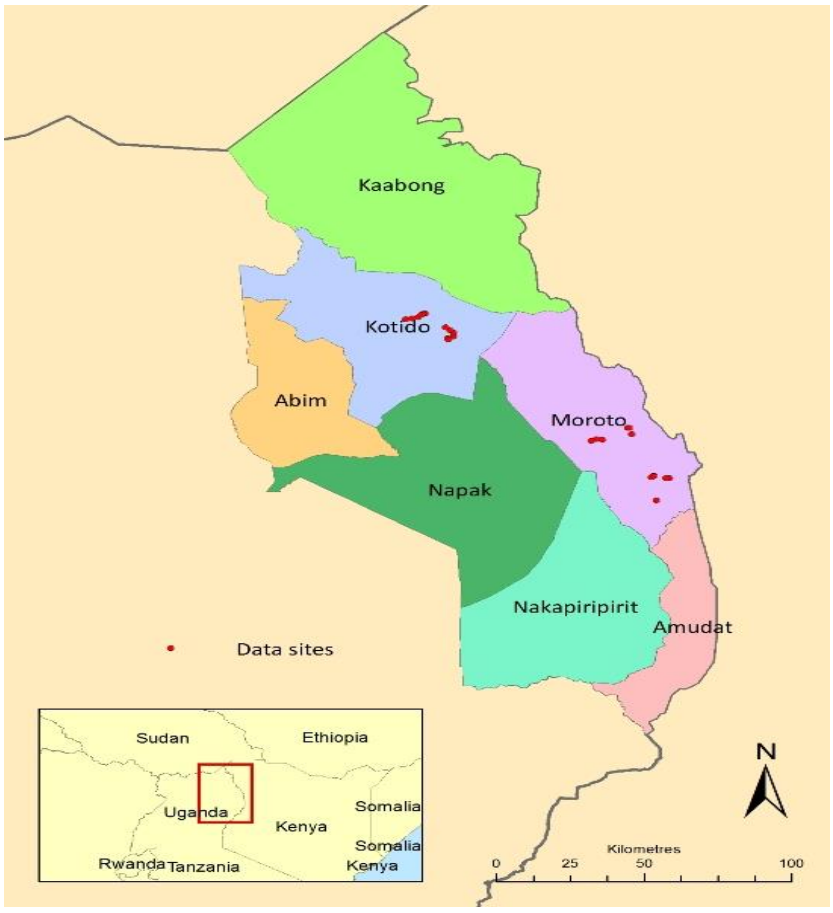

Figure 3.1: Map of Karamoja Region showing the study sites (red circles) in the districts of Moroto and Kotido. The region is sub-divided into seven districts. The study was conducted in four sub-counties, two in each district. Inset is the map of Uganda and neighbouring countries, highlighting the location of the study area.

3.3.3 Data collection

3.3.3.1 Clearance, training and administration for the study

Ethical clearance for the study was obtained from the Research Ethics Committees (REC) of the National Agricultural Research Organisation (NARO), Uganda (no. 1416), and the Faculty of Humanities, University of Pretoria (no. GW20150211). The REC of NARO is accredited by the Accreditation Committee of Uganda National Council for Science and Technology (UNCST). The RECs review and approve research projects with the aim of protecting rights and welfare of human research participants. The Agricultural Technology and Agribusiness Advisory Services (ATAAS) project, under which this study falls, was approved by UNCST. Data collection progressed only after consent was given by the participants. To avoid bias during mobilisation, we introduced the general topic of the study as cattle health and management, rather than specifically TBDs. Methodological training that consisted of PE techniques, interviewing skills, and in-class practice of selected PE techniques (Catley, 2005) was conducted for the team members.

Data were collected between October and December 2013 using PE techniques as described by Catley et al. (2012). Data collection tools consisted of focus group discussions, key informant interviews, review of surveillance data and researcher observations. Discussions with the participants were conducted in the local language (Ngakarimojong), by two teams of four members: a team leader who asked most of the questions and facilitated the discussion, a community mobiliser, a recorder and a translator. In each team, there was one veterinarian and two assistant veterinarians. The checklist of topics and PE techniques were pre-tested and adjusted in one location in Moroto District, which was not included in the final study. Each team conducted one or two discussions per day, normally in the mid-morning, with discussions taking an average of 90 minutes. Within each method, cross-checking and probing were done to verify internal consistency of information, make sure that the informants understood the different items to be scored or ranked, and to gather more detailed information on a particular subject. Efforts were made to ensure all members of the discussion group expressed their opinions, and that discussion was open and not dominated by one or a few individuals. The informants were given time to discuss and reach consensus. Ranking, scoring and other visualisation activities were done on flip charts that were laid on the ground. The PE activities were repeated with all 24 informant groups (12 in Moroto, 12 in

Kotido) representing 24 *manyattas*. In the afternoon, interview teams met and reviewed the records of the discussions to ensure these were accurate.

3.3.3.2 Participatory epidemiology tools

Semi-structured interviews and participatory mapping for groups

Semi-structured interviews were used to gather qualitative data by guided conversation on pre-determined topics, using open-ended questions. The topics included husbandry and grazing systems, description of major diseases affecting cattle, control options for ticks and TBDs, and timing and frequency of tick control. Other topics were veterinary services, use and source of veterinary drugs, and constraints to the control of ticks and TBDs. The informants listed the diseases that affected cattle by their own description and local names. The researchers and district veterinarians knew the local disease names, and the description by the informants was collated with confirmation from previous clinical and post-mortem findings, and laboratory diagnosis (field veterinarians, personal communication), and then available literature.

Participants drew a map of their area using coloured pens. The map showed the location of roads, wet and dry season grazing areas, watering points (including rivers, ponds and dams), veterinary services and drug sources, and physical features like hills and settlements. Participants highlighted areas associated with high intensity of ticks, wildlife habitats, and distribution of haematophagous insects. Following the map, semi-structured questions were asked to obtain specific information about the epidemiology of TBDs.

Simple ranking for tick control options and drugs used

Cards were used to represent tick control options and drugs. A symbol that depicted the item to be ranked, as suggested by the participants, was drawn on a card. Informants then arranged items vertically in an order based on defined criteria: effectiveness, cost, frequency and simplicity of use. The cards were arranged on the ground against one criterion at a time. The first and last cards in the sequence were given the highest and lowest scores, respectively. The item with the highest score was then ranked number one.

Pairwise ranking and comparison for cattle diseases and constraints to the control of TBDs

Pairwise ranking was done by comparing each item individually with all the other items one-by-one. The informants were asked to list and discuss the five most important cattle diseases with respect to cattle productivity and impact on their livelihoods. The recorder sketched a 5 x 5 grid. Two cards that represented two diseases were then randomly picked, and the informants were asked to compare the two diseases and mention the reasons why one disease was more important than the other. The recorder documented the disease that was indicated as more important in the corresponding box. The total score (0-5) for each disease in a group was noted. The median scores for each disease in the 24 groups were then used to establish an overall ranking. The exercise was repeated for constraints to the control of ticks and TBDs, and the participants discussed ways of improving TBD control. The process of pairwise ranking for diseases was used to generate indicators for disease matrix scoring (see next section).

Matrix scoring for cattle diseases

The matrix scoring for the diseases was conducted as described by Catley and Mohammed (1996). In each district, the five top-ranked cattle diseases, based on the pairwise ranking, were scored against clinical and production-related indicators mentioned during the pairwise comparisons. The diseases were represented using cards, which were placed along the top *x*-axis of the matrix and the indicators were illustrated on cards placed along the *y*-axis. Other diseases outside the top five were categorised as 'others'. An indicator (e.g. mortality rate) was picked at random to score. The informant group was given 30 stones to divide among the disease cards, the stones indicating that the disease was associated with the indicator being scored. The number of stones was chosen because five stones per disease should be sufficient to show differences between diseases but not so large as to be difficult to handle or divide (Catley and Mohammed, 1996). The number of stones allocated to each disease was then counted and recorded. The scoring procedure was repeated until all the indicators had been scored against each disease.

Proportional piling for disease incidence

Proportional piling was used to estimate the incidence, mortality and case fatality rate of the five most important cattle diseases, using the method described by Catley et al. (2014). Each informant group was provided with a pile of 100 stones that represented the number of cattle in a herd. First, the informant group was asked to divide the stones into two, a pile representing the proportion of the herd that got sick and those that remained healthy during the past one year. The pile representing the proportion of animals that became ill was further sub-divided into six piles corresponding to the proportion of animals that got each of the five diseases and a sixth group representing 'other diseases' during the past one year. This activity provided estimates of the annual incidences of the diseases. Subsequently, each group was asked to remove some of the already-allocated stones representing the sick, to indicate the proportion of dead animals for each of the five prioritised diseases and the group of 'other diseases' during the past one year. We cross-checked the pattern of surviving in relation to dead, and asked about the proportion of survivors treated, the type of treatment used, and proportion of survivors still in the herd. We also asked for the durations of illness within the pile that represented the dead animals. This provided estimates of the mortalities and case fatality rates due to the diseases. The group of 'other diseases' was not included in the estimation of case fatality rates.

Key informant interviews, review of surveillance data, direct observations, and clinical examinations

Key informant interviews (n = 30) were held independently before or after the group sessions, with district veterinary officers (DVOs) and CAHWs. The interviews followed a checklist of important topics to guide the discussion, including husbandry and grazing systems, major cattle diseases, control options for ticks and TBDs, acaricides and chemotherapeutic drugs sources and use, and constraints to the control of ticks and TBDs. Information from the veterinarians on diseases was based on previous clinical examinations and post-mortem findings, and surveillance data provided to the DVOs from the Institute for International Cooperation and Development (IICD). The IICD is an Italian non-profit organisation with objectives that include improving livestock production and productivity in Karamoja Region, and has a Veterinary Diagnostic Laboratory. The DVOs regularly (between three months and

one year) request the IICD to provide or conduct disease surveillance. The informants also estimated distances between key features, which had been mentioned in group discussions.

To seek confirmation of information gathered during the PE study, we assessed a published surveillance report (IICD, 2010) and other literature (Jost et al., 1998). We also analysed unpublished data from disease surveillance that had been carried out by IICD in four districts of Karamoja Region, including Moroto and Kotido, two months before this study. We observed pastoralists' activities regarding tick and TBD control in some herds, and analysed the surrounding environment as we walked through the villages. In 18 of the 24 *manyattas* visited, we found sick animals (including calves) that had not been driven out for grazing (n = 82). We took case histories, conducted clinical examinations of the animals, and provided treatment and/or gave advice. To avoid bias, treatment of cases was only done after group discussions in a sub-location were complete. We examined blood samples from eight clinical cases of TBDs at University of Pretoria (Onderstepoort, South Africa) by reverse line blot hybridisation assay.

3.3.4 Statistical analyses

Non-parametric tests and descriptive statistical procedures (frequencies, median and range) were used to analyse the PE data, using Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM SPSS, 2011). The level of agreement between informant groups was assessed using Kendall's coefficient of concordance (*W*). A *p*-value was assigned to *W*. Evidence of agreement between informant groups was categorised as 'weak' ($W < 0.26$, $p > 0.05$), 'moderate' ($W = 0.26 - 0.38$, $p < 0.05$) and 'strong' ($W > 0.38$, $p < 0.01$) as previously described by Catley (2006).

3.4 Results

3.4.1 Composition of the groups

The number of informants in the focus groups ranged from 8 to 12, and they were all male. The social setting of the communities in Karamoja Region makes it difficult to interview women, reportedly due to high domestic workload for women, but also due to cultural norms. By our observation, women were preoccupied with activities including construction and

repair of houses and fences around the *manyattas*. It was also the role of women to avail food in the homes. Nearly all the informants were illiterate. No group declined to participate in the study, and all informants readily participated in the interviews.

3.4.2 Grazing systems and water sources

Participatory mapping revealed that animals graze close to the settlement areas (within about 5 km) during the wet season (Figures 3.2 and 3.3). During the dry season, animals migrate and share grazing and watering points with communities from neighbouring districts and countries (mainly with Turkana from Kenya). The participants indicated that it would take them up to two days to reach the dry season grazing areas. When animals graze far away from *manyattas*, animal health care services become more difficult. The informants mentioned that tick infestations and the risk of TBDs were higher in the wet season and communal grazing areas. Haematophagous arthropods, which may transmit some TBDs, were prevalent in the grazing areas. Wild animals (e.g. buffaloes and small antelopes) shared grazing with livestock, mostly in dry season grazing areas. In Kotido, dry grazing areas were in proximity to Kidepo Valley National Park.

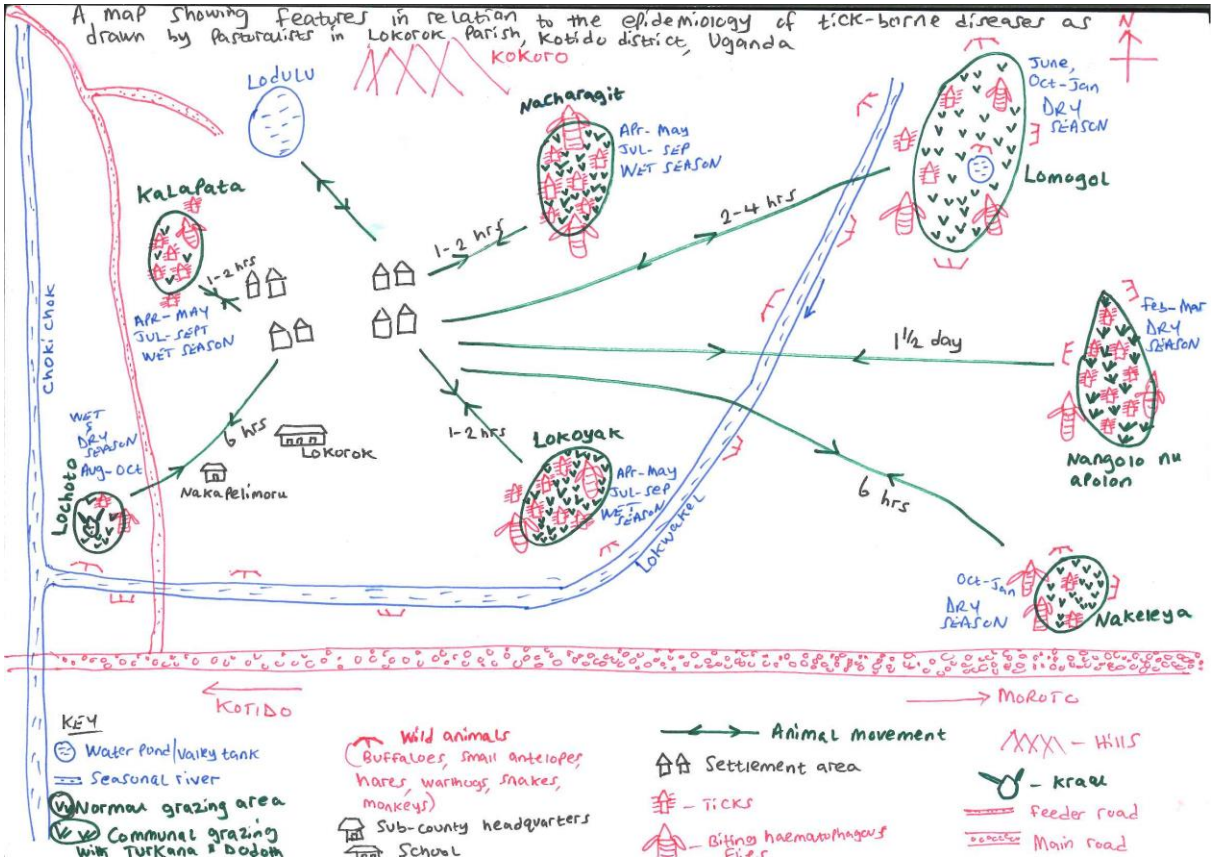

Figure 3.2: Map showing physical features, seasonal grazing areas, watering points, and distribution of ticks, wildlife and haematophagous arthropods in grazing areas around Lokorok parish, Kotido District, Uganda. This map was constructed by livestock keepers during a participatory epidemiology study regarding tick-borne diseases in November, 2013. Dry season grazing areas were located far (up to 1.5 days walk) from the settlement areas. Tick intensities were higher in the communal and wet season grazing areas.

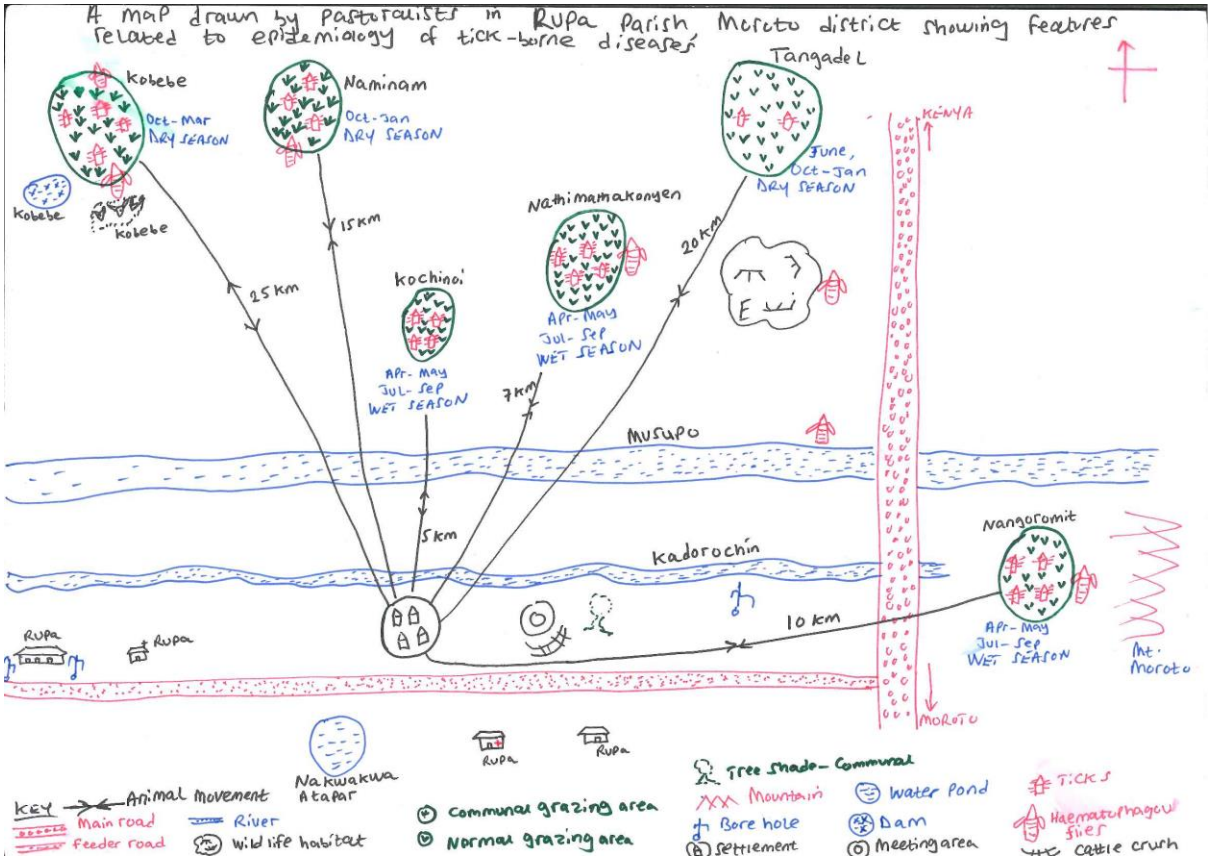

Figure 3.3: Map showing physical features, seasonal grazing areas, watering points, and distribution of ticks, wildlife and haematophagous arthropods in grazing areas around Rupa parish, Moroto District, Uganda. This map was constructed by livestock keepers during a participatory epidemiology study regarding tick-borne diseases in December, 2013. Wet season grazing areas were located near settlement areas (on average 7 km). Tick intensities were higher in the communal and wet season grazing areas.

3.4.3 Cattle diseases

3.4.3.1 Description of cattle diseases

The semi-structured interviews showed that the informants understood and demonstrated good knowledge of clinical signs, post-mortem findings and epidemiological features of cattle diseases which conform to the literature (Blood and Radostits, 1989). This knowledge is orally passed on from one generation to the next. The common cattle diseases were given literal meanings in the local language which correspond to specific disease entities. Anaplasmosis and babesiosis were recognised by all Karamojong livestock keepers as *lopid* (bile disease) and *lokulam* (urinary bladder disease), respectively. The participants mentioned that anaplasmosis was characterised by 'enlarged gall bladder', 'hard dry dung', 'yellowish discolouration of the outer membranes and internal organs', and the disease mostly affected adult cattle. Through the years, the livestock keepers have learnt to associate particular

lesions in internal organs or tissues of dead animals with specific diseases. Cattle affected by babesiosis 'pass out reddish urine', 'urination is difficult' and 'blood is watery'. East Coast fever was known by the cattle keepers as *lokit* (ear disease), an association with the enlargement of the parotid lymphnodes, which are found behind the ears, in the early stages of the disease. East Coast fever was also characterised by lachrymation, blindness, inappetence and swelling of the prescapular lymphnodes, and the disease affected mostly the calves. Heartwater is recognised as *lokou* (head disease), characterised mainly by animals moving in circles. The cattle become 'aggressive to humans' and 'fluids accumulate in the chest cavity and around the heart'. The participants emphasised that ECF was associated with grazing animals on pasture where they pick up the ticks that transmit the disease; however, the informants were uncertain about the causes of anaplasmosis, heartwater and babesiosis. Nonspecific diarrhoea was known locally as *loleo* which literally means 'severe diarrhoea'. It refers to a condition where sick animals have profuse diarrhoea, lose weight and have dry skin. The problem of overgrown hooves (*emara*) led to lameness in animals, wounds and foot rot. Blackquarter is called *ekicumet* in Moroto and *lokicumet* in Kotido. Both names mean 'speared/pierced from one side', and the disease led to sudden death of apparently health animals, swelling, stiffness, paralysis, hotness, and lameness of the affected limbs. The disease *emitina* (mange), meaning 'itching spots', was reportedly more common in calves, characterised by hairless areas around body parts. *Ediit* (trypanosomosis), literally 'tsetse fly', was associated with bites from tsetse flies, the principal vectors of the disease. Trypanosomosis led to extreme emaciation in animals. Contagious bovine pleuropneumonia (CBPP) was recognised as *loukoi* (lung disease), characterised by 'laboured, fast breathing and cough', and at slaughter, 'lungs are attached to the thoracic cavity' and 'fluids and pus accumulate in the lungs'. The livestock keepers mentioned that overcrowding of animals in the enclosures ("kraals") and at watering points contributed to the spread of CBPP. Cattle affected by CBPP had mucous discharge from the nostrils and froth in the mouth, and moved slower in a group of animals. A disease referred to by the cattle keepers as *lotidae*, which reportedly affected humans, and led to skin lesions after contact with blood from affected animals, often leading to death, was later defined by the DVOs as anthrax. Clinical manifestations of mixed infections were described by the observation of concurrent clinical signs and/or post-mortem features of the respective diseases. We did not encounter

conflicting information among informant groups and individuals regarding the perception and knowledge of livestock diseases in the two districts.

3.4.3.2 Most important cattle diseases as determined from pairwise ranking

As shown in Table 3.1, informants in Moroto ranked the five most important cattle diseases as ECF, anaplasmosis, CBPP, heartwater and babesiosis in that order. The most important diseases in Kotido were ECF and anaplasmosis (tied), trypanosomosis, CBPP and nonspecific diarrhoea in that order. Prioritisation and ranking of disease pairs by the cattle keepers were based on morbidity and mortality rates, rates of transmission, treatment costs, difficulty in accessing the correct treatment, difficulty of control, rapid fatality, and lack of knowledge to manage the disease. East Coast fever, anaplasmosis, and CBPP were listed in the pairwise ranking activity in every informant group in both districts. Kendall's coefficient of concordance (W) for informant groups for the diseases indicated strong agreement in Moroto ($W = 0.682$, $p < 0.001$, n = 12) and Kotido ($W = 0.568$, $p < 0.001$, n = 12).

Table 3.1: Pairwise ranking: overall ranking of cattle diseases among groups of livestock keepers in Karamoja Region, Uganda, October to December 2013

Disease	Moroto			Kotido		
	n	Median score	Rank	n	Median score	Rank
East Coast fever	12	4.0	1	12	3.0	2
Anaplasmosis	12	3.0	2	12	3.0	2
Trypanosomosis	0	-	-	12	1.5	3
CBPP	12	2.0	3	12	1.0	4
Nonspecific diarrhoea	2	0	8	7	0.5	5
Heartwater	9	0	4	1	0	7
Blackquarter	4	0	6	3	0	6
Mange	3	0	7	1	0	8
Babesiosis	5	0	5	0	-	-
Overgrown hooves/foot rot	2	0	9	0	-	-
Anthrax	1	0	10	1	0	9

n = number of informant groups.

3.4.3.3 Matrix scoring for cattle diseases

The results of matrix scoring for the five most important diseases and a group of 'other' diseases by informants' criteria in Moroto and Kotido are summarised in Tables 3.2 and 3.3, respectively. Disease indicators were identical between districts. In both districts, there was strong agreement between informant groups for the eight disease indicators ($W = 0.38 - 0.66$, $p < 0.05$ to $p < 0.001$). ECF and anaplasmosis had the highest scores for all the indicators.

Table 3.2: Summarised matrix scoring of cattle diseases by pastoralists' criteria in Moroto District, Uganda, October to December 2013

Indicator	Disease					
	ECF *Lokit*	Anaplasmosis *Lopid*	CBPP *Loukoi*	Heartwater *Lokou*	Babesiosis *Lokulam*	Others
High morbidity (*W* = 0.61***)	10.5 (3, 15)	8 (7, 15)	3.5 (1, 11)	2 (0, 4)	0 (0, 4)	2 (0, 13)
High mortality (*W* = 0.52***)	10 (0, 30)	8 (0, 14)	3 (0, 6)	2 (0, 8)	0 (0, 5)	2.5 (0, 16)
Rapid fatality (*W* = 0.50***)	8 (1, 17)	8 (1, 13)	4 (1, 8)	2 (0, 6)	0 (0, 4)	3 (0, 20)
High treatment cost (*W* = 0.62***)	11 (1, 16)	9 (5, 18)	3 (1, 11)	2 (0, 4)	0 (0, 3)	3 (0, 11)
Difficult to treat (*W* = 0.66**)	12 (1, 14)	9.5 (7, 19)	2 (0, 8)	2.5 (1, 5)	0 (0, 5)	1.5 (0, 7)
Difficult to access correct drug (*W* = 0.47**)	8 (0, 13)	9.5 (0, 16)	3.5 (2, 30)	3 (0, 9)	0 (0, 1)	1.5 (0, 13)
Inadequate knowledge for effective treatment (*W*=0.54***)	9 (2, 11)	9 (2, 14)	3 (1, 6)	2 (0, 9)	0 (0, 6)	4 (2, 12)
Rate of transmission[a] (*W* = 0.46*)	8.5 (0, 14)	8 (1, 13)	8 (2, 18)	0.5 (0, 3)	0 (0, 5)	2 (0, 6)

Number of informant groups = 12. *W* = Kendall's coefficient of concordance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *W* values vary from 0 to 1; the higher the value, the higher the level of agreement between the informant groups.

Median scores (number of stones that were assigned) are shown in each cell. Minimum and maximum values are shown in parentheses. More counters represent a stronger positive association.

[a]Rate of transmission was the rate at which other animals in the herd acquired a particular disease from the time the first case(s) was detected.

Table 3.3: Summarised matrix scoring of cattle diseases by pastoralists' criteria in Kotido District, Uganda

Indicator	Disease					
	ECF *Lokit*	Anaplasmosis *Lopid*	Trypanosomosis *Ediit*	CBPP *Loukoi*	Non-specific diarrhoea *Loleo*	Others
High morbidity (*W* = 0.55***)	8 (6, 13)	7 (4, 14)	3 (2, 11)	4 (2, 6)	2 (0, 8)	3 (0, 8)
High mortality (*W* = 0.46***)	8 (4, 15)	11 (5, 17)	2.5 (1, 7)	2.5 (1, 6)	1.5 (0, 9)	3 (0, 7)
Rapid fatality (*W* = 0.55***)	8 (5, 13)	8 (5, 12)	2 (1, 8)	3 (1, 5)	2 (0, 7)	3 (1, 14)
High treatment cost (*W* = 0.65***)	8 (6, 18)	7.5 (4, 12)	2.5 (1, 5)	3.5 (1, 7)	2.5 (0, 5)	4 (0, 6)
Difficult to treat (*W* = 0.61***)	8 (5, 14)	10 (7, 13)	2 (1, 4)	2 (1, 5)	1 (0, 8)	4 (0, 9)
Difficult to access correct drug (*W* = 0.61**)	8 (5, 12)	10 (5, 14)	3 (1, 5)	4 (1, 5)	1 (0, 5)	5 (1, 9)
Inadequate knowledge for effective treatment (*W* = 0.42*)	7 (6, 15)	8.5 (1, 14)	3.5 (1, 7)	4 (1, 8)	2.5 (0, 4)	2.5 (0, 6)
Rate of transmission[a] (*W* = 0.38**)	7.5 (3, 11)	6.5 (2, 11)	3 (2, 5)	5 (3, 7)	4 (0, 8)	3 (1, 11)

Number of informant groups = 12. *W* = Kendall's Coefficient of Concordance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *W* values vary from 0 to 1; the higher the value, the higher the level of agreement between the informant groups.

Median scores (number of stones that were assigned) are shown in each cell. The minimum and maximum values are shown in parentheses. More counters represent a stronger positive association.

[a]Rate of transmission was the rate at which other animals in the herd acquired a particular disease from the time the first case(s) was detected.

3.4.3.4 Incidence of cattle diseases

The overall median proportion of cattle that became ill during the past one year, with the range, was 65.5% (range: 39, 93). Overall median deaths from all diseases was 44% (39, 93) in Moroto and 53% (45, 84) in Kotido. The overall median scores for annual incidence, mortality and case fatality rates of cattle estimated by Karamojong cattle keepers and attributed to the five top ranked and 'other diseases', during proportional piling, are illustrated in Tables 3.4 (Moroto) and 3.5 (Kotido). East Coast fever had the highest estimated incidence score in Moroto (18% [2, 33]) and Kotido [21% [6, 32]), followed by anaplasmosis (17.5% [8, 32], Moroto; 17% [10, 33], Kotido). Tick-borne diseases (ECF, anaplasmosis, heartwater and babesiosis) were responsible for 69.7% of the overall illness and 79.3% of deaths in Moroto. Fifty-six point one percent (56.1%) of the total illnesses and 60.7% of the total deaths reported in Kotido were due to ECF and anaplasmosis. Most animals that suffered from TBDs in the study area died, as the median scores for case fatality rates were at least 66% for each disease.

Table 3.4: Median standardised scores of annual incidence, mortality and case fatality estimates for the five top ranked and other cattle diseases in Moroto District of Karamoja pastoral Region, Uganda

	Median scores for diseases (%)					
	ECF	Anaplasmosis	CBPP	Heartwater	Babesiosis	Others
Sick	18 (2, 33)	17.5 (8, 32)	9 (1, 21)	5 (1, 10)	4 (1, 13)	7 (1, 23)
Dead	14 (1, 30)	17.5 (6, 32)	3.5 (1, 17)	2 (1, 19)	4 (0, 6)	2.5 (0, 23)
CFR	89.5 (42, 100)	82.8 (63, 100)	41 (19, 100)	66.7 (20, 100)	85.7 (0, 100)	

Results obtained by incidence scoring technique (number of informant groups = 12)
Other diseases: mange, blackquarter, anthrax, foot rot/overgrown hooves, nonspecific diarrhoea and lumpy skin disease; CFR, case fatality rate; Numbers in brackets are minimum and maximum values.

Table 3.5: Median standardised scores of annual incidence, mortality and case fatality estimates for the five top ranked and other cattle diseases in Kotido District of Karamoja pastoral Region, Uganda

	Median scores for diseases (%)					
	ECF	Anaplasmosis	Trypano-somosis	CBPP	Nonspecific diarrhoea	Others
Sick	21 (6, 32)	17 (10, 33)	8 (2, 18)	6.5 (4, 18)	12 (4, 17)	5.5 (3, 10)
Dead	16.5 (4, 28)	10 (7, 30)	2 (0, 17)	2.5 (1, 13)	11 (2, 17)	2.5 (0, 7)
CFR	81.7 (44, 100)	70.7 (48, 100)	41.7 (14, 100)	38.9 (22, 100)	86.7 (40, 100)	

Results obtained by incidence scoring technique (number of informant groups = 12)
Other diseases: heartwater, mange, blackquarter, anthrax and lumpy skin disease.
CFR, case fatality rate; Numbers in brackets are minimum and maximum values.

3.4.4 Control of ticks and tick-borne diseases

3.4.4.1 Tick control practices

The conventional methods for controlling ticks were hand spraying and pour-on acaricides. Other methods included picking off ticks by hand, bush burning and the use of plants. For all 24 informant groups, daily picking of ticks by hand and irregular hand spraying with acaricides were the most common tick control practices. Hand picking was a common practice because there is no monetary cost, it does not require special skills or knowledge, and can be done at any time. However, the pastoralists indicated that the practice was cumbersome, it was difficult to restrain the animals, and it was risky for animal handlers. Furthermore, hand picking caused bleeding and created wounds on the body of the animal. Ticks were killed by throwing them into a fire or crushing them between stones.

The purchase of acaricides was determined by availability of supplies and money. Quantities of acaricides bought were often insufficient, leading to spraying at irregular and infrequent intervals. Spraying was done with hand sprays. Only two groups (19 participants) used spray pumps. Some cattle keepers indicated that they applied acaricides by swabbing the body of the animals with fibres of shrubs soaked in acaricides. About two-thirds of herders did the dilution using uncalibrated materials. They described the quantities of acaricide used in terms of bottle lids. From our observation and information from the key informants, cattle keepers used insufficient acaricide wash (about one litre per animal). However, apart from one group in Kotido, the informants noted that the efficacy of the acaricides was good to control ticks.

The most common acaricides were amitraz group formulations (mainly Amitix®), indicated by 215 informants in 23 groups. Ivermectin, flumethrin, pye-grease and Supona Extra® (chlorfenvinphos pour-on) were used by few participants (31, 65, 31 and 37 individuals in 3, 7, 3 and 4 *manyattas*, respectively). Livestock keepers preferred amitraz acaricides to other groups, as amitraz drugs were cheaper. One group (9 informants) in Moroto used deltamethrin (Decatix®).

The frequency of spraying ranged from twice a week to once a year. Half (12) of the informant groups (n = 117 participants) applied acaricide weekly in wet and dry seasons (if acaricides were available). Acaricides were applied to only those animals with high tick infestations. Spraying was generally more frequent in the wet than dry season, with some informants indicating that they sprayed twice a week in the wet season. The pastoralists noted that ticks survived and multiplied better in the wet season, leading to higher intensity which required higher frequency of spraying. Furthermore, there was a perception that acaricides were washed off in the rain and this prompted them to spray their animals again. Tick control in calves commenced when ticks were seen on the animals, about two weeks after birth.

Bush burning in the dry season as a method to control ticks was mentioned by 17 (n = 162 participants) of the 24 groups, with the aim to destroy ticks and their habitats (grass). However, the informants believed the method was less effective since the ticks burrowed underground or climbed trees to escape the fire. Traditional medicine including the use of plants is practiced (68 pastoralists in 7 groups) to control ticks; however, the informants said the method was less effective, and that plants were used in desperation to control ticks when conventional acaricides were not available. Two informant groups indicated that they used a mixture of ash and urine to wash the animal against ticks.

3.4.4.2 Drugs and drug use practices in treating TBDs

All group participants admitted that they were treating cases of TBDs. Oxytetracycline was the drug most commonly used and it was used indiscriminately for various diseases. All the participants in the 24 *manyattas* indicated they used a 10% concentration of oxytetracycline and five groups (n = 51 participants) also used a 20% concentration. From our observation,

oxytetracyclines are more available and cheaper than alternatives, and are perceived by cattle keepers to have a broad spectrum of activity against a number of diseases.

Some participants occasionally used other drugs including parvaquone (e.g. Parvexon®) and buparvaquone (e.g. Butalex®) for ECF (9 groups, n = 89 participants), Imizol® for anaplasmosis (3 groups, n = 33 participants), diminazene aceturate (4 groups, n = 42 participants) and penicillin-streptomycin formulations (7 groups, n = 67 participants). The participants could distinguish the different brands and concentrations of commonly-used drugs only by their prices and packaging. Identity of the drugs was obtained through probing, combined with the experiences of CAHWs and community mobilisers. At times, the informants showed the drugs that were kept for use or empty bottles which previously contained the drugs. The cattle keepers did not necessarily take into account the weights of the animals and dose regimens were not properly followed.

Traditional herbs were also reported as a treatment for TBDs. These included *Aloe vera*, tobacco and red pepper for ECF. Burning of lymphnodes with hot iron was mentioned by three informant groups (n = 29 participants) as a treatment for ECF. All informants indicated that treatment against TBDs was not very effective leading to low recovery rates, probably due to under dosing and the use of incorrect drugs.

3.4.4.3 Constraints to the control of ticks and TBDs

The informants ranked the major constraints that hindered the control of ticks and TBDs as inadequate knowledge, inadequate veterinary services, limited availability of drugs, and shortage of money in that order. Other constraints in order of importance were high costs of drugs, lack of equipment, lack of cattle crushes, and insecurity from cattle raids.

Inadequate veterinary services were blamed on the small number of local veterinarians (one or two per district) who could seldom reach the communities. Non-government organisations (NGOs) and CAHWs complemented the local veterinary services; however, they had limited inputs for animal health care, and in some cases CAHWs were not residents of the respective communities. In the absence of CAHWs, the cattle keepers bought drugs and treated their

animals. The informants indicated that due to inadequate veterinary services, they lacked enough information to determine proper dosage and correct treatment for diseases.

Drug outlets were often lacking among communities; therefore, livestock keepers walked up to 45 km (distance estimated by key informants) in search of drugs. As a result, the cattle keepers could not control diseases in time, resulting in high morbidity and case fatality rates. Shortage of money, as expressed by two-thirds of the informant groups, meant that the cattle keepers had difficulty to buy drugs and equipment, and pay for the services of CAHWs. From our observation, the practice of selling animals for profit does not seem to apply in this region. Livestock keepers only sell animals when they need cash for basic needs such as food and medicines. One group in Moroto (11 participants) said that inaccessibility to diagnostic facilities hampered the effective treatment of diseases. There was strong agreement from pairwise ranking between informant groups for constraints to the control of TBDs [(Moroto, $W = 0.47$, $p < 0.001$), (Kotido $W = 0.35$, $p < 0.001$)].

3.4.5 Key informant interviews, review of surveillance data, direct observations, and clinical examinations of animals

Data collected showed agreement between key informants and cattle herders, regarding the control of ticks and TBDs in the area. There was also agreement from the key informants about the most important cattle diseases. A review of surveillance data for cattle in Moroto and Kotido Districts from 2009, revealed prevalences of 17-28% (anaplasmosis), 13-18% (babesiosis), 38-41% (ECF) by microscopic examination of blood and lymph node smears (DVOs, personal communication; IICD, 2010). On clinical examination of the sick animals that we found in 18 of the 24 *manyattas*, we observed cases of TBDs: on average two cases of anaplasmosis and one of ECF per *manyatta*, and one case of heartwater in each of two *manyattas* in Moroto District. The signs of ECF that were observed during the clinical examinations included enlarged parotid and prescapular lymph nodes, lachrymation, difficulty in breathing (dyspnoea), anaemia (white discolouration of conjunctiva, oral mucosa and vulvar mucosa), fever (>40°C rectal temperature), lung oedema (through thoracic auscultation) and anorexia. For anaplasmosis, the clinical signs were constipation, jaundice (visible as yellowish colouration of the oral and vulvar mucous membranes) and anorexia. Heartwater was observed mainly by nervous signs including ataxia, chewing movements,

circling and aggression. Animals suffering from babesiosis had haemoglobinuria and anaemia. We analysed unpublished data from disease surveillance that had been carried out by IICD in four districts of Karamoja Region, including Moroto and Kotido, two months before this study. The laboratory and field staff at IICD carried out disease surveillance to ascertain the health status of cattle populations in 61 herds in Nakapiripirit, Napak, Kotido and Moroto Districts of Karamoja Region. The surveillance team collected blood samples and lymph node biopsies and conducted clinical examinations on sick cattle in the 61 herds. Out of the 61 herds, 16 were from the 24 *manyattas* included in this study. The samples were analysed in the laboratory for *Brucella* spp., *Babesia* spp., *Anaplasma* spp. and *Theileria* spp. Tick-borne parasites were observed in Giemsa-stained smears (thin and thick) under microscope. The surveillance team recorded data from clinical examination for each animal alongside results from microscopic examination of Giemsa-stained blood and lymph node smears. Ninety-one percent (of 44 cases in 61 herds) of the suspected ECF cases from clinical examinations were confirmed positive by analysis of blood and lymph node biopsy smears for piroplasms and schizont-infected lymphocytes, while 67% (of 52 cases in 61 herds) of the suspected clinical cases of anaplasmosis were confirmed positive by laboratory analysis. Three out of five cases of babesiosis that were diagnosed during clinical examination were confirmed by laboratory analysis. *Anaplasma* parasites were observed as dense, homogeneously staining blue-purple inclusions in infected red blood cells. In this study, we did not repeat the confirmation of clinical cases by microscopic examination since this activity had been carried out in most of the study herds, in the same study area, just two months before this study, by field and laboratory staff from IICD. We think we would not get different data in this study from those recorded from the surveillance activities. Four field and laboratory staff at IICD that had participated in the previous surveillance exercise constituted the research team for the present study. We used the logistics and facilities at the IICD laboratory in Moroto to collect and process field samples. Nevertheless, the reverse line blot hybridisation assay confirmed the presence of tick-borne infections in blood samples we collected from clinical cases found in the present study: *Anaplasma marginale* (five anaplasmosis cases), *Theileria parva* and *A. marginale* (one ECF case), and *T. parva* (two ECF cases).

Although Moroto and Kotido districts were previously categorised as pastoral and agro-pastoral zones respectively (Anderson and Robinson, 2009), from our observation, and

information from key informants and livestock keepers, livestock herding was the main livelihood activity in both districts. The degree of dependence on livestock (social, cultural and economic), and livestock migration was similar in the two districts.

3.5 Discussion

We used PE methods to define and prioritise cattle diseases, evaluate current control activities for TBDs, and identify constraints to the control of TBDs. Participants also proposed solutions to service delivery, disease control and surveillance. Most pastoral areas of Africa, including Karamoja, are physically remote and characterised by mobility of herds. When combined with apparent reluctance of herders to provide accurate information on herd size to outsiders, disease estimates (e.g. mortality) determined by conventional research methods may be questionable due to invalid denominators (Catley et al., 2014). For individual interviews with conventional questionnaires, local people answer questions posed by outsiders, which may be of limited interest to them. In contrast, PE methods seem to offer lower risk of informants consciously offering incorrect or misleading information because the topic under discussion is often a local priority (Catley et al., 2012). Karamoja communities are clustered in *manyattas* where groups of cattle keepers have similar social, economic and political objectives, and manage and share resources (including animals, water and grazing) together. In such settings, any discussion on livestock is a concern for groups of cattle keepers, with consent of the *manyatta* leader, rather than individuals. For these reasons, we believe that PE methods have significant advantages over conventional household surveys in this setting.

Our PE results indicated that Karamoja cattle keepers regard TBDs, especially ECF and anaplasmosis, as a major constraint to cattle keeping in the area. This perception relates to the high incidence rate, high case fatality rates, and treatment costs associated with the diseases. The estimated incidence scores for TBDs were similar in the two districts, indicating that the diseases are widespread among cattle in Karamoja Region. Our findings are consistent with previous studies in Tanga Region, Tanzania (Swai et al., 2009) and Machakos District, Kenya (Wesonga et al., 2010) where ECF and anaplasmosis were among the most important diseases of cattle in terms of economic losses resulting from mortality and costs of

treatment. Our findings are also consistent with those of Kasozi et al. (2014) who reported widespread occurrence of *T. parva* (47.4%) and *Anaplasma* spp. (14.4%) among cattle in 15 districts of western and central Uganda. In another study, Jost et al. (1998) demonstrated that anaplasmosis was the second most important disease, while ECF was fourth, in Karamoja Region. In this study, the overall mortality estimates (44-53%) in cattle from all diseases are similar to that reported among livestock keepers in South Sudan (39%; Malak et al., 2012), but differ from those in Ethiopia (8% in Shiferaw et al., 2010; 6%-18% in Catley et al., 2014). The variations in the mortality may be due to differences in local epidemiologic conditions as well as control strategies and veterinary services. Our values for mortality are consistent with the Global Alliance for Livestock Veterinary Medicines (GALVmed) estimates that indicated that 60% of Karamojong pastoralists' calves die of ECF (GALVmed, 2014). In other reports from Karamoja, it was estimated that on average 100 cattle (out of about 3,000) die per month in communal camps, mostly due to disease (equating to 40% annual mortality; Anderson and Robinson, 2009), and the average herd sizes have markedly reduced in the last decade (field veterinarians, personal communication; IICD, 2010) from around 100 to around 40 (field veterinarians, personal communication). However, although there has been reduction in herd sizes, the number of herds has increased, corresponding to the increase in the human population. This has maintained and/or increased the overall cattle population in the region (Anderson and Robinson, 2009; UBOS, 2014), despite the high rate of losses to disease.

A low carrier state of *T. parva* (18%) in cattle was reported in some parts of Karamoja Region using nested PCR (Kabi et al., 2014), which may reduce the likelihood of the development of acquired immunity, and therefore result in outbreaks of ECF. In other parts of Uganda, *T. parva* prevalence was up to 21% in a crop-livestock system of Tororo District (Muhanguzi et al., 2014a; Muhanguzi et al., 2014b). Although indigenous zebu cattle usually show endemic stability to anaplasmosis in areas with little tick control (Perry and Young, 1995), clinical cases were observed and a high incidence reported by the informants. In endemic areas where many different *A. marginale* strains are present, persistent infections occur in cattle due to superinfection with *A. marginale* antigenic variants. The variants evade the immune response to the primary strain or infect the truly naïve animals which results in acute bacteraemia (Palmer and Brayton, 2013). The other TBDs, babesiosis and heartwater, are also responsible for economic losses in eastern Africa (Kivaria, 2006; Ocaido et al., 2009b).

Within-method and across-method triangulation were used, demonstrating consistency in the findings. The information from cattle keepers was supported by researchers' observations, review of published surveillance reports (Jost et al., 1998; IICD, 2010), information from key informants, laboratory diagnosis and tick infestation.

This study showed that wildlife share common grazing land with cattle. Wildlife especially Cape buffalo (*Syncerus caffer*) act as a reservoir for many of the important tick borne pathogens of cattle (Oura et al., 2011), indicating a potential to spread and increase parasite burdens in cattle.

The pastoralists applied under-strength acaricide, often at irregular intervals, and used very little or no acaricide wash, which is consistent with other studies among pastoralists in East Africa (Mugisha et al., 2005; Mbassa et al., 2009; Swai et al., 2009; Wesonga et al., 2010). The correct frequency for acaricide application depends on the acaricidal agent, tick species involved, breed of cattle, season, and level of tick infestation, but is on average twice per week (Mugisha et al., 2005; Mbassa et al., 2009). In the present study, tick control on calves commenced about two weeks after birth, when ticks were seen on the animals, a practice that reduces exposure to infection when animals are still protected by maternal antibodies, and may therefore leave animals susceptible to infection at a later stage. There was widespread use of oxytetracyclines to treat TBDs in Karamoja Region, which is consistent with previous reports among pastoralists in East Africa (Chenyambuga et al., 2010; Kairu-Wanyoike et al., 2014; Kasozi et al., 2014). These inappropriate practices lead to increased haemoparasite burdens and animal losses, due to endemic instability (Mbassa et al., 2009), drug resistance and maintenance of chronic carriers (APUA, 2010; Kasozi et al., 2014).

Like other pastoral areas in East Africa (Wesonga et al., 2010; Onono et al., 2013; Kasozi et al., 2014), the constraints to the control of TBDs were inadequate extension services, inadequate knowledge to give the right treatment and limited availability of drugs. Due to insufficient/poor amenities, the region is not attractive to veterinarians, consistent with other rural pastoral areas in Africa (Shiferaw et al., 2010). The local government veterinary offices lacked basic diagnostic and sampling facilities, and power supply for a cold chain was

unreliable or lacking. Resources for transport were lacking or inappropriate with regard to expected workload. The diagnostic facility at IICD was inadequate for the entire region. These constraints limited the effective and timely diagnosis and control of diseases, and may have led to the high disease-related mortality.

From our findings, together with those of previous studies, we are able to gain insight into future strategies for controlling TBDs and improving livestock productivity in marginal pastoral areas. The Karamojong livestock keepers identified locally appropriate veterinary service delivery systems, disease surveillance, and control options as being essential to achieving relevant and sustained benefits. The informants suggested that veterinary service delivery can be improved if government provides incentives to attract more veterinarians. Veterinary services are necessary to assist livestock keepers on the right choice of acaricides, correct dilution, effective spraying techniques, and frequency of application. Furthermore, the capacity and effectiveness of CAHWs, whose services have become a common component of tropical veterinary programmes in pastoral communities (Jost et al., 1998; Swai et al., 2014), can be enhanced by continuous training, and subsidising and improving on the supply of drugs, vaccines and equipment. CAHWs can play a significant role, but appropriate policies must be in place to strengthen their activities (Swai et al., 2014). Government-community partnership is essential for establishment and maintenance of dip tanks. The pastoralists also emphasised the need to rationalise and improve water supply to match preferred animal movements. Immunisation can be a viable option for control of common TBDs in cattle. Currently in eastern Africa, immunisation is done only against ECF, using the Muguga cocktail infection and treatment method (ITM), coordinated by the Global Alliance for Livestock Veterinary Medicines [GALVmed] (Di Giulio et al., 2009).

In pastoral systems of East Africa, including Karamoja, dipping facilities are frequently not operational because of lack of finances for refurbishment of dip tanks and provision of acaricides. In the past, the dip tanks in Karamoja Region have been destroyed or vandalised. Water supply is also a challenge. Dipping in the East African region is not compulsory, and is consequently inconsistent (Mugisha et al., 2005; Kivaria, 2006; Mbassa et al., 2009). However, a study in eastern Tanzania demonstrated an improvement in the organisational skills of farmers, through cooperative societies, which addressed the problem of funding; thus

providing a sustainable community-based model using cattle dips to reduce losses due to ticks and TBDs and increase cattle productivity (Mbassa et al., 2009).

We did not carry out laboratory confirmation of most cases of TBDs that were observed by physical examination of sick animals, and this was a short coming in this study. However, confirmation of clinical cases by microscopic examination had been carried out in a previous surveillance exercise, in most of the study herds, and in the same study area, just two months before this study.

In this study we could not interview women regarding the study objectives. Although women do not own livestock in Karamoja (Anderson and Robinson, 2009), they do participate in some livestock-related activities including construction and cleaning of sheds for small ruminants and calves, hand picking and burning of ticks, milking cows, and processing traditional medicines. This may indicate that women can contribute to the improvement of livestock health and production in pastoral areas. It is therefore important that future livestock development programmes should consider both genders, so as to ensure equity and empower women, thus reducing their vulnerability and improving their livelihoods.

Although animal milk is an important component in children's diets in Karamoja (Stites and Mitchard, 2011), the effect of TBDs on milk production was not mentioned as an indicator in the matrix scoring exercises. A possible explanation is that men may place less emphasis on this aspect of production because milking of cows is predominantly women's business (field veterinarians, personal communication) and the participants were all men. Alternative explanations for the omission of effects of milk production (other than gender bias) may be: households rely more on purchased milk as a source for children (Stites and Mitchard, 2011), and livestock keepers may have difficulty in quantifying relative decreases in milk production due to different diseases (given the small quantity of milk already produced). Therefore, in developing PE methods, researchers may include additional indicators of disease impacts (such as milk production in this case), which are not mentioned by informants. However, this may reduce the primary advantage of participatory approaches over more structured surveys; namely, that the impacts of disease and the problems associated with their control are identified by livestock keepers themselves.

The approaches used in this study are limited by the possibility of mixed parasite infections in cattle in endemic areas that result in the combined or simultaneous effects of different parasites, and which may present difficulties during scoring. In all groups visited, the informants described the possibility of mixed infections, due to concurrent clinical signs of TBDs, especially of anaplasmosis and ECF. In cases of mixed diseases, cases were allocated to the disease whose manifestation was more pronounced. This may result in underestimation of the burden of those diseases which, when present as a mixed infection, tend to be masked by a more severe concurrent infection. Teasing apart the relative contribution of co-infections would however require extensive confirmatory laboratory testing, a costly exercise that would negate many of the advantages of the participatory approach employed in our study.

Our findings, together with previous studies, suggest that TBDs impact on the livelihoods of pastoralists in East Africa. There is urgent need for an integrated approach for control of ticks and TBDs, including further work on vaccines for cattle and improved policy. There is also need to improve drug usage and extension services. Research should also target the contribution of multiple host species, including wildlife, multiple strains of tick-borne pathogens, and acaricide resistance. Research in anti-tick vaccines offers the advantage of controlling both tick numbers and disrupting the tick vector-pathogen interface.

3.6 Conclusions

We investigated the management practices and relative importance of TBDs among transhumant zebu cattle in Karamoja Region, Uganda. Tick-borne diseases, in particular ECF and anaplasmosis, were regarded by participants as the most important due to high morbidity, mortality and treatment costs. Control of ticks was done mostly by daily hand picking of ticks and irregular hand spraying with acaricides. The main constraints to the control activities were inadequate knowledge to manage the diseases, inadequate veterinary services and limited availability of drugs.

3.7 References

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

APUA, 2010. Antibiotics in food animal production: A forty year debate. Alliance for the Prudent Use of Antibiotics Newsletter, Boston MA, 28 (2), 27 pp. www.tufts.edu/med/apua/news/newsletter_33_3555326098.pdf (accessed March 24, 2015).

BLOOD, D.C., RADOSTITS, O.M., 1989. Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 7th edn, Baillière Tindall, 24-28 Oval Road, London NWI 7DX, England, 1502 pp.

CATLEY, A., 2005. Participatory Epidemiology: A guide for trainers. African Union/Interafrican Bureau for Animal Resources, Nairobi, 52 pp.

CATLEY, A., 2006. Use of participatory epidemiology to compare the clinical veterinary knowledge of pastoralists and veterinarians in East Africa. Tropical Animal Health and Production 38, 171-184.

CATLEY, A., ADMASSU, B., BEKELE, G., ABEBE, D., 2014. Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. Disasters 38, 500-516.

CATLEY, A., ALDERS, R.G., WOOD, J.L.N., 2012. Participatory epidemiology: Approaches, methods, experiences. The Veterinary Journal 191, 151-160.

CATLEY, A., OSMAN, J., MAWIEN, C., JONES, B.A., LEYLAND, T.J., 2002. Participatory analysis of seasonal incidences of diseases of cattle, disease vectors and rainfall in southern Sudan. Preventive Veterinary Medicine 53, 275-284.

CATLEY, A.P., MOHAMMED, A.A., 1996. The use of livestock-disease scoring by a primary animal-health project in Somaliland. Preventive Veterinary Medicine 28, 175-186.

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. Livestock Research for Rural Development Volume 22 (7), Article # 135. Retrieved May 2, 2013, from http://www.lrrd.org/lrrd22/7/chen22135.htm.

DI GIULIO, G., LYNEN, G., MORZARIA, S., OURA, C., BISHOP, R., 2009. Live immunization against East Coast fever - current status. Trends in Parasitology 25, 85-92.

GALVMED, 2014. Uganda's ECF Progress. GALVmed Newsletter, Global Alliance for Livestock Veterinary Medicines, Pentlands SciencePark, Bush Loan, Edinburgh EH26 OPZ, UK, pp. 4. www.galvmed.org/wp-content/uploads/2014/03/GALVmed-Newsletter-March-2014.pdf (accessed December 5, 2014).

IBM SPSS, 2011. IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY.

IICD, 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities, Ruspantini, C. (Ed.), European Commission, Government of Uganda and Institute for International Co-operation and Development (IICD) Uganda, 67 pp. www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/C&DPublicationLivestockDiseaseSurveillance2.pdf. Accessed March 24, 2015.

JOST, C., STEM, C., RAMUSHWA, M., TWINAMASIKO, E., MARINER, J., 1998. Comparative ethnoveterinary and serological evaluation of the Karimojong Community Animal Health Worker Program in Uganda. Annals of the New York Academy of Sciences 849, 327-337.

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, 414.

KAIRU-WANYOIKE, S.W., KIARA, H., HEFFERNAN, C., KAITIBIE, S., GITAU, G.K., MCKEEVER, D., TAYLOR, N.M., 2014. Control of contagious bovine pleuropneumonia: Knowledge, attitudes, perceptions and practices in Narok district of Kenya. Preventive Veterinary Medicine 115, 143-156.

KASOZI, K.I., MATOVU, E., TAYEBWA, D.S., NATUHWERA, J., MUGEZI, I., MAHERO, M., 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. Open Journal of Veterinary Medicine 4, 220-231.

KIVARIA, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production 38, 291-299.

MAAIF, 2011. Statistical Abstract, Agricultural Planning Department, Ministry of Agriculture, Animal Industry and Fisheries. www.agriculture.go.ug/userfiles/Statistical Abstract 2011.pdf. Accessed June 7, 2013.

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, 216-223.

MBASSA, G.K., MGONGO, F.O.K., MELAU, L.S.B., MLANGWA, J.E.D., SILAYO, R.S., BIMBITA, E.N., HAYGHAIMO, A.A., MBIHA, E.R., 2009. A financing system for the control of tick-borne diseases in pastoral herds: The Kambala (Tanzania) model. Livestock Research for Rural Development 21 (3), Article #44. http://www.lrrd.org/lrrd21/3/mbas21044.htm. Accessed May 20, 2015.

MUGISHA, A., MCLEOD, A., PERCY, R., KYEWALABYE, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

MUHANGUZI, D., PICOZZI, K., HATENDORF, J., THRUSFIELD, M., WELBURN, S.C., KABASA, J.D., WAISWA, C., 2014a. Prevalence and spatial distribution of *Theileria parva* in cattle under crop-livestock farming systems in Tororo District, Eastern Uganda. Parasites & Vectors 7, 91.

MUHANGUZI, D., PICOZZI, K., HATENDORF, J., THRUSFIELD, M., WELBURN, S.C., KABASA, J.D., WAISWA, C., 2014b. Collateral benefits of restricted insecticide application for control of African trypanosomiasis on *Theileria parva* in cattle: a randomised controlled trial. Parasites & Vectors 7, 432.

NORVAL, R.A.I., 1992. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

OCAIDO, M., MUWAZI, R.T., OPUDA, J.A., 2009a. Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. Tropical Animal Health and Production 41, 731-739.

OCAIDO, M., OTIM, C.P., KAKAIRE, D., 2009b. Impact of major diseases and vectors in smallholder cattle production systems in different agro-ecological zones and farming systems in Uganda. Livestock Research for Rural Development Volume 21 (9), Article #155. Retrieved November 29, 2013, from http://www.lrrd.org/lrrd21/9/ocai21155.htm.

ONONO, J.O., WIELAND, B., RUSHTON, J., 2013. Constraints to cattle production in a semiarid pastoral system in Kenya. Tropical Animal Health and Production 45, 1415-1422.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

PALMER, G.H., BRAYTON, K.A., 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15, 1969-1975.

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, 107-120.

SHIFERAW, T.J., MOSES, K., MANYAHILISHAL, K.E., 2010. Participatory appraisal of foot and mouth disease in the Afar pastoral area, northeast Ethiopia: implications for understanding disease ecology and control strategy. Tropical Animal Health and Production 42, 193-201.

STITES, E., MITCHARD, E., 2011. Milk Matters in Karamoja: Milk in Children's Diets and Household Livelihoods. Feinstein International Center, Tufts University, 200 Boston Ave., Suite 4800, Medford, MA 02155, USA, 35 pp. (http://www.fao.org/fileadmin/user_upload/drought/docs/MM-Karamoja.pdf. Accessed October 10, 2015.

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, 34-42.

SWAI, E.S., MASAAZA, S., DABORN, C.J., 2014. Evaluation of community animal health delivery systems in Simanjiro, Tanzania. Livestock Research for Rural Development Volume 26 (8), Article # 138. Retrieved September 20, 2014, from http://www.irrd.org/Irrd26/8/swai26138.htm.

UBOS, 2014. Statistical Abstract. Uganda Bureau of Statistics. www.ubos.org/onlinefiles/uploads/ubos/statistical_abstracts/Statistical_Abstract_2014.pdf. Accessed June 21, 2015.

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. Livestock Research for Rural Development Volume 22 (6), Article #111. Retrieved December 13, 2013, from http://www.irrd.org/Irrd22/6/weso22111.htm.

CHAPTER 4

4. Endemic status of tick-borne infections and tick species diversity among transhumant Zebu cattle in Karamoja Region, Uganda

4.1 Abstract

We conducted a study to investigate tick species diversity, seroprevalence of antibodies to *Anaplasma marginale* and *Theileria parva*, and the risk factors for these infections among cattle under a transhumant production system in Karamoja Region, Uganda, from November 2013 through January 2014. Twenty herds were randomly selected from 20 purposively-selected superherds. Semi-structured interviews and piling for annual proportion of tick-borne disease (TBD) cases in different age groups, with pastoralist groups, clinical examinations and field observations were employed to obtain information related to the epidemiology of TBDs. Ticks were collected and identified from whole body inspections of at least seven systematically selected cattle in each herd. Concurrently, serum was collected from 397 cattle. Antibodies to *A. marginale* were detected by MSP-5 competitive inhibition enzyme-linked immunosorbent assay, and to *T. parva* by indirect fluorescent antibody test. Clinical examinations and informant interviews showed that TBDs affected all age groups of cattle. Tick species that have not been reported in recent studies from other parts of Uganda were collected, namely *Amblyomma lepidum*, *Hyalomma truncatum*, *Amblyomma gemma*, and *Rhipicephalus pulchellus*. Out of the 10,923 ticks collected, *Rhipicephalus appendiculatus* (54.4%) was the most abundant species followed by *Rhipicephalus decoloratus* (17.7%), *Amblyomma variegatum* (12%) and *A. lepidum* (11.6%). Two-thirds of the sampled cattle were infested with moderate (37.3%, 11-50 ticks) to abundant (28.6%, > 50 ticks) numbers of ticks. The true seroprevalence of *A. marginale* was high (95.1%, 95% confidence interval [CI] 91.3% - 98.5%), while that of *T. parva* was low (16.5%, 95% CI 12.9% - 19.6%). Univariate analysis

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showed that cattle of 5-12 months (18.3%, odds ratio [OR] = 4.1) and 13-24 months (30.3%, OR = 8.0) were more likely to be seropositive for *T. parva* than those > 24 months. For *A. marginale*, cattle of 13-24 months (92.4%, OR = 2.7) and > 24 months (89.7%, OR = 2.0) were more likely to be seropositive than those 5-12 months. There was a significant difference ($p < 0.001$, OR = 6.5) in the proportion of *T. parva* seropositive animals between Moroto (24.5%) and Kotido Districts (4.8%), but not for *A. marginale*. However, the mixed effect logistic regression analysis showed that no variable was associated with *T. parva* or *A. marginale* seropositivity ($p > 0.05$). In conclusion, the low seroprevalence of antibodies to *T. parva*, possibly due to limited exposure of calves to the pathogen from infected ticks, may suggest a high likelihood of ECF in cattle. High seroprevalence for *A. marginale* suggests that a high proportion of cattle were exposed to infection. The findings provide knowledge of epidemiology of TBDs in Karamoja cattle and support for strategic control and improvement of cattle productivity.

4.2 Introduction

Ticks are among the most important vectors of pathogenic micro-organisms affecting livestock (Jongejan and Uilenberg, 2004). Moreover, ticks can cause severe toxic conditions such as paralysis and toxicosis, irritation and allergy, damage to hides and skins, loss of teats, lameness, and tick-bite wounds that can become secondarily infected with bacteria (Jongejan and Uilenberg, 2004). Tick-borne diseases (TBDs) are responsible for significant losses among cattle and impact the livelihoods of resource-poor communities in sub-Saharan Africa (Kivaria, 2006; Ocaido et al., 2009a; Thumbi et al., 2013).

Previous studies show that East Coast fever (ECF) and anaplasmosis are among the most economically important diseases of cattle in East Africa (Ocaido et al., 2009b; Chenyambuga et al., 2010; Onono et al., 2013; Chapter 3 of this thesis). East Coast fever, caused by the protozoan *Theileria parva* and transmitted by the ixodid tick *Rhipicephalus appendiculatus*, is endemic in eastern, central and southern Africa including Uganda (Norval et al., 1992; Thumbi et al., 2013; Kabi et al., 2014; Muhanguzi et al., 2014). The disease is responsible for 40-80% of annual mortality in calves (Kivaria, 2006; Lynen et al., 2006; Thumbi et al., 2013). Cattle can

become long-term asymptomatic carriers of *T. parva* following treatment or spontaneous recovery, thereby maintaining the parasite population (Dolan, 1986; Kabi et al., 2014). Bovine anaplasmosis is caused by *Anaplasma marginale*, which is the most prevalent tick-borne pathogen of cattle in many parts of the world (Aubry and Geale, 2011). The disease is responsible for high morbidity and mortality in susceptible animals in subtropical and tropical regions (Aubry and Geale, 2011) and is endemic in Uganda (Magona et al., 2011a). Anaplasmosis is typically more severe in animals older than two years of age. *Anaplasma marginale* is transmitted biologically by several tick species, and mechanically through haematophagous arthropods such as *Tabanus*, *Stomoxys* and mosquitoes, and blood-contaminated fomites (Aubry and Geale, 2011). In East Africa, *A. marginale* is transmitted mainly by the tick *Rhipicephalus decoloratus* (Chenyambuga et al., 2010; Magona et al., 2011a). Cattle surviving infection become lifetime carriers and reservoirs of the infection (Aubry and Geale, 2011).

Transhumant pastoralism is a livestock production system characterised by seasonal and cyclical movement of herds from areas of pasture and water scarcity to areas where forage and water are found (Egeru et al., 2014). Because they live in climatically marginalised environments, mobile pastoralists depend on livestock to sustain livelihoods (Onono et al., 2013). Cattle in Karamoja Region are of the short-horned East African zebu type (*Bos indicus*), and are grazed extensively on communal land (Anderson and Robinson, 2009). Cattle diseases have increased in the past decade resulting in high mortalities and reduced herd sizes (Anderson and Robinson, 2009; IICD, 2010).

In eastern Africa, several factors encourage persistent infections and carrier states of TBDs in cattle herds, including production systems and management practices, inadequate veterinary infrastructure and resources, and bimodal annual rainfall (Perry and Young, 1995). Control practices against ticks and TBDs affect the patterns of exposure to tick-borne infections in cattle and establishment of immunity at individual or herd levels (Mugisha et al., 2005; Kivaria, 2006; Wesonga et al., 2010). A state of endemic stability implies an ecological balance between cattle, tick, parasite and the environment where calves are challenged by infected ticks when they are still protected by innate and/or colostral immunity. This results in a high

level of immunity in adult cattle with a consequent low incidence of clinical diseases and low case-fatality of the few infections (Perry and Young, 1995; Jonsson et al., 2012; Gachohi et al., 2013). Endemic instability occurs in indigenous breeds of cattle kept by pastoralists (Norval et al., 1992; Perry and Young, 1995) in situations where cases of TBDs are not treated effectively, which results in high case fatality. This is because the proportion of cattle that are carriers declines with increased mortality rates of infected cattle, which leads to low seropositivity or low population immunity amongst cattle (Dolan, 1986; Perry and Young, 1995). On the other hand, open grazing systems are characterised by unrestricted tick exposure. Continuous exposure of cattle, especially in indigenous breeds, to infected ticks under open grazing in endemic areas may facilitate the development of immunity, decreasing susceptibility to disease in cattle. However, even in high tick-infested areas, low infection rates in ticks may not allow sufficient exposure of calves to tick-borne pathogens, and therefore acquired immunity may not develop. In semi-arid and arid areas, population immunity to tick-borne pathogens is low or variable, characterised by periodic unsuitability of climate for the survival and development of ticks, for example *R*. *appendiculatus* (Perry and Young, 1995). Given the impact of TBDs, there is need to understand the prevalence and dynamics of ticks and TBDs, in order to predict disease outbreaks, develop effective control strategies, and increase livestock productivity in pastoral areas. Various studies have been conducted in eastern Africa regarding exposure patterns for TBDs and associated risk factors, but mostly under mixed crop-livestock production systems (Rubaire-Akiiki et al., 2006; Kabi et al., 2008; Swai et al., 2009; Magona et al., 2011a; Malak et al., 2012; Gachohi et al., 2013; Wesonga et al., 2015). There is a lack of data from a systematic study on the tick species diversity, seroprevalence of antibodies to TBDs, and associated risk factors for cattle under the pastoral system in Karamoja Region.

The objectives of this study were to: (i) investigate the proportion of TBD cases in different age groups in the cattle population using a participatory approach, (ii) determine the diversity and abundance of ticks infesting cattle, (iii) determine the seroprevalance of antibodies to *A*. *marginale* and *T*. *parva*, and (iv) determine risk factors for seropositivity in cattle. The proportion of TBD cases in different age groups, and the diversity and abundance of ticks are important indicators of the likelihood of endemic stability, and thus provide the biological

evidence to choose an appropriate approach for control of TBDs, for example dipping, immunisation or the enhancement of innate resistance, or no intervention (Norval et al., 1992; Perry and Young, 1995; Jonsson et al., 2012). Antibody prevalence has been used as an indicator of the existence of endemic stability for babesiosis and anaplasmosis (Perry and Young, 1995; Jonsson et al., 2012). For *T. parva*, the association between antibody prevalence and endemic stability is not absolute, as it is complicated by other factors (Perry and Young, 1995). For example, in production systems with intensive tick control, ECF incidence is low and this is generally accompanied by low antibody prevalence. However, the case-fatality rate of the few infections that occur is high, as all animals in this population are highly susceptible (Perry and Young, 1995). On the other hand, in the indigenous Zebu cattle kept in endemic areas under extensive management with little or no acaricide application, ECF incidence is also low and antibody prevalence is high. This results in a high population immunity, and the case-fatality rate of the few infections that become clinically apparent is low (Perry and Young, 1995).

4.3 Materials and Methods

4.3.1 Ethics approval

The study was approved by the relevant Ethics Committees at the University of Pretoria (Animal [protocol no. V026-14] and Humanities [no. GW20150211]), and by the National Agricultural Research Organisation of Uganda (no. 1416). Permission was obtained to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa; reference number 12/11/1/1). A veterinary import permit (permit number 13/1/1/30/2/0-201408003716, Department of Agriculture, Forestry and Fisheries, Republic of South Africa) was obtained to transport the serum samples from Uganda to South Africa. The samples were heat inactivated against foot and mouth disease virus on arrival in South Africa. Standard techniques were followed during the collection of blood samples and ticks. All participating pastoralists gave informed verbal consent before the study commenced.

4.3.2 Study design and sample size methodology

The study was a cross-sectional design conducted in two purposively-selected districts, Moroto and Kotido, from November 2013 through January 2014 (Map shown in Chapter 3). During this period, the settlement areas and livestock herds are more accessible as compared to the wet season when roads are muddy and often blocked by seasonal rivers. One herd was randomly selected from each of 20 purposively-selected superherds. A superherd is a group of herds identified as sharing grazing and watering areas during the wet season and belonging to the same cattle camp during the dry season. Management practices of herds within superherds are homogenous. The average herd size in the Karamoja Region is around 40 head of cattle (field veterinarians, personal communication). It was difficult to ascertain the exact number of herds and superherds, to construct a complete sampling frame, due to lack of comprehensive information on the number and distribution of cattle populations. There is also unpredictable inflow and outflow of cattle from raids, seasonal migration of animals and erratic reductions in numbers due to diseases. Furthermore, the cultural belief that does not allow one to count and reveal the exact number of herds or animals in herds makes estimates difficult (field veterinarians, personal communication). Therefore, we estimated the number of herds and superherds using records from a previous vaccination exercise. Ten out of about 63 superherds in Kotido and 10 out of about 72 superherds in Moroto were purposively selected for the study. The effective sample size of cattle to estimate seroprevalence of antibodies to *T. parva* and *A. marginale* was calculated by taking into account the design effect, which is the loss of effectiveness by the use of cluster sampling, instead of simple random sampling. The design effect is basically the ratio of the actual variance, under the sampling method actually used, to the variance computed under the assumption of simple random sampling. The design effect was estimated using the formula below (Killip et al., 2004):

$$DE = 1 + \delta (n-1)$$

where DE is the design effect, δ is the intracluster correlation coefficient for the being seropositive for antibodies for *T. parva* and *A. marginale*, and, n is the average herd size. No pilot study was conducted to calculate the intracluster correlation coefficient, therefore a δ

value of 0.03 was assumed. A value of 0.03 is interpreted to mean that the cattle in a herd (cluster) are about only 3% more likely to have the same chance of exposure than if the two animals were chosen at random in the serosurvey. A relatively large within-cluster variance is expected within a herd in the study area because groups of herds (10 to 20) graze together over a large area, which is expected to greatly reduce the chance that animals from the same herd are equally exposed to infected ticks. The average herd size was 40 cattle and a DE value of 2.17 was calculated. Therefore, the sample variance is 2.17 times bigger than it would be if the survey were based on the same sample size but selected randomly. An effective sample size of 369 cattle, taking into consideration the design effect, was calculated using the formula below (Killip et al., 2004):

$$ESS = MK/DE$$

where ESS = effective sample size, m = average number of cattle in a herd, k = number of herds, and DE = design effect. The number was increased by 7% to take care of any loss of samples during transportation and processing. We therefore decided to collect serum samples from 397 cattle for this study. The number of cattle selected per herd was determined by proportional allocation depending on the size of the herd. In a herd, animals were grouped by age category, and then sub-grouped by sex. At first, one animal was selected at random in a sub-group, and then other animals were picked using pre-determined sampling intervals. Proportional allocation was used to determine the number of cattle sampled in each sub-group. Between 17 and 24 cattle, representing each sex and age group (5-12 months, 13-24 months and > 24 months) were chosen in each herd.

4.3.3 Semi-structured interviews for management practices and proportional piling exercises

In each participating superherd, a group of 8-12 herdsmen and herd owners was interviewed to obtain information regarding the transmission and maintenance of TBDs. The informant data was gathered to determine the extent to which the herders' assessments of herd health are consistent with the findings from clinical examination and sero-survey. As in other pastoral areas in eastern Africa, the herds in Karamoja are not regularly tested because the

use of conventional approaches to research and disease surveillance is constrained by the mobility of herds, and lack of resources (Anderson and Robinson, 2009; Shiferaw et al., 2010; Wesonga et al., 2010; Catley et al., 2014). The cattle herds are also less accessible during the wet season. Therefore, researchers have to make best use of local knowledge and experience of the pastoralists, who are valuable sources of disease information (Catley et al., 2014). The interviews were conducted in the local language (Ngakarimojong) and followed a pre-tested checklist of topics. The topics included: prevalent cattle diseases, tick and TBD control practices, acaricide usage, timing and frequency of tick control, and grazing systems. Using a series of proportional piling exercises, each informant group also estimated the proportion of ECF and anaplasmosis cases in different age groups among cattle during the past one year. Proportional piling is a method whereby informants listed the cattle diseases that affected production and impacted their livelihoods, by their own description and names. If ECF and/or anaplasmosis occurred during the past one year, the diseases were illustrated on separate cards using symbols suggested by the informants. Informants were asked to classify cattle into different age groups according to their production system. The cards representing the TBDs were placed along the *y*-axis of a matrix on the ground, and the age groups illustrated along the *x*-axis. Each TBD was picked at random to score. An informant group was given 15 stones to divide among the age groups, the stones indicating that the age group was associated with the TBD being scored. The amount of stones was chosen because five stones per age group should be sufficient to show differences between age groups but not so large as to be difficult to handle or divide by the informants (Catley and Mohammed, 1996). Probing and cross-checking were done to make sure that the informants understood the topics of discussion during the interviews and agreed on the scoring. The number of stones allocated to each age group was then counted and recorded as a proportion of the total number of stones (15) for each TBD.

4.3.4 Clinical examinations, field observations, and collection and identification of ticks

Clinical examinations of sick cattle were conducted among herds. We also observed the surrounding environment with regard to TBDs and assessed tick and TBD control activities. Tick species diversity and abundance were determined by collecting all attached ticks from the whole body of at least seven systematically-sampled cattle in each herd. A total of 161

cattle from the 20 herds were sampled for ticks to assess the potential risk of transmission of TBDs. The ticks were preserved separately for each animal in labelled vials containing 70% ethanol, and thereafter identified and categorised (up to genus level for larvae and nymphs and species level for adults) using a standard stereoscopic microscope as previously described (Walker et al., 2013). The tick burden (adult, nymph and larva) on each animal was categorised as: none, few (1-10 ticks), moderate (11-50 ticks), and abundant (> 50 ticks) (Magona et al., 2011b).

4.3.5 Serological analysis

Serum samples

Blood was collected into 5 ml plain vacuum tubes (Becton Dickinson Vacutainer Systems Europe, UK) from the jugular vein of selected animals. Blood samples were stored in ice boxes while in the field, and later refrigerated overnight to allow the blood to clot. Sera were then aspirated using a Pasteur pipette and kept in 2 ml aliquots in cryotubes (Greiner Bio One, Germany) at -20°C. Frozen sera were transported to Onderstepoort Veterinary Institute - Agricultural Research Council (ARC-OVI), Pretoria, South Africa for serological analysis.

Indirect fluorescent antibody test for Theileria parva

A standard indirect fluorescent antibody test (IFAT) [sensitivity 96%, specificity 95%] was used to test serum for *T. parva* antibodies at ARC-OVI [Laboratory Manual Serology Volume II. OVI, Protozoology Division] as described previously by Goddeeris et al. (1982), with slight modifications. A standard Dulbecco phosphate-buffered saline solution (PBS) was used to make dilutions of test sera, and positive and negative controls at 1:80 and 1:160 in 96 well micro-titre plates. The 1:80 dilution is the standard cut-off titre at the ARC-OVI for positive reactions. The sera were then incubated at 37^{o}C for one hour with an antigen in tissue culture monolayer fixed on glass slides. Each slide contained known positive (from infected cattle as determined by quantitative real-time PCR and IFAT) and negative controls. The antigen was prepared at ARC-OVI, and contained live lymphoblastoid cells infected with *T. parva* macro-schizonts propagated in cell culture medium. Unbound antibodies were removed by washing them off. Bound antibodies were revealed by incubating the antigen-antibody complexes with rabbit anti-bovine IgG antibodies conjugated to a fluorescence compound - fluorescein

isothiocyanate (FITC) (Sigma-Aldrich, Life Sciences, South Africa). The conjugated anti-bovine IgG was first mixed with Evan's blue (0.02%) to give a dark background. Unbound FITC-conjugate was washed away leaving only the FITC-conjugate bound to test serum antibody-antigen complex. In the presence of specific antibodies, fluorescing antibody-antigen complexes were observed as bright fluorescence with a fluorescent microscope at 50 x and 100 x objective. No fluorescence was observed in a negative reaction.

Competitive inhibition enzyme-linked immunosorbent assay for Anaplasma

Antibodies for *A*. *marginale* were detected using the *Anaplasma*-specific competitive inhibition enzyme-linked immunosorbent assay (cELISA) (sensitivity 96%, specificity 95%) at the ARC-OVI, as described by Knowles et al. (1996). The test uses a monoclonal antibody against the *A*. *marginale* major surface protein (MSP) 5 that is highly conserved and reactive to *A*. *marginale*, *A*. *marginale* subp. *centrale* (*A*. *centrale*) and *A*. *ovis* (infective for sheep and goats). A recombinant MSP5 was coated on 96-well plates and incubated with the sample sera for 30 minutes at 37^{o}C. Then, monoclonal antibody ANAF16C1 conjugated to horseradish peroxidase was added to each well and incubated for one hour at 37^{o}C. After washing with phosphate buffer saline and Tween 20 solution, a goat anti-mouse IgG antibody conjugated to horseradish peroxidase was added to the wells followed by incubation for 30 minutes at 37^{o}C. Subsequently, a substrate TMB-CORE+ (BIO-RAD®) was added to produce a colour change for the negative samples. The plates were then incubated for an additional 5 minutes at 37^{o}C. An ELISA microplate reader Multiskan EX and a computer software program Ascent Software Version 2.6 (Thermo Scientific™) were used to measure and record optical density (OD) at 620 nm filter. Each plate included four known negative and positive sera (determined by cELISA). Percent inhibition (%I) was calculated as 100 - [(OD of test sera/Mean OD of negative reference sera) x 100]. A cutoff of 20% inhibition was used to classify samples. Samples with <20% inhibition were considered negative, and samples with greater than or equal to 20% inhibition were considered positive.

4.3.6 Statistical analyses

The mean for the proportions of TBD cases (from proportional piling) in each age group were calculated, along with 95% confidence intervals. The means were compared between ECF and

anaplasmosis and among age groups, for each disease, using the independent samples t-test, analysis of variance (ANOVA) for overall differences, and Tukey's test for pairwise comparisons. The level of agreement among informant groups for the proportions of cases, for each TBD, in the three age groups was assessed using Kendall's coefficient of concordance (*W*), and a *p*-value was assigned. Evidence of agreement among informant groups was categorised as weak for $W < 0.26$, $p > 0.05$; moderate for $W = 0.26\text{-}0.38$, $p < 0.05$ and strong for $W > 0.38$, $p < 0.01$ (Catley, 2006). The Chi-squared test was employed to determine significant differences in proportion of animals infested with various tick species between the districts.

Apparent seroprevalence estimates at individual level, defined as the number of seropositive animals over the number of animals tested, were derived for each age category, sex and district. A herd was considered positive if at least one animal was found positive by serological testing. The confidence intervals for seroprevalence of antibodies were estimated by performing descriptive statistics with 1000 bootstrap replications and sampling by clustering in the Statistical Package for the Social Sciences (SPSS) and using quantiles formation of the normal distribution (qnorm) with 'library (MASS)' package using R Console and RStudio. Bootstrapping was used to estimate the sampling distribution of the prevalence by assigning a measure of accuracy, in this case confidence interval. Inference about the cattle population from the sample data was modelled by resampling from original sample data by using sampling with replacement. The true prevalence was estimated from the apparent prevalence by taking into consideration the sensitivity and specificity of the laboratory tests used, to account for variability and uncertainty in the performance of the diagnostic tests at individual-animal level, using the formula below (Greiner and Gardner, 2000):

$$\text{True prevalence} = AP + Sp - 1/Se + Sp - 1$$

where Ap is the apparent prevalence, Sp the specificity of the diagnostic procedure and Se the sensitivity of the diagnostic procedure.

A conceptual framework was developed to select candidate variables identified as exposure factors for tick-borne infections for the production system of Karamoja Region. Univariate analysis of associations using the Chi-squared test was carried out for each exposure variable, with the serological test status of the animal considered as a binary outcome (positive or negative). The level of significance was set at $p \leq 0.05$. The exposure variables considered were age (5-12 months, 13-24 months, > 24 months), sex (male or female), district (Moroto or Kotido) and presence of tick vectors for *T. parva* (*R. appendiculatus*) and *A. marginale* (*R. decoloratus*). Tick control practices were not included in the analysis because spraying was mostly at irregular intervals in the herds and was done on a few animals in each herd. Prevalence ratios, odds ratios (OR) and *p*-values for the exposure variables were obtained. Intra-cluster correlations within the herds were also tested for the serological status for *T. parva* and *A. marginale*, by performing log ratio tests between a model 'herd' as random effect and a null model. This was followed by a mixed effect logistic regression in which the herd was included as a random effect and other variables were fixed effects. The analytical procedures were performed using SPSS version 23.0 (IBM SPSS, 2014), and R Console version 3.2.1 (R Console, 2015) and RStudio version 3.2.1 at 5% level of significance.

4.4 Results

4.4.1 Management practices and age distribution of TBD cases

In all sampled herds (n = 20), spraying (using hand sprayers) was done on a few cattle with little acaricide wash, incorrect dilutions, and at irregular intervals. All informants in the 20 groups indicated that tick spraying and hand picking of ticks in calves started about two weeks after birth, when ticks were seen. The Karamojong pastoralists categorised their cattle into three exclusive age groups as: 'Ngimanang' (calves < 6 months), 'Ngamayoi' (young 6-12 months), and 'Ngitobain' (adult > 12 months). Figure 4.1 shows the proportion of annual ECF and anaplasmosis cases in the three age groups of cattle as estimated by the pastoralists. The mean proportion of ECF (19%) from all informant groups was significantly higher ($t = 4.0$, $p < 0.001$) in calves of < 6 months of age compared to the same age group for anaplasmosis (2%). There was strong agreement among informant groups regarding the proportions of annual cases in the three age groups of cattle for anaplasmosis ($W = 1.0$, $p < 0.001$) and moderate

agreement for ECF (W = 0.33, p = 0.001). Disease scoring indicated a significant difference (p < 0.05) in the means for proportion of ECF cases among the three age groups. However, when pairwise comparisons were done, there was a significant difference (p < 0.05) in the mean proportion of ECF cases between calves < 6 months (18.9%) and adults (> 12 months) (50.9%), but similar (p > 0.05) between calves < 6 months and young cattle of 6-12 months (29.9%). The mean proportion of anaplasmosis was significant (p < 0.05) with multiple and pairwise comparisons among the age groups.

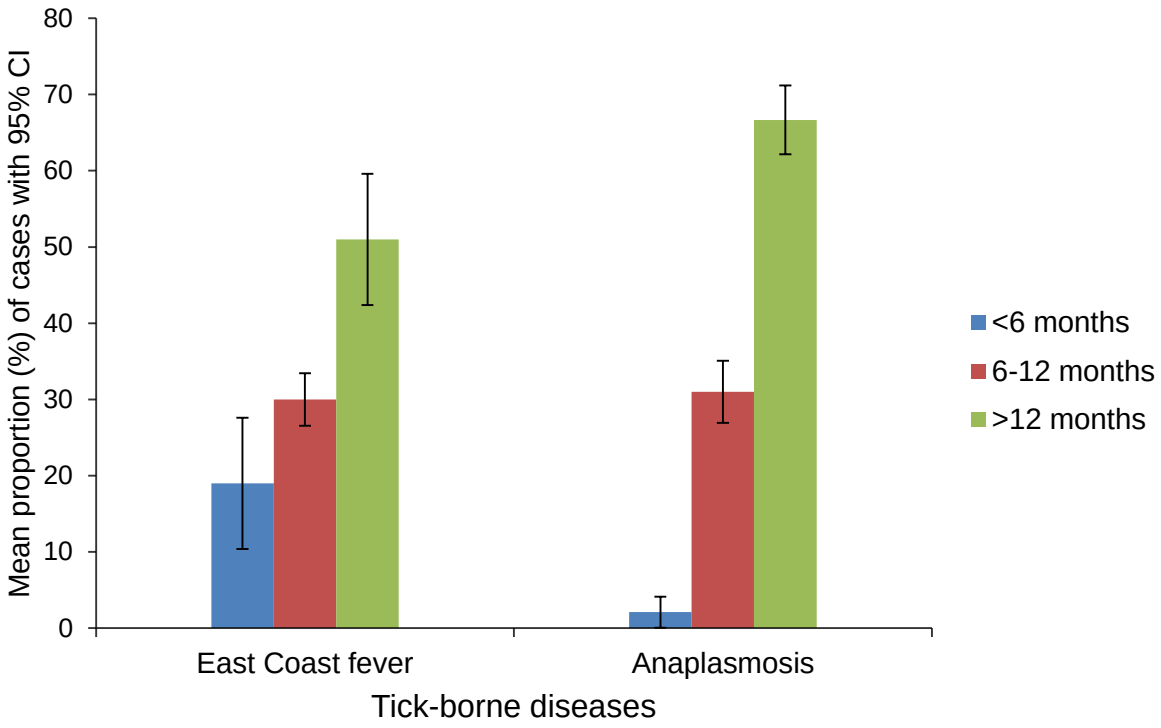

Figure 4.1: Bar chart with 95% confidence intervals illustrating the proportion of annual cases of East Coast fever and anaplasmosis distributed across three age groups, as estimated by 20 groups of Karamojong pastoralists, using a series of proportional piling exercises, from November 2013 through January 2014.

4.4.2 Clinical findings and tick infestation

Clinical examinations demonstrated seven cases of ECF (three in calves < 6 months and four in cattle > 12 months) in 6 of the 20 herds sampled, while anaplasmosis (21 cases) was diagnosed from various age groups of cattle in nearly all herds. Heartwater (two cases) was diagnosed in two herds and babesiosis (one case) in one herd. The signs of ECF that were observed during clinical examinations include enlarged parotid and prescapular lymph nodes,

lachrymation, difficulty in breathing (dyspnoea), anaemia (white discolouration of conjunctiva, oral and vulvar mucosa), fever (>40°C rectal temperature), lung oedema (through thoracic auscultation) and anorexia. For anaplasmosis, the clinical signs were constipation, jaundice (visible as yellowish colouration of the oral and vulvar mucous membranes) and anorexia. Heartwater was observed mainly by nervous signs including ataxia, chewing movements, circling and aggression. Animals with babesiosis had haemoglobinuria and anaemia. The species and numbers of ticks (adult) collected from 161 cattle in 20 herds, and the proportion of animals infested, are summarised in Table 4.1. A total of 10,923 ticks belonging to eight species were collected. *Rhipicephalus appendiculatus* was the most abundant species on cattle (54.4%), followed by *Rhipicephalus decoloratus* (17.7%), *Amblyomma variegatum* (12%), and *Amblyomma lepidum* (11.6%). We collected tick species that are either rare in Uganda (*A. lepidum*, *Hyalomma truncatum* and *Amblyomma gemma*) or were not known to be present in the country (*Rhipicephalus pulchellus*). Most cattle were infested with *A. lepidum* (78.9%) and *R. appendiculatus* (77%). Overall, the tick burden on cattle from the study area was high. Two-thirds of the animals sampled were infested with moderate (37.3%, 11-50 ticks) to abundant (28.6%, > 50 ticks) numbers of ticks, while 32.3% were infested with few ticks (1-10), and only 1.9% of cattle had no visible ticks on the body. The proportion of cattle with abundant tick infestation was significantly higher ($p < 0.05$) in Moroto (53.7%) than in Kotido (2.5%) (Figure 4.2).

Table 4.1: Identification and distribution of adult ticks collected from cattle in 20 herds in Moroto and Kotido Districts of Karamoja Region, Uganda, during a cross-sectional study, November 2013 through January 2014

Tick species	No. of ticks collected (%)	No. of cattle infested (%)		
		Moroto	Kotido	Total
Total	10,923 (100)	82 (100)	79 (100)	161 (100)
*A. variegatum***	1,313 (12)	51 (62.2)	28 (35.4)	79 (49.1)
A. lepidum	1,272 (11.6)	65 (79.3)	62 (78.5)	127 (78.9)
*R. appendiculatus***	5,947 (54.4)	72 (87.8)	52 (65.8)	124 (77)
*H. truncatum***	402 (3.7)	43 (52.4)	15 (19.0)	58 (36)
R. decoloratus	1,929 (17.7)	48 (58.5)	38 (48.1)	86 (53.4)
A. gemma	9 (0.08)	1 (1.2)	2 (2.5)	3 (1.86)
R. pulchellus	2 (0.02)	2 (2.4)	0 (0.0)	2 (1.24)
*R. evertsi evertsi**	49 (0.4)	3 (3.7)	11 (13.9)	14 (8.7)

*$p < 0.05$, **$p < 0.01$, the proportion of cattle infested with tick species was significantly different (Chi-squared test) between districts.

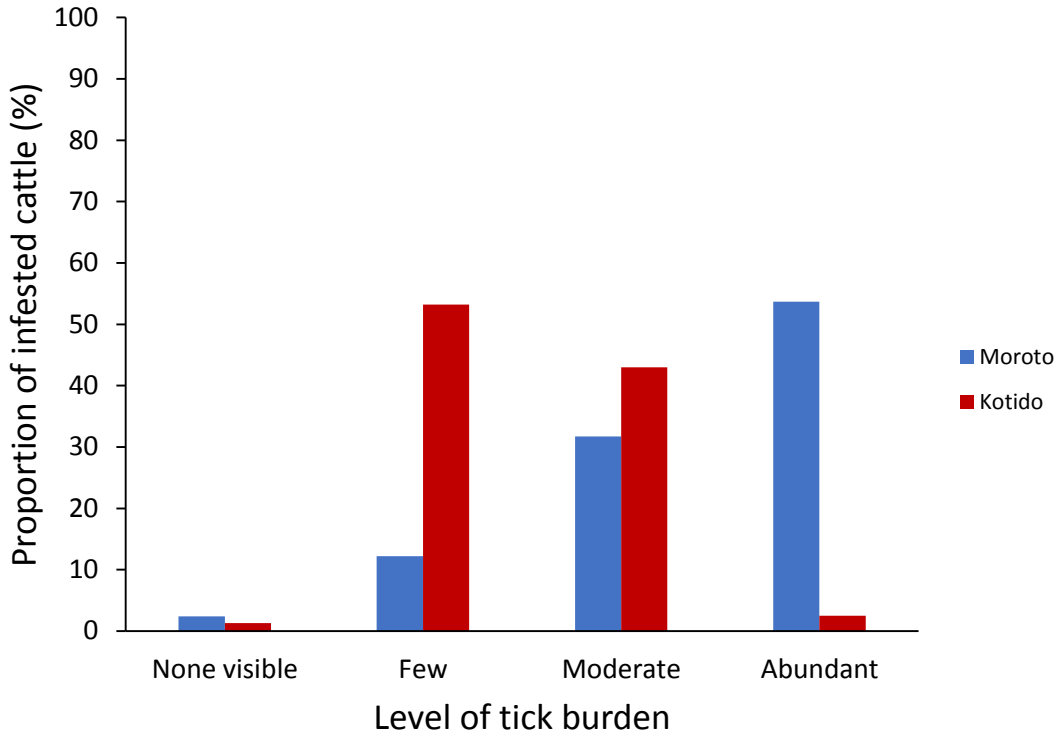

Figure 4.2: Bar chart showing the distribution of different levels of tick burden among 161 cattle in 20 herds in two districts of Karamoja Region, Uganda during a cross-sectional study, from November 2013 through January 2014.

4.4.3 *Theileria parva* and *Anaplasma marginale* seroprevalences and risk factors for seropositivity

Serum samples were collected from 20 herds and 397 individual cattle (17-24 per herd). Most cattle were female (74.3%). The numbers of cattle sampled were 208 for Moroto and 189 for Kotido. By age group, cattle sampled were: 175 (44.1%) for 5-12 months, 66 (16.6%) for 13-24 months, and 156 (39.3%) for > 24 months.

Overall, *T. parva* apparent seroprevalence was low (15.1%, 95% CI 11.8 – 17.9%) while that of *A. marginale* was high (86.6%, 95% CI 83.1 – 89.7%). The true prevalences were 16.5% (95% CI 12.9 – 19.6%) for *T. parva* and 95.1% (95% CI 91.3 – 98.5%) for *A. marginale*. All herds sampled had at least one animal seropositive for *A. marginale*, while 75% (15/20) of herds were seropositive for *T. parva*. Univariate analysis showed that the age of cattle was significantly associated with seropositivity for both *T. parva* and *A. marginale* ($p < 0.05$). Cattle of 5-12 months (18.3%, odds ratio [OR] = 4.1) and 13-24 months (30.3%, OR = 8.0) were more

likely to be seropositive for *T. parva* than those > 24 months (Table 4.2). For *A. marginale*, cattle of 13-24 months (92.4%, OR = 2.7) and > 24 months (89.7%, OR = 2.0) were more likely to be seropositive than those 5-12 months (Table 4.3). There was a statistically significant difference (Chi-squared test, $p < 0.001$, OR = 6.5) in the proportion of *T. parva* positive animals between Moroto (24.5%) and Kotido Districts (4.8%) (Table 4.2). Out of the 60 *T. parva* positive cattle in this study, 44 were from five herds from an area in Moroto, which was located closest to Kenya, but which also had more than half of the cattle in the 13-24 months age group. Although the difference in proportion of *A. marginale* positive cattle between Moroto and Kotido was not significantly different (Chi-squared test, $p > 0.05$) (Table 4.3), the five herds in Moroto had higher proportions (each ≥ 95%) compared to other herds. The sex of animal and presence of the tick vectors for *T. parva* and *A. marginale* were not associated with seropositivity ($p > 0.05$). The log ratio test for herd effect showed that *T. parva* and *A. marginale* serological status was clustered within herds ($p < 0.05$). When mixed effect logistic regression was conducted, no variable was associated with *T. parva* or *A. marginale* seropositivity ($p > 0.05$).

Table 4.2: Descriptive statistics and univariable associations between potential individual-level risk factors and *Theileria parva* serological test status in cattle in Karamoja Region, Uganda

Variable	Categories	No. +ve/total (%)	*p*-value
Age	5-12 months	32/175 (18.3)	< 0.001
	13-24 months	20/66 (30.3)	
	> 24 months	8/156 (5.1)	
District	Moroto	51/208 (24.5)	< 0.001
	Kotido	9/189 (4.8)	
Gender	Female	45/295 (15.3)	0.89
	Male	15/102 (14.7)	
R. appendiculatus presence	Yes	12/120 (10.0)	0.52
	No	2/37 (5.4)	

Results from 397 cattle in a cross-sectional study carried out from November 2013 through January 2014

Table 4.3: Descriptive statistics and univariable associations between potential individual-level risk factors and *Anaplasma marginale* serological test status in cattle in Karamoja Region, Uganda

Variable	Categories	No. +ve/total (%)	*p*-value
Age	5-12 months	143/175 (81.7)	0.03
	13-24 months	61/66 (92.4)	
	> 24 months	140/156 (89.7)	
District	Moroto	184/208 (88.5)	0.27
	Kotido	160/189 (84.7)	
Gender	Female	256/295 (86.8)	0.89
	Male	88/102 (86.3)	
R. (*B*.) *decoloratus* presence	Yes	73/83 (88.0)	0.44
	No	68/74 (91.9)	

Results from 397 cattle in a cross-sectional study carried out from November 2013 through January 2014

4.5 Discussion

To our knowledge, this study is the first to provide a systematic assessment of serum antibody prevalence to *T*. *parva* and *A*. *marginale* infections and tick species diversity among cattle under a pastoral transhumant production system in Karamoja Region, Uganda. Previous studies were conducted in other parts of Uganda under mixed farming systems (Rubaire-Akiiki et al., 2006; Kabi et al., 2008; Magona et al., 2011a).

Eight different species of ticks were found on cattle in the study area. The dominance of *R*. *appendiculatus*, *A*. *variegatum* and *R*. *decoloratus* on cattle is consistent with previous reports for other parts of Uganda (Rubaire-Akiiki et al., 2006; Chenyambuga et al., 2010; Magona et al., 2011a) and elsewhere in eastern Africa (Kivaria et al., 2012). Before this study, *R*. *pulchellus* (zebra tick) had not been reported from vegetation or animals in Uganda. The only record of *R*. *pulchellus* from Uganda was of one unattached female tick, which was collected from a tourist in Queen Elizabeth National Park who may have carried the tick from a Kenyan or Tanzanian game park, which lie within the normal distribution of *R*. *pulchellus* (Matthysse and Colbo, 1987). *Rhipicephalus pulchellus* may be spreading into Karamoja Region from neighbouring countries (especially Kenya), possibly due to uncontrolled animal movements and sharing of grazing areas with cross-border communities. Although the distribution of the tick species *A*. *lepidum*, *A*. *gemma*, and *H*. *truncatum* includes Uganda (Walker et al., 2013),

these ticks have not been reported in recent studies from other parts of the country (Rubaire-Akiiki et al., 2006; Chenyambuga et al., 2010; Magona et al., 2011a). *Amblyomma gemma*, *A. lepidum*, *R. pulchellus*, and *H. truncatum* were previously identified on cattle in Ethiopia (Kumsa et al., 2015) and Kenya (Kariuki et al., 2012). The high tick species diversity in the study area may affect the transmission dynamics of *A. marginale* whose biological transmission is effected by over 20 tick species (Aubry and Geale, 2011). The presence of *A. lepidum* may increase the risk of heartwater (Walker and Olwage, 1987), *Theileria mutans*, and *Theileria velifera* infections (Walker et al., 2013).

In this study, the tick burden was high, with about two-thirds of the cattle sampled moderately to highly infested. The tick numbers for *R. appendiculatus*, *R. decoloratus* and *A. variegatum* were twice, four times and five times, respectively, higher than those observed among cattle in Soroti District (Magona et al., 2011a), and Mbale and Sironko Districts in Uganda (Rubaire-Akiiki et al., 2006). Zebu cattle, which is the breed type reared in Karamoja Region, are known to be relatively resistant to tick infestation (Latif, 1993) due to their ability to mount an immune response to tick infestation (Mattioli et al., 2000). High tick-burden on the Zebu cattle in Karamoja may be attributed to: (i) communal grazing and extensive movement of cattle which increase the risk of exposure to tick-infested areas (Fyumagwa et al., 2007; Madder et al., 2011), (ii) the presence of wild animals including buffalo, which share grazing areas with cattle, and reportedly permit the persistence of tick populations (Walker et al., 2014), and (iii) inappropriate tick control practices including the use of little acaricide wash, wrong dilutions and irregular spraying observed in the area. Furthermore, host and environmental stress factors such as concurrent infections, poor nutrition and fatigue due to movements can reduce the resistance to ticks in the animals (Latif, 1993). A significantly higher proportion of cattle was abundantly infested with ticks in Moroto than Kotido. This may be explained by the observation that difficulty to access acaricide supplies as a constraint to tick control was more pronounced in Moroto than Kotido.

In this study, most of the ticks collected were adults (79-82%), followed by nymphs (13-18%) and a few were larvae (0-5%). There was a positive correlation between the proportions of adult and immature stages among the different tick species. Tick species with a higher number

of adults also had more immature stages. Our findings are consistent with the significant positive correlations of counts between species for each instar observed by Kaiser et al. (1982) in southern Uganda, indicating that population sizes are to some extent synchronised. In tropical regions of eastern Africa, several overlapping generations of ticks can be completed annually and no clear pattern of seasonal abundance may be evident, with all instars occurring simultaneously on cattle (Kaiser et al., 1982; Walker et al., 2013). Therefore, the period of sampling for this study may have no implications in terms of which tick stages might be favoured or missed. Given the relatively high diversity of tick species encountered in this study, their potential as vectors of diseases of livestock, wildlife and humans should be further investigated, by testing the ticks for pathogens and conducting transmissibility studies. Future studies should also evaluate the efficacy of potential control methods on the tick species present, including the use of acaricides (various compositions, spraying intervals and application methods) and vaccines, which may contribute to the development of cost-effective methods for control of ticks and TBDs.

There was a low true seroprevalence for *T. parva* (16.5%), which may suggest a high likelihood of ECF in animals at the time of peak exposure to *T. parva* and increased likelihood of disease following a single exposure (Jonsson et al., 2012). The *T. parva* seroprevalence was lower than reported previously in similar age groups of cattle, but under mixed farming systems, in eastern Africa: 100% for Soroti, Uganda (Kabi et al., 2008), 19% for Mbeere District, Kenya (Gachohi et al., 2010), 40.9% in Machakos County, Kenya (Wesonga et al., 2015), 70% for central Equatoria State, South Sudan (Malak et al., 2012), and 44.3% for Tanga Region, Tanzania (Swai et al., 2009). The low seroprevalence for *T. parva* in this study may be due to low infection challenge from ticks, supported by the fact that there was no association between presence of *R. appendiculatus* on cattle and seropositivity, and yet most cattle (77%) were infested. However, we did not establish the infection rate in ticks. Cycles of drought occur in Karamoja Region, and these may interrupt the survival and development of *R. appendiculatus*, a three-host tick which spends at least 90% of its lifecycle on the ground and in the vegetation, making the tick sensitive to extremes of temperature. This in turn may affect the propagation of *T. parva* (Perry and Young, 1995). Furthermore, since *T. parva* is

transstadially transmitted, the tick loses infection after transmission and across generations (Uilenberg, 2006).

Furthermore, cases of ECF were not treated effectively, which resulted in high case fatality. The proportion of cattle that are carriers declines with increased mortality rates of infected cattle. This leads to low seropositivity/population immunity among cattle (Dolan, 1986; Perry and Young, 1995). There was higher *T. parva* seroprevalence in cattle of 13-24 months compared to those 5-12 months; however, the difference was not statistically significant when the mixed effect logistic regression analysis was done. Most *T. parva* positive cattle were from five herds from an area in Moroto, which also had more than half of the cattle in the 13-24 months age group. This probably is the explanation why we did not find any significance of age in the mixed effect logistic regression analysis. The effect of age in the univariate approach was therefore due to the unbalanced proportion of age between the herds. A previous study in Kenya showed that increase in age, up to 24 months, is associated with increased *T. parva* seroprevalence (Gachohi et al., 2012). This is because age is a proxy for exposure time, and antibodies for *T. parva* persist in the circulation for as long as six months (Gachohi et al., 2012). The mean proportion of ECF cases in calves < 6 months (from proportional piling) was higher than that in the same age group for anaplasmosis. East Coast fever is more severe in young calves (Jonsson et al., 2012; Thumbi et al., 2013), and the disease does not feature inverse age immunity like babesiosis and anaplasmosis (Jonsson et al., 2012). The pastoralists reported a higher proportion of ECF cases in adult cattle (> 12 months) compared to calves of up to 12 months. In this study area, the calves are grazed around settlement areas, and this may reduce the exposure rate of the calves to ticks infected with *T. parva*.

High true seroprevalence for anaplasmosis (95.1%) may suggest that a high proportion of cattle were exposed to infection. Our findings are in contrast with those from previous studies in eastern Africa, in similar age groups of cattle but under mixed farming systems, that showed lower seroprevalence for *A. marginale*: 57% for Soroti District, Uganda (Kabi et al., 2008), 58% for Mbeere District, Kenya (Gachohi et al., 2010), 50% in central Equatoria State, South Sudan (Malak et al., 2012), and 41.1% for Tanga Region, Tanzania (Swai et al., 2009).

The most likely explanation for the high seroprevalence in this study is high persistent infections from the tick vectors. A high proportion of cattle (53.4%) were infested with *R*. *decoloratus*, the main vector of *A*. *marginale* in eastern Africa; however, the level of infection in the ticks was not established. Once infected, animals remain carriers for life. The persistent infections then result in host antibody responses which can be detected by the MSP5 cELISA (Knowles et al., 1996; Castañeda-Ortiz et al., 2015). However, there was no significant association between *A*. *marginale* seropositivity and presence of *R*. *decoloratus*. This may suggest either that the actual ticks that infected the animals, and caused them to mount an immune response, were no longer present on the cattle at the time of collection, or that the pathogen was also transmitted by other mechanisms. *Anaplasma marginale* can be transmitted mechanically by haematophagous arthropods and contaminated fomites, and through the placenta (Aubry and Geale, 2011). Haematophagous arthropods such as the *Culicoides* midges were abundant in the grazing areas, and the livestock keepers often used disposable needles on more than one animal. The seropositivity for anaplasmosis increased with age, which is consistent with the concept of reverse age immunity for anaplasmosis (Aubry and Geale, 2011). However, the difference in seropositivity to *A*. *marginale* was not statistically significant from the mixed effect logistic regression analysis.

False positivity for *A*. *marginale* may occur due to cross reactivity of *A*. *marginale* with *A*. *centrale*, *A*. *phagocytophilum* and *A*. *ovis* (Knowles et al., 1996), and with *Ehrlichia* spp. (Al-Adhami et al., 2011). However, cattle are not susceptible to infection with *A*. *ovis*, and low levels (< 5%) of other *Anaplasma* and *Ehrlichia* spp. have been reported in cattle populations in Uganda (Muhanguzi et al., 2010; Oura et al., 2011). In this study, as described in Chapter Five of this thesis, the prevalence of *A*. *centrale* (12.1% by quantitative PCR [qPCR]) was much lower than that of *A*. *marginale* (82.9% by qPCR).

Endemic stability for ECF is defined as the state in which the majority (> 70%) of the cattle population becomes infected and immune by six months of age, and little or no clinical disease occurs. Endemic instability, on the other hand, describes a state in which only a small proportion (< 30%) of cattle in the population become infected and immune by six months of age (Gachohi et al., 2013). Norval et al. (1983) classified endemic status for TBDs as: stable

endemic situation (81-100% positive sera), a situation approaching stability (61-80%), and an unstable situation (≤ 60%). Recently, Jonsson et al. (2012) suggested that if 75% of calves were exposed to infection (indicated by 75% seroprevalence) before nine months of age, then clinical tick-borne disease should be rare. The low seroprevalence for *T. parva* combined with clinical cases of ECF reported by informant groups and diagnosed in this study, suggest endemic instability of ECF in Karamoja Region. However, high seroprevalence may not always indicate endemic stability of TBDs. There should be a high level of challenge of calves by infected ticks with a consequent acquired immunity in adult animals and low incidence of clinical disease (Norval et al., 1992; Jonsson et al., 2012). Endemic stability may therefore be difficult to achieve due to variation in climate and management practices which affect exposure of animals to infection (Jonsson et al., 2012). Moreover, seropositive animals may not all be solidly protected against immunologically diverse heterologous strains. Different genotypes might differ in pathogenicity, and the immunological and genetic heterogeneity among hosts might influence susceptibility of infected animals to disease (Jonsson et al., 2012). Therefore, despite high seropositivity, clinical examinations and focus group discussions from this study revealed clinical cases of anaplasmosis in various age groups of cattle. Genetic and antigenic diversity among *T. parva* isolates has been demonstrated in Uganda (Oura et al., 2011; Muwanika et al., 2016) and Tanzania (Mwega et al., 2015), and many strains of *A. marginale* have been identified from various geographical areas around the world (Cabezas-Cruz and de la Fuente, 2015). Although antibody responses against the schizont stage of *T. parva* are detectable in recovering animals, and provide the most reliable evidence of exposure to the parasite, these mechanisms are not considered to play a role in protection (McKeever and Morrison, 1990). Immunity to *T. parva* in cattle is mediated predominantly by cellular mechanisms directed at the schizont-infected cell. This is supported by evidence that there is lack of correlation between the anti-schizont antibody and protection (McKeever and Morrison, 1990). In endemic stable areas, persistent infections occur in cattle due to superinfection with *A. marginale* antigenic variants (Castañeda-Ortiz et al., 2015). The variants evade the immune response to the primary strain or infect the truly naïve animals which results in acute bacteraemia (Palmer and Brayton, 2013). Therefore, further studies need to assess the possibility of *A. marginale* superinfection among cattle in Karamoja and evaluate the incidence of anaplasmosis in animals infected with diverse

parasite genotypes. The pastoralists reported an increase in the proportion of anaplasmosis cases with age. Although cattle of all ages can become infected with *A*. *marginale*, the occurrence and severity of anaplasmosis increases with age of animals (Aubry and Geale, 2011).

Although a single cross-sectional study may be less powerful to determine the endemic status of TBDs, the epidemiological information from this study may provide a useful indicator of the endemic status of TBDs in Karamoja Region, and therefore a scientific basis for planning future strategies for control. It may not be feasible to conduct longitudinal studies in this area because of the difficulty to follow-up particular animals or herds. An effective control of ticks and TBDs can be achieved by integrating the strategic use of acaricides and application of vaccines (Norval et al., 1992; Minjauw and McLeod, 2003). Given the high proportion of cattle that are not exposed to *T*. *parva* in Karamoja Region, immunisation is essential to increase the proportion of immune animals (Gachohi et al., 2012). However, a high level of challenge from naturally infected *R*. *appendiculatus* ticks is required to sustain the immunity (Gachohi et al., 2012; Gachohi et al., 2013).

4.6 Conclusions

Tick species that have not been reported in recent studies in Uganda were found in Karamoja Region. This may suggest that the ticks are spreading into Karamoja Region from neighbouring countries (especially Kenya), possibly due to uncontrolled animal movements and sharing of grazing areas with cross-border communities. The low seroprevalence for *T*. *parva* in this study may be attributed to low *T*. *parva* infection challenge from ticks. High seroprevalence for *A*. *marginale* may be attributed to biological and mechanical transmission leading to high persistent infections, and subsequent seropositivity. The epidemiological data from this study may support future control programmes for ticks and TBDs.

4.7 References

AL-ADHAMI, B., SCANDRETT, W.B., LOBANOV, V.A., GAJADHAR, A.A., 2011. Serological cross-reactivity between *Anaplasma marginale* and an *Ehrlichia* species in naturally and experimentally infected cattle. Journal of Veterinary Diagnostic Investigation 23, 1181-1188.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

CABEZAS-CRUZ, A., DE LA FUENTE, J., 2015. *Anaplasma marginale* major surface protein 1a: A marker of strain diversity with implications for control of bovine anaplasmosis. Ticks and Tick-borne Diseases 6, 205-210.

CASTAÑEDA-ORTIZ, E.J., UETI, M.W., CAMACHO-NUEZ, M., MOSQUEDA, J.J., MOUSEL, M.R., JOHNSON, W.C., PALMER, G.H., 2015. Association of *Anaplasma marginale* strain superinfection with infection prevalence within tropical regions. PLoS One 10 (3), e0120748. doi: 10.1371/journal.pone.0120748.

CATLEY, A., 2006. Use of participatory epidemiology to compare the clinical veterinary knowledge of pastoralists and veterinarians in East Africa. Tropical Animal Health and Production 38, 171-184.

CATLEY, A., ADMASSU, B., BEKELE, G., ABEBE, D., 2014. Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. Disasters 38, 500-516.

CATLEY, A.P., MOHAMMED, A.A., 1996. The use of livestock-disease scoring by a primary animal-health project in Somaliland. Preventive Veterinary Medicine 28, 175-186.

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. Livestock Research for Rural Development Volume 22 (7), Article # 135. Retrieved May 2, 2013, from http://www.lrrd.org/lrrd22/7/chen22135.htm.

DOLAN, T.T., 1986. Chemotherapy of East Coast fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. Journal of Comparative Pathology 96, 137-146.

EGERU, A., WASONGA, O., KYAGULANYI, J., MAJALIWA, G.J.M., MACOPIYO, L., MBURU, J., 2014. Spatio-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda. Pastoralism: Research, Policy and Practice 4, 6.

FYUMAGWA, R.D., RUNYORO, V., HORAK, I.G., HOARE, R., 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. South African Journal of Wildlife Research 37, 79-90.

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, 194.

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, 103-113.

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, 208-223.

GODDEERIS, B.M., KATENDE, J.M., IRVIN, A.D., CHUMO, R.S., 1982. Indirect fluorescent antibody test for experimental and epizoological studies on East Coast fever (*Theileria parva* infection in cattle). Evaluation of a cell culture schizont antigen fixed and stored in suspension. Research in Veterinary Science 33, 360-365.

GREINER, M., GARDNER, I.A., 2000. Application of diagnostic tests in veterinary epidemiologic studies. Preventive Veterinary Medicine 45, 43-59.

IBM SPSS, 2014. IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY.

IICD, 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities, Ruspantini, C. (Ed.), European Commission, Government of Uganda and Institute for International Co-operation and Development (IICD) Uganda, 67 pp. www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/C&DPublicationLivestockDiseaseSurveillance2.pdf. Accessed March 24, 2015.

JONGEJAN, F., UILENBERG, G., 2004. The global importance of ticks. Parasitology 129, S3-S14.

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, 85-89.

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. Journal of Protozoology Research 18, 61-70.

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, 414.

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, 63-74.

KARIUKI, E.K., PENZHORN, B.L., HORAK, I.G., 2012. Ticks (Acari: Ixodidae) infesting cattle and African buffaloes in the Tsavo conservation area, Kenya. Onderstepoort Journal of Veterinary Research 79, 1-4.

KILLIP, S., MAHFOUD, Z., PEARCE, K., 2004. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers. The Annals of Family Medicine 2, 204-208.

KIVARIA, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production 38, 291-299.

KIVARIA, F.M., KAPAGA, A.M., MBASSA, G.K., MTUI, P.F., WANI, R.J., 2012. Epidemiological perspectives of ticks and tick-borne diseases in South Sudan: Cross-sectional survey results. Onderstepoort Journal of Veterinary Research 79, 1-10.

KNOWLES, D., TORIONI DE ECHAIDE, S., 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, 2225-2230.

KUMSA, B., SOCOLOVSCHI, C., RAOULT, D., PAROLA, P., 2015. New *Borrelia* species detected in ixodid ticks in Oromia, Ethiopia. Ticks and Tick-borne Diseases 6, 401-407.

LATIF, A.A., 1993. Sustainable control methods of ticks and tick-borne diseases in Africa. In: Future of livestock industries in East and Southern Africa, KATEGILE, J.A., MUBI, S. (Eds.), Proceedings of the Workshop held at Kadoma Ranch Hotel, Zimbabwe 20-23 July 1992. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia, 227 pp.

LYNEN, G., DI GIULIO, G., HOMEWOOD, K., REID, R., MWILAWA, A., 2006. Deployment of a live ECF vaccine in pastoral areas: lessons learned from Tanzania. In: The role of biotechnology in animal agriculture to address poverty in Africa: opportunities and challenges. Proceedings of the 4th All African Conference on Animal Agriculture and the 31st Annual Meeting of the Tanzania Society for Animal Production (TSAP), 20-24

September, 2005, REGE, J.E.O., NYAMU, A.M., SENDALO, D. (Eds.), Arusha, Tanzania, All Africa Society for Animal Production (AASAP), Nairobi, Kenya, 193-201.

MADDER, M., THYS, E., ACHI, L., TOURÉ, A., DE DEKEN, R., 2011. *Rhipicephalus* (*Boophilus*) *microplus*: a most successful invasive tick species in West-Africa. Experimental and Applied Acarology 53, 139-145.

MAGONA, J.W., WALUBENGO, J., KABI, F., 2011a. Response of Nkedi Zebu and Ankole cattle to tick infestation and natural tick-borne, helminth and trypanosome infections in Uganda. Tropical Animal Health and Production 43, 1019-1033.

MAGONA, J.W., WALUBENGO, J., OLAHO-MUKANI, W., JONSSON, N.N., WELBURN, S.W., EISLER, M.C., 2011b. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55, 203-213.

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, 216-223.

MATTHYSSE, J.G., COLBO, M.H., 1987. The Ixodid Ticks of Uganda. Entomological Society of America, College Park, Maryland, pp. xii + 426 pp.

MATTIOLI, R.C., PANDEY, V.S., MURRAY, M., FITZPATRICK, J.L., 2000. Immunogenetic influences on tick resistance in African cattle with particular reference to trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Gobra zebu (*Bos indicus*) cattle. Acta Tropica 75, 263-277.

MCKEEVER, D.J., MORRISON, W.I., 1990. *Theileria parva*: the nature of the immune response and its significance for immunoprophylaxis. Scientific and Technical Review of the Office International des Epizooties (Paris) 9, 405-421.

MINJAUW, B., MCLEOD, A., 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in

India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

MUGISHA, A., MCLEOD, A., PERCY, R., KYEWALABYE, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

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, 91.

MUWANIKA, V., KABI, F., MASEMBE, C., 2016. Population genetic structure of *Theileria parva* field isolates from indigenous cattle populations of Uganda. Ticks and Tick-borne Diseases 7, 291-297.

MWEGA, E., HASAN, S.D., MOSES, N., ELPIDIUS, R., SKILTON, R., GWAKISA, P., 2015. Genetic and antigenic diversity of *Theileria parva* in cattle in eastern and southern zones of Tanzania. A study to support control of East Coast fever. Parasitology 142, 698-705.

NORVAL, R.A.I., FIVAZ, B.H., LAWRENCE, J.A., DAILLECOURT, T., 1983. Epidemiology of tick-borne diseases of cattle in Zimbabwe. I. Babesiosis. Tropical Animal Health and Production 15, 87-94.

NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S., LAWRENCE, J.A., MUKHEBI, A.W., BISHOP, R.P., MCKEEVER, D.J., SHAW, M.K., 1992. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

OCAIDO, M., MUWAZI, R.T., OPUDA, J.A., 2009a. Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. Tropical Animal Health and Production 41, 731-739.

OCAIDO, M., OTIM, C.P., KAKAIRE, D., 2009b. Impact of major diseases and vectors in smallholder cattle production systems in different agro-ecological zones and farming systems in Uganda. Livestock Research for Rural Development Volume 21 (9), Article #155. Retrieved November 29, 2013, from http://www.lrrd.org/lrrd21/9/ocai21155.htm.

ONONO, J.O., WIELAND, B., RUSHTON, J., 2013. Constraints to cattle production in a semiarid pastoral system in Kenya. Tropical Animal Health and Production 45, 1415-1422.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

PALMER, G.H., BRAYTON, K.A., 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15, 1969-1975.

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, 107-120.

R Console, 2015. "World-Famous Astronaut". Copyright © 2015. The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit).

RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

RUBAIRE-AKIIKI, C.M., OKELLO-ONEN, J., MUSUNGA, D., KABAGAMBE, E.K., VAARST, M., OKELLO, D., OPOLOT, C., BISAGAYA, A., OKORI, C., BISAGATI, C., ONGYERA, S., MWAYI, M.T., 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, 251-266.

SHIFERAW, T.J., MOSES, K., MANYAHILISHAL, K.E., 2010. Participatory appraisal of foot and mouth disease in the Afar pastoral area, northeast Ethiopia: implications for understanding disease ecology and control strategy. Tropical Animal Health and Production 42, 193-201.

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, 34-42.

THUMBI, S.M., BRONSVOORT, M.B., KIARA, H., TOYE, P.G., POOLE, J., NDILA, M., CONRADIE, I., JENNINGS, A., HANDEL, I.G., COETZER, J.A.W., STEYL, J., HANOTTE, O., WOOLHOUSE, M.E.J., 2013. Mortality in East African shorthorn zebu cattle under one year: predictors of infectious-disease mortality. BMC Veterinary Research 9, 175.

UILENBERG, G., 2006. *Babesia* - A historical overview. Veterinary Parasitology 138, 3-10.

WALKER, A.R., BOUATTOUR, A., CAMICAS, J.-L., ESTRADA-PEÑA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G., PRESTON, P.M., 2013. Ticks of domestic animals in Africa: a guide to identification of species. International Consortium on Ticks and Tick Borne Diseases (ICTTD-2), 221 pp.

WALKER, J.B., OLWAGE, A., 1987. The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. The Onderstepoort Journal of Veterinary Research 54, 353-379.

WALKER, J.G., KLEIN, E.Y., LEVIN, S.A., 2014. Disease at the wildlife-livestock interface: Acaricide use on domestic cattle does not prevent transmission of a tick-borne pathogen with multiple hosts. Veterinary Parasitology 199, 206-214.

WESONGA, F.D., GACHOHI, J.M., KITALA, P.M., GATHUMA, J.M., NJENGA, M.J., 2015. *Theileria parva* infection seroprevalence and associated risk factors in cattle in Machakos County, Kenya. Tropical Animal Health and Production 47, 93-101.

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. Livestock Research for Rural Development Volume 22 (6), Article #111. Retrieved December 13, 2013, from http://www.irrd.org/Irrd22/6/weso22111.htm.

CHAPTER 5

5. Molecular investigation of tick-borne haemoparasite infections among transhumant Zebu cattle in Karamoja Region, Uganda

5.1 Abstract

Tick-borne diseases (TBDs) are a major constraint to cattle production in pastoral areas in Africa. Although information on tick-borne infections is important to prioritise control approaches, it is limited for transhumant Zebu cattle in Karamoja, Uganda. We conducted a study to determine the occurrence and level of tick-borne infections among cattle in Karamoja Region. A total of 240 cattle were selected for blood collection using systematic sampling in 20 randomly-selected herds in two districts. The hypervariable V4 region of the 18S rRNA gene for *Theileria*/*Babesia* and the V1 region of the 16S rRNA gene for *Ehrlichia*/*Anaplasma* were amplified and hybridised to genus- and species-specific oligonucleotide probes on a reverse line blot (RLB) membrane. A duplex quantitative real-time polymerase chain reaction (qPCR) assay based on *msp1β* and *groEL* genes was used for the detection of *Anaplasma marginale* and *A. centrale*, while monoplex qPCR assays were used for the detection of *Ehrlichia ruminantium* (226 bp fragment of the pCS20 region) and *Theileria parva* (18S rRNA gene). The RLB hybridisation assay demonstrated the presence of tick-borne haemoparasites in all but one sample (99.6%), mostly as mixed infections (97.5%). The most frequently detected species were *Theileria mutans* (88.3%, 95% confidence interval: 84.6-92.1%), *A. marginale* (73.8%: 68.3-79.2%), *T. velifera* (71.3%: 65.8-76.7%) and *Anaplasma* sp. Omatjenne (63.3%: 57.5-68.8%). Other virulent pathogens, namely *Babesia bigemina* (5.0%) and *T. parva* (2.9%), were also detected with RLB, but not *E. ruminantium*. The proportions of qPCR positive samples were 82.9% (*A. marginale*), 12.1% (*A. centrale*), 3.3% (*T. parva*), and 1.7% (*E. ruminantium*). The full-length 18S rRNA genes from 6 out of 47 samples that were positive on RLB for the *Babesia* genus-specific probe and not for any of the *Babesia* species-specific probes were amplified, cloned and sequenced. The sequences were used to construct

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phylogenetic trees. Variations (5 to 9 nucleotides) in the 18S rRNA gene sequences of *B. bigemina* were identified, when compared with *B. bigemina* sequences from other parts of the world. Three nucleotide differences in the *B. bigemina* probe region may explain the failure of the RLB hybridisation assay to detect *B. bigemina* in some samples. *Theileria mutans* and *B. bigemina* sequences grouped in separate clades from previously published sequences. In conclusion, this study demonstrated high and widespread occurrence, and sequence variation of tick-borne haemoparasites among cattle in the pastoral area of Karamoja, which is useful for diagnosis and control of TBDs.

5.2 Introduction

In pastoral areas of sub-Saharan Africa, cattle are regularly exposed to tick-borne haemoparasites of the genera *Theileria*, *Anaplasma*, *Babesia* and *Ehrlichia* (Gachohi et al., 2010; Kasozi et al., 2014). Tick-borne diseases (TBDs) inflict significant economic losses on cattle production and resource use, thereby impacting the livelihoods of pastoralists (Kivaria, 2006; Ocaido et al., 2009a). Losses directly attributed to TBDs include mortality, production losses, and the costs of veterinary diagnosis, treatment and tick control (Kivaria, 2006). Previous studies in Uganda showed that 75.4% of losses in cattle were attributable to ticks and TBDs (Ocaido et al., 2009b), and the costs for controlling ticks and TBDs constituted 85% of the total costs for disease control in cattle (Ocaido et al., 2009a). Tick-borne diseases also constrain the improvement of the local breeds of cattle due to the high morbidity and mortality in exotic (*Bos taurus*) and crossbred cattle (Muhanguzi et al., 2010b).

The most pathogenic and economically significant tick-borne haemoparasites of cattle in East Africa are *Theileria parva*, *Anaplasma marginale*, *Babesia bigemina* and *Ehrlichia ruminantium*. *T. parva*, an apicomplexan protozoan parasite of cattle, is transmitted by *Rhipicephalus appendiculatus* and causes East Coast fever (ECF), an acute, usually fatal, lymphoproliferative disease (Norval et al., 1992). In Africa, bovine babesiosis is caused mainly by the intra-erythrocytic protozoan parasites, *B. bigemina* and *B. bovis* (Uilenberg, 2006).

Babesia bigemina is reported to be the main cause of bovine babesiosis in Uganda and is transmitted by *R*. *decoloratus* (Rubaire-Akiiki et al., 2004; Magona et al., 2011b). *A*. *marginale*, an intra-erythrocytic rickettsia, is the main cause of bovine anaplasmosis, a tick-borne, haemolytic disease that is endemic across much of the globe (Aubry and Geale, 2011). In Uganda, *A*. *marginale* is transmitted by *R*. *decoloratus* (Rubaire-Akiiki et al., 2006; Magona et al., 2011a; Magona et al., 2011b). Other forms of transmission for *A*. *marginale* include mechanical transfer of contaminated blood through haematophagous arthropods and fomites. Placental transmission during pregnancy can also occur (Costa et al., 2016). The obligate intracellular rickettsia, *E*. *ruminantium*, is transmitted by ticks of the genus *Amblyomma* and causes heartwater disease in ruminants (Allsopp, 2010). *Amblyomma variegatum* is the reported tick vector of heartwater in Uganda (Magona et al., 2011a).

The development of molecular biological techniques has resulted in the improved detection, identification and genetic characterisation of many haemoparasites. Highly specific and sensitive species-specific quantitative real-time polymerase chain reaction (qPCR) and PCR-based reverse line blot (RLB) hybridisation assays have been developed and used for the detection of tick-borne infections (Gubbels et al., 1999; Bekker et al., 2002; Decaro et al., 2008; Sibeko et al., 2008; Steyn et al., 2008; Muhanguzi et al., 2010a; Muhanguzi et al., 2010b; Njiiri et al., 2015). The RLB assay enables the identification of mixed infections and has proven to be a valuable tool in the identification of novel parasites (Nijhof et al., 2005; Oosthuizen et al., 2008). The RLB assay has previously been used to detect tick-borne haemoparasites in cattle samples from Uganda; the most common haemoparasites detected were: *T*. *parva*, *T*. *mutans*, *T*. *taurotragi*, *T*. *velifera*, *A*. *marginale*, *A*. *centrale*, *A*. *bovis* and *E*. *ruminantium* (Muhanguzi et al., 2010a; Muhanguzi et al., 2010b; Oura et al., 2011; Asiimwe et al., 2013).

Karamoja Region in north-eastern Uganda is characterised by a semi-arid climate, and communities rely on livestock as the main source of livelihood (Anderson and Robinson, 2009; IICD, 2010). Like other pastoral groups in eastern Africa, the Karamojong practice mobile livestock herding (Egeru et al., 2014). The major drivers of transhumant pastoralism are

drought conditions, which result from climatic variability, and consequent seasonal pasture and water scarcity, and animal diseases. The practice is therefore a key coping mechanism for pastoralists to avoid cattle losses and ensure livestock productivity (Anderson and Robinson, 2009; Egeru et al., 2014). Cattle in Karamoja Region are of the short-horned East African zebu type (*Bos indicus*) (Anderson and Robinson, 2009). Despite the social and economic importance of cattle in Karamoja communities, little is known about tick-borne haemoparasite infections in the Karamoja Region. A recent study showed that the Karamojong pastoralists perceive TBDs to be the most important diseases impacting cattle production (Byaruhanga et al., 2015b) [Chapter 3 of this thesis]. It is important to have knowledge of tick-borne infections in cattle populations from particular production systems so as to prioritise and develop appropriate control measures against TBDs.

The objectives of this study were to determine which tick-borne pathogens are present among cattle in the transhumant production system of Karamoja, Uganda, the prevalence of infections as indicated by RLB hybridisation and qPCR methods, and the molecular characteristics of these pathogens by sequencing and phylogenetic analyses.

5.3 Materials and Methods

5.3.1 Ethics statement

The study was approved by the Animal Ethics Committee of the University of Pretoria, South Africa (V026-14) and the National Agricultural Research Organisation, Uganda (no. 1416). Permission was obtained to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa; reference number 12/11/1/1). A veterinary import permit (permit number 13/1/1/30/2/0-201408003716, Department of Agriculture, Forestry and Fisheries, Republic of South Africa) was obtained to transport the blood samples from Uganda to South Africa. Standard techniques were followed in collecting blood samples for laboratory examination.

5.3.2 Study area

This study was conducted in Moroto and Kotido Districts of Karamoja Region, north-eastern Uganda (Map shown in Chapter 3 of this thesis), from November 2013 through January 2014.

5.3.3 Study design and study animals

Twenty herds, 10 from each district, were randomly selected from 20 purposively-selected superherds for the study (see Map in Chapter 3). The effective sample size of cattle was calculated by taking into account the design effect, which is the loss of effectiveness by the use of cluster sampling, instead of simple random sampling. The design effect is basically the ratio of the actual variance, under the sampling method actually used, to the variance computed under the assumption of simple random sampling. The design effect was estimated using the formula below (Killip et al., 2004):

$$DE = 1 + \delta (n-1)$$

where DE is the design effect, δ is the intracluster correlation coefficient for being positive to tick-borne pathogens, and n is the average cluster size. No pilot study was conducted to calculate the intracluster correlation coefficient, therefore a δ value of 0.03 was assumed. A value of 0.03 is interpreted to mean that the cattle in a herd (cluster) are about only 3% more likely to have the same chance of exposure than if the two animals were chosen at random in the survey. A relatively large within-cluster variance is expected within a herd in the study area because groups of herds (10 to 20) graze together over a large area, which is expected to greatly reduce the chance that animals from the same herd are equally exposed to infected ticks. The average herd size was 40 cattle and a DE value of 2.17 was calculated. Therefore, the sample variance is 2.17 times bigger than it would be if the survey were based on the same sample size but selected randomly. An effective sample size of 369 cattle was calculated using the formula below (Killip et al., 2004):

$$ESS = MK/DE$$

where m = average number of cattle in a herd, k = number of herds, ESS = effective sample size, and DE = design effect. However, given the available laboratory logistics, a total of 240 cattle were sampled for blood collection. In each herd, 12 cattle of various age groups and both sexes were selected by systematic sampling to represent about 30% of a herd. In a herd, animals were grouped by age category, and then sub-grouped by sex. At first, one animal was selected at random in a sub-group, and then other animals were picked using pre-determined sampling intervals. Proportional allocation was used to determine the number of cattle sampled in each category.

5.3.4 Blood sample collection and DNA extraction

Blood was collected aseptically from the jugular vein of each animal into 5 ml vacuum tubes (Becton Dickinson Vacutainer systems Europe, UK) with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. About 125 µl of each blood sample was spotted on FTA® Classic Card (Whatman®, Whatman International Ltd, Maidstone, England) and left to dry for one hour, and then transported to the Department of Veterinary Tropical Diseases, University of Pretoria, South Africa. Five discs of 0.75 mm each were excised from each FTA card blood spot, and DNA was extracted from the discs using the QIAamp® DNA Mini Kit (QIAGEN, Southern Cross Biotechnology Pty Ltd, Cape Town, South Africa). The DNA was stored at -20°C until further analyses.

5.3.5 Reverse line blot (RLB) hybridisation assay

The RLB hybridisation assay was performed as previously described (Gubbels et al., 1999; Bekker et al., 2002; Nijhof et al., 2003; Nijhof et al., 2005), to simultaneously detect and differentiate various pathogenic and non-pathogenic tick-borne parasite species that could possibly be present in the blood of carrier and sick cattle. Furthermore, co-infections with tick-borne haemoparasites can be determined by RLB. Separate PCR master mixes were prepared for the amplification of *Theileria*/*Babesia* species (Nijhof et al., 2003; Nijhof et al., 2005) and *Ehrlichia*/*Anaplasma* species (Bekker et al., 2002) using Platinum® Quantitative PCR

SuperMix-UDG (Invitrogen, The Scientific Group, South Africa). The primers RLB-F2 (5'-GAC ACA GGG AGG TAG TGA CAA G-3') and RLB-R2 (biotin-5'-CTA AGA ATT TCA CCT CTG ACA GT-3') were used to amplify a 460 to 540 bp fragment of the 18S rRNA gene spanning the V4 hypervariable region conserved for *Theileria* and *Babesia* species. For *Ehrlichia* and *Anaplasma* species, a 492 to 498 bp fragment of the hypervariable V1 region of the 16S rRNA gene was amplified using primers Ehr-F (5'-GGA ATT CAG AGT TGG ATC MTG GYT CAG-3') and Ehr-R (biotin-5'-CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT-3'). Each reaction (25 µl) contained 2.5 µl genomic DNA, 0.1 µM of each primer, 3 mM $MgCl_2$, 200 µM each of dGTP, dATP and dCTP, 400 µM dUTP, 0.75 U Platinum® Taq DNA polymerase, 0.5 U Uracil-DNA glycosylase (UDG) and 9.5 µl nuclease-free water. A touchdown thermal cycling program was used as previously described (Nijhof et al., 2005). DNA extracted from *B. bovis* and *A. centrale* blood vaccines (Onderstepoort Biological Products, Pretoria, South Africa) were used as positive controls for the 18S rRNA PCR and 16S rRNA PCR, respectively. The negative control contained PCR reagents but no template DNA. Table 5.1 shows the *Anaplasma*, *Ehrlichia*, *Theileria* and *Babesia* genus- and species-specific probes that were covalently linked to a Biodyne C blotting membrane (Pall Biosupport, Ann Arbor, USA) used in the RLB hybridisation assay. PCR products were applied to the membrane and hybridised to the probes using an MN45 mini blotter apparatus (Immunetics, Cambridge, UK) as previously described (Nijhof et al., 2005). Hybridised PCR products were detected as a probe-PCR-streptavidin complex by enhanced chemiluminescence (ECL).

Table 5.1: Genus- and species-specific reverse line blot hybridisation oligonucleotide probes that were used for the detection of tick-borne haemoparasites in blood samples of cattle from Karamoja Region, Uganda, November 2013 through January 2014

Oligonucleotide probe name	Sequence (5' to 3')
Ehrlichia/*Anaplasma* group-specific ("E/A catch-all")	GGG GGA A AG ATT TAT CGC TA
Anaplasma centrale	TCG AAC GGA CCA TAC GC
Anaplasma marginale	GAC CGT ATA CGC AGC TTG
Anaplasma phagocytophilum	TTG CTA TAA AGA ATA ATT AGT GG
Ehrlichia ruminantium	AGT ATC TGT TAG TGG CAG
Anaplasma bovis	GTA GCT TGC TAT GAG AAC A
Ehrlichia chaffeensis	ACC TTT TGG TTA TAA ATA ATT GTT
Anaplasma sp. (Omatjenne)	CGG ATT TTT ATC ATA GCT TGC
Ehrlichia canis	TCT GGC TAT AGG AAA TTG TTA
Theileria/*Babesia* group-specific ("T/B catch-all")	TAA TGG TTA ATA GGA RCR GTT G
Theileria genus-specific ("T catch-all")	ATT AGA GTG TTT CAA GCA GAC
Babesia genus-specific 1 ("B catch-all 1")	ATT AGA GTG TTT CAA GCA GAC
Babesia genus-specific 2 ("B catch-all 2")	ACT AGA GTG TTT CAA ACA GGC
Babesia felis	TTA TGC TTT TCC GAC TGG C
Babesia divergens	ACT RAT GTC GAG ATT GCA C
Babesia microti	GRC TTG GCA TCW TCT GGA
Babesia bigemina	CGT TTT TTC CCT TTT GTT GG
Babesia bovis	CAG GTT TCG CCT GTA TAA TTG AG
Babesia rossi	CGG TTT GTT GCC TTT GTG
Babesia canis	TGC GTT GAC GGT TTG AC
Babesia vogeli	AGC GTG TTC GAG TTT GCC
Babesia bicornis	TTG GTA AAT CGC CTT GGT C
Babesia caballi	GTG TTT ATC GCA GAC TTT TGT
Babesia leo	ATC TTG TTG CTT GCA GCT T
Babesia gibsoni	CAT CCC TCT GGT TAA TTT G
Babesia sp. (sable)	GCG TTG ACT TTG TGT CTT TAG C
Theileria sp. (kudu)	CTG CAT TGT TTC TTT CCT TTG
Theileria sp. (sable)	GCT GCA TTG CCT TTT CTC C
Theileria bicornis	GCG TTG TGG CTT TTT TCT G
Theileria annulata	CCT CTG GGG TCT GTG CA
Theileria buffeli	GGC TTA TTT CGG WTT GAT TTT
Theileria sp. (buffalo)	CAG ACG GAG TTT ACT TTG T
Theileria mutans	CTT GCG TCT CCG AAT GTT
Theileria parva	GGA CGG AGT TCG CTT TG
Theileria taurotragi	TCT TGG CAC GTG GCT TTT
Theileria velifera	CCT ATT CTC CTT TAC GAG T
Theileria equi	TTC GTT GAC TGC GYT TGG
Theileria lestoquardi	CTT GTG TCC CTC CGG G
Theileria ovis	TTG CTT TTG CTC CTT TAC GAG
Theileria annae	CCG AAC GTA ATT TTA TTG ATT TG
Theileria separata	GGT CGT GGT TTT CCT CGT

The degenerate position R denotes either A or G, W denotes either A or T and Y denotes C or T.

5.3.6 Quantitative real-time PCR (qPCR) assays

Samples that had been analysed by RLB were also analysed with qPCR assays for specific detection of the more pathogenic tick-borne pathogens (n = 240 samples each for *A. marginale*, *A. centrale* and *T. parva*; n = 120 samples for *E. ruminantium*). The qPCR assay is expected to be more sensitive than RLB hybridisation assay, and may therefore detect DNA of pathogens from samples previously diagnosed as negative by the RLB assay. A duplex qPCR assay was used for simultaneous detection and quantification of *A. marginale* and *A. centrale* DNA (Carelli et al., 2007; Decaro et al., 2008). The PCR was performed on a LightCycler® 2.0 (Roche Diagnostics, Mannheim, Germany) using a LightCycler® FastStart TaqMan® Kit (Roche Diagnostics). The primers AM-For (5'-TTG GCA AGG CAG CAG CTT-3') and AM-Rev (5'-TTC CGC GAG CAT GTG CAT-3') were used to amplify a 95 bp fragment of the *msp1β* gene for *A. marginale*, while the AC-For (5'-CTA TAC ACG CTT GCA TCT C-3') and AC-Rev (5'-CGC TTT ATG ATG TTG ATG C-3') primers were used to amplify a 77 bp fragment of the *groEL* gene for *A. centrale*. A TaqMan probe labelled with the fluorescent reporter dye 6-carboxy-fluoroscein and with blackhole quencher 1 (5' 6-FAM-TCG GTC TAA CAT CTC CAG GCT TTC AT-BHQ1-3') was used for the detection of *A. marginale*, while a TaqMan probe labelled with the fluorescent reporter dye LightCycler® Red 610 and with blackhole quencher 2 (5'-LC610-ATC ATC ATT CTT CCC CTT TAC CTC GT-BHQ2-3') was used for *A. centrale*. Mastermix pure grade water was used as a negative control while known *A. marginale* and *A. centrale* positive samples (confirmed by qPCR and species-specific gene sequence analysis) were included to serve as positive controls. Each PCR reaction (20 µl) contained 0.4x of FastStart mix, 0.6 µM of each *A. marginale* primer, 0.9 µM of AC-For primer, 0.6 µM of AC-Rev primer, 0.2 µM of each probe, 1 U (final concentration 0.025x) of UDG, 8 µl of water, and 2.5 µl test sample DNA or positive/negative control. Thermal cycling started with UDG activation at 40°C for 10 min, followed by activation of iTaq DNA polymerase at 95°C for 10 min and 40 cycles of denaturing at 95°C for 1 min and annealing-extension at 60°C for 1 min. The cooling step was at 40°C for 30 sec. Fluorescence data for all cycles was analysed using LightCycler® Software Version 4.1 (Roche Diagnostics).

The pCS20 qPCR for *E. ruminantium* was performed as previously described (Steyn et al., 2008), using amplification primers CowF (5'-CAA AAC TAG TAG AAA TTG CAC A-3') and CowR (5'-TGC ATC TTG TGG TGG TAC-3') to amplify a 226 bp fragment of the conserved pCS20 region, and a TaqMan probe Cow™ (5'-6FAM-TCC TCC ATC AAG ATA TAT AGC ACC TAT TA XT-PH-3') for the detection of the amplicons. The PCR was performed in a Rotor-Gene Q system (QIAGEN) using a LightCycler FastStart DNA Master Hybridisation Probe Kit (Roche Diagnostics). Mastermix pure grade water was used as a negative control and DNA extracted from an *in vitro* culture of the Welgevonden stock of *E. ruminantium* was included as the positive control. Fluorescence data for the amplification cycles was analysed using Rotor-Gene Q Series Software (QIAGEN). The *T. parva* qPCR was performed as previously described (Sibeko et al., 2008), using LightCycler FastStart DNA Master^{Plus} Hybridisation Probes mix on a LightCycler® 2.0 (Roche Diagnostics). A 167 bp fragment from the V4 variable region of the *T. parva* 18S rRNA gene was amplified using primers Parva-F (5'- CTG CAT CGC TGT GTC CCT T-3') and *Theileria*-R (5'-ACC AAC AAA ATA GAA CCA AAG TC-3'), and hybridisation probes were used to detect the amplicons: *T. parva* LC640 (5'-LCRed640-TCG GAC GGA GTT CGCT-PH-3') and *T. parva* Anchor (5'-GGG TCT CTG CAT GTG GCT TAT-FL-3'). Positive controls consisted of DNA from a known infected buffalo [KNP102, Onderstepoort Veterinary Institute] (Sibeko et al., 2008) while the negative controls contained nuclease free water. The florescent data was analysed using LightCycler® Software Version 4.1.

5.3.7 Amplification, cloning and sequencing of the 18S rRNA gene of *Theileria* and *Babesia* species

The near full-length (~1,600 bp) 18S rRNA gene was amplified from six selected DNA samples that displayed positive RLB *Babesia* genus-specific signals without species-specific signals; the amplicons were characterised by cloning and sequencing. The primers used for amplification were as previously described (Oosthuizen et al., 2008). Expand High-Fidelity PCR Master mix (Roche Diagnostics) was used, and amplification was performed in an automated thermocycler (Perkin-Elmer, Foster City, CA). The amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN) and then ligated into the pGEM®-T vector (Promega, Madison,

USA) and transformed into JM 109 High Efficiency Competent cells (Promega, Madison, USA), according to the manufacturers' instructions. At least five colonies per sample were selected and screened by colony PCR using primers RLB-F2 and RLB-R2 (Nijhof et al., 2003) and PCR mixture conditions as described for the RLB hybridisation assay, except that individual colonies were used as template. The primers used for sequencing were: RLB-F2, RLB-R2, Nbab_1F, Nbab_1R, BT18S_2F, BT18S_3F, BT18S_4F and BT18S_4R as previously described (Oosthuizen et al., 2008). Sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa).

5.3.8 Sequence and phylogenetic analyses

Sequence reads were processed and assembled using the CLC Genomics Workbench version 7.5.1 (CLC Bio, Boston, MA, USA). Near full-length 18S rRNA gene consensus sequences were obtained from 30 clones. The Basic Local Alignment Search Tool [BLAST] (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search for homologous sequences from GenBank using the blastn program. The assembled sequences were aligned with published sequences of related *Theileria* and *Babesia* species using the Multiple Alignment using Fast Fourier Transform (MAFFT) [version 7] program (Katoh and Standley, 2013). The alignment was then manually truncated to the length of the shortest sequence using BioEdit version 7.2.5 (Hall, 1999). The evolutionary history was inferred using the maximum likelihood based on the General Time Reversible model (Nei and Kumar, 2000). Phylogenetic trees were constructed by the neighbour-joining, maximum likelihood and maximum parsimony methods as implemented by the Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6) software package (Tamura et al., 2013). In each method, bootstrap analysis was done for the percentage of replicate trees in which the associated taxa clustered together in 1000 replicates (Felsenstein, 1985). Bootstrap test of phylogeny is one of the most commonly used methods to test the reliability of an inferred tree and its branching patterns (groupings). The method creates pseudoreplicates of trees by random sampling, with replacement, at sites (columns) in a multiple sequence alignment, such that each pseudoreplicate is the same size as the original dataset. The frequency with which a group or taxa is found among all

pseudoreplicates is a measure of the bootstrap support. If a group or taxa is found in all pseudoreplicate trees it is said to have a 100% bootstrap support. The genetic distances between the sequences were estimated by determining the number of base differences between sequences using MEGA6 (Tamura et al., 2013).

5.3.9 Nucleotide sequence accession numbers and statistical analysis

Representative nucleotide sequences reported in this chapter are available in the GenBank™ database under the accession numbers: **KU206291** and **KU206292** (*B. bigemina*); **KU206301** (*T. velifera*); **KU206308** (*Theileria* sp. strain MSD); **KU206309**, **KU206311** and **KU206317** (*T. mutans*).

The RLB data were used to estimate the population prevalences. The 95% confidence intervals for prevalence of tick-borne infections were estimated by performing descriptive statistics with 1000 bootstrap replications and sampling by clustering using the Statistical Package for the Social Sciences (SPSS) and using quantiles formation of the normal distribution (qnorm) with 'library (MASS)' package using R Console and RStudio. Bootstrapping was used to estimate the sampling distribution of the prevalence by assigning a measure of accuracy, in this case confidence interval. Inference about the cattle population from the sample data was modelled by resampling from original sample data by using sampling with replacement. A conceptual framework was developed to select candidate variables identified as exposure factors for tick-borne infections for the production system of Karamoja Region. Univariate analysis of associations, using the Chi-squared test, was carried out to determine the association between tick-borne pathogen infection and district (Moroto and Kotido) and age group of cattle (5-12 months, 13-24 months, and > 24 months), with the RLB test status of the animal considered as a binary outcome (positive or negative). Prevalence ratios, odds ratios (OR) and *p*-values for the exposure variables were obtained. Intra-cluster correlations within the herds were tested for the RLB test status for each of the tick-borne pathogens, by performing log ratio tests between a model 'herd' as random effect and a null model. This was followed by a mixed effect logistic regression in which the herd was included as a random

effect and other variables were fixed effects. The Generalised Linear Mixed Model based on the -2 log pseudo likelihood was used to compare models. The Chi-squared and Cramer's V tests were used to assess the significance and strength of association, as indicators of protective effects, between benign *Theileria* co-infections (*T. mutans* and *T. velifera*) and *T. parva*. To determine whether positive or negative outcomes for co-infections occur, the observed and expected frequencies of joint occurrence were compared. If the observed frequency was greater than the expected, then the association is positive, if it is less than expected, then the association is negative. Cohen's kappa (*k*) test was used to determine the level of agreement (Landis and Koch, 1977) between qPCR and RLB results for *A. marginale*, *A. centrale*, *T. parva* and *E. ruminantium*. The data were analysed using SPSS version 23.0 (IBM SPSS, 2014), and R Console version 3.2.1 (R Console, 2015) and RStudio version 3.2.1 (RStudio Team, 2015).

5.4 Results

5.4.1 Distribution of sampled cattle by location, sex and age group

A total of 20 herds and 240 individual cattle (12 per herd) were sampled for whole blood. An equal number of animals (120) was sampled in Moroto and Kotido Districts. Most (77.5%) cattle were female. By age group, the sampled cattle were: 82 (34.2%) for 5-12 months, 37 (15.4%) for 13-24 months, and 121 (50.4%) for > 24 months.

5.4.2 Tick-borne haemoparasite prevalence by RLB hybridisation assay

The RLB results demonstrated the presence of tick-borne infections in all but one sample (99.6%). Most cattle sampled had mixed infections with two or more species (97.5%), while single infections were found in only 2.1% of the samples. Figure 5.1 shows a representative reverse line blot demonstrating hybridisation of PCR products generated from samples collected in this study with RLB probes (Table 5.1).

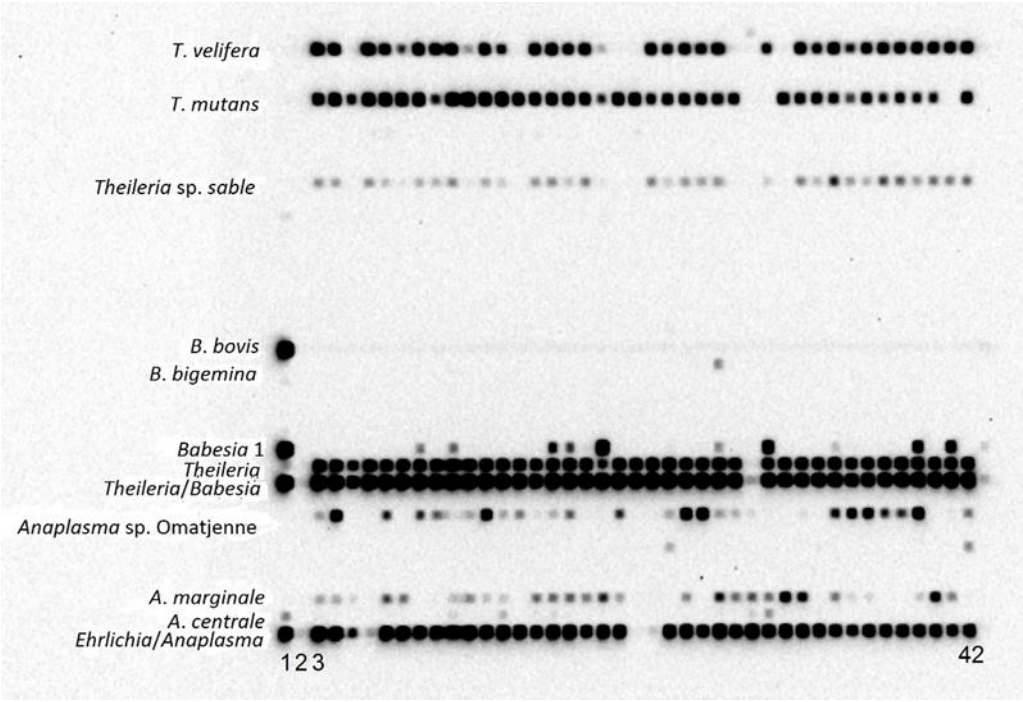

Figure 5.1: Representative reverse line blot showing tick-borne haemoparasite infection status of 40 cattle sampled in Karamoja Region, Uganda from November 2013 through January 2014. Genus/species-specific oligonucleotides were applied in horizontal rows, and PCR products from the test samples and controls in vertical lanes. Lane 1, positive controls (*Anaplasma centrale* and *Babesia bovis*); lane 2, negative control (no DNA template); lanes 3 to 42, PCR products from 40 cattle. The black spots on the X-ray film indicate a positive signal caused by hybridisation of the PCR product to the probe immobilised on the membrane; the absence of black spots indicates negative signals. Dots across the *Theileria* sp. (sable) lane were most likely due to cross-reactions with *T. velifera* (Mans et al., 2011).

The distribution of the tick-borne parasite species in the study area is summarised in Table 5.2. The RLB data were used to estimate the population prevalences. The 95% confidence intervals for prevalence of tick-borne infections were estimated by performing descriptive statistics with 1000 bootstrap replications and sampling by clustering. Chi-squared tests showed that the only significant associations between haemoparasite prevalence and district were for *A. centrale* and *A. bovis*. Animals in Moroto were more likely to be positive for *A. centrale* (odds ratio = 2.2 [95% CI: 1.1-4.2], $p < 0.05$) and *A. bovis* (OR = 3.5 [0.9-13.2], $p < 0.05$) infections than those in Kotido. The influence of age group of cattle on infection was significant ($p < 0.05$) only for *A. centrale*, *A. bovis* and *B. bigemina*. The prevalence of *A. centrale* decreased with age: 35.4% (n = 29) for 5-12 months, 16.2% (n = 6) for 13-24 months,

and 11.6% (n = 14) for > 24 months. Prevalence of infection with *A*. *bovis* increased from 5-12 months (6.1%, n = 5) to 13-24 months (13.5%, n = 5), and then decreased for animals > 24 months of age (2.5%, n = 3). Most of the *B*. *bigemina* positive cattle were 5-12 months of age (9.8%, n = 8), followed by > 24 months (3.3%, n = 4); no animals 13-24 months of age were positive. To test whether tick-borne infections were clustered within herds, we performed a Log Ratio test between a model with 'herd' as random effect and a null model. The test showed that all the tick-borne infections were clustered within herds ($p < 0.05$). Therefore, a mixed effect logistic regression model was tested with 'herd' included as a 'random effect' and 'age group' and 'district' as fixed effects. The Generalised Linear Mixed Model based on the -2 log pseudo likelihood was used to compare models. The model fit was good (80.4 to 90%). The mixed effect logistic regression showed that the influence of age group of cattle on infection was significant ($p < 0.05$) only for *A*. *centrale* and *B*. *bigemina*. There was no significant influence ($p > 0.05$) of district on any of the tick-borne pathogens detected by the RLB assay. The presence of different tick-transmitted pathogens within the same host concurrently, provides an opportunity for interaction that could generate positive or negative outcomes for a parasite population. Benign *Theileria* co-infections may result in protective effects against *T*. *parva*. We tested for the significance and strength of association between benign *Theileria* infections (*T*. *mutans* and *T*. *velifera*) and *T*. *parva*. There was a negative association between co-infection with *T*. *mutans* and *T*. *parva* (observed frequency = 6.0, expected frequency = 6.2), which however was not statistically significant (Fisher's Exact test, $p > 0.05$; Cramer's V = 0.014, $p > 0.05$). Co-infection between *T*. *velifera* and *T*. *parva* was neither positive nor negative (equal values of observed and expected frequencies, 5.0), and the association was not statistically significant (Fisher's Exact test, $p > 0.05$; Cramer's V = 0.01, $p > 0.05$).

Table 5.2: The prevalence of tick-borne haemoparasites in blood samples of cattle from two districts in Karamoja Region, Uganda, as determined by reverse line blot (RLB) hybridisation assay, from November 2013 through January 2014

Type of infection	Number of RLB positive cattle (%)			
	Moroto (n=120)	Kotido (n=120)	Total (n=240)	95% CI
*Anaplasma centrale**	32 (26.7)	17 (14.2)	49 (20.4)	15.8-25.4
Anaplasma marginale	93 (77.5)	84 (70.0)	177 (73.8)	68.3-79.2
Anaplasma phagocytophilum	1 (0.8)	0 (0.0)	1 (0.4)	0.0-1.3
*Anaplasma bovis**	10 (8.3)	3 (2.5)	13 (5.4)	2.9-8.3
Anaplasma sp. Omatjenne	77 (64.2)	75 (62.5)	152 (63.3)	57.5-68.8
Babesia bigemina	9 (7.5)	3 (2.5)	12 (5.0)	2.5-7.5
Theileria mutans	107 (89.2)	105 (87.5)	212 (88.3)	84.6-92.1
Theileria parva	6 (5.0)	1 (0.8)	7 (2.9)	1.3-5.0
Theileria taurotragi	2 (1.7)	0 (0.0)	2 (0.8)	0.0-2.1
Theileria velifera	89 (74.2)	82 (68.3)	171 (71.3)	65.8-76.7
Ehrlichia/Anaplasma genus-specific only	6 (5.0)	6 (5.0)	12 (5.0)	2.5-7.5
Theileria/Babesia genus-specific only	2 (1.7)	2 (1.7)	4 (1.7)	0.4-3.3
Theileria genus-specific only	1 (0.8)	2 (1.7)	3 (1.3)	0.0-2.9
Babesia genus-specific 1 only	25 (20.8)	15 (12.5)	40 (16.7)	12.1-21.3
Babesia genus-specific 2 only*	7 (5.8)	0 (0.0)	7 (2.9)	1.3-5.0

CI, confidence intervals based on 1,000 bootstrap samples.

*There was a significant difference, by Pearson's chi-squared test, in the prevalence of parasite between districts ($p < 0.05$).

5.4.3 Comparison between RLB and qPCR results

Table 5.3 shows the comparison between the results from RLB hybridisation and qPCR assays, for *A. marginale*, *A. centrale*, *T. parva* and *E. ruminantium*. Despite the discordance between the two assays for *T. parva*, the qPCR assay confirmed the low prevalence of *T. parva* among cattle. The results from RLB and qPCR assays emphasise that *A. centrale* prevalence in this pastoral area is lower than that of *A. marginale*.

Table 5.3: Comparison of results from qPCR and RLB hybridisation assays for *Anaplasma marginale*, *Anaplasma centrale*, *Theileria parva* and *Ehrlichia ruminantium* in blood samples of cattle from Karamoja Region, Uganda, November 2013 through January 2014

	Number and percentage of positive samples				
	RLB	qPCR	Both RLB and qPCR	Cohen's kappa (*k*) and 95% CI	*p*-value
A. marginale (n = 240)	177 (73.7%)	199 (82.9%)	170 (70.8%)	0.56 (0.43-0.68)**	< 0.001
A. centrale (n = 240)	49 (20.4%)	29 (12.1%)	28 (11.7%)	0.67 (0.53-0.79)***	< 0.001
T. parva (n = 240)	7 (2.9%)	8 (3.3%)	2 (0.8%)	0.24 (-0.03-0.52)*	< 0.001
E. ruminantium (n = 120)	0 (0.0%)	2 (1.7%)	0 (0.0%)	No statistic	

n, number of cattle samples tested; RLB, reverse line blot; qPCR, quantitative real-time polymerase chain reaction; CI, confidence interval based on 1,000 bootstrap samples; Cohen's kappa, proportion of agreement over and above the agreement expected by chance (range -1 to +1); *, fair agreement, **moderate agreement, ***, substantial agreement; $p < 0.001$, kappa value was significant (not due to chance).

5.4.4 18S rRNA gene sequence analysis

Thirty near full-length 18S rRNA gene sequences were obtained from the six bovine samples selected in this study; BLAST analysis indicated that 23 were most closely related to other *Theileria* 18S rRNA gene sequences, while seven were *Babesia* sequences. Twelve sequences, from five samples, were closely related (99% identity) to a published sequence of *T. mutans* Intona from cattle in Kenya [AF078815] (Chae et al., 1999). The 12 *T. mutans* sequences from this study differed from the *T. mutans* Intona sequence by 4 to 5 bp (99 to 100% query cover), and differed from each other by up to 2 bp (Table 5.4). One sequence (MT9) was closely related (99% identity) to *Theileria* sp. strain MSD (AF078816) from South Africa (Chae et al., 1999) with a difference of 11 nucleotides (99% query cover). Ten sequences were closely related (99% identity) to the sequences of *T. velifera* (AF097993) from cattle in Tanzania (Gubbels et al., 1999). Micro-heterogeneity of up to 3 bp was observed in *T. velifera* sequences from this study with related sequences from cattle and African buffalo (Table 5.4)

[100% query cover]. Seven sequences showed 99% identity to *B*. *bigemina* BRC02 (FJ426361, 1647 bp) from Brazil (Criado-Fornelio et al., 2009), and the sequences differed from *B*. *bigemina* BRC02 by 5 bp and from *B*. *bigemina* 563 HQ840960 (from China, 1689 bp) by 9 bp (Table 5.5).

Table 5.4: Estimates of evolutionary divergence between *Theileria mutans* and *Theileria* sp. strain MSD sequence variants, and previously published sequences of *T. mutans* and *Theileria* sp. strain MSD. The sequences were identified in blood samples from cattle in Karamoja Region, Uganda from November 2013 through January 2014

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*T. mutans*_AF078815																	
2	*T. mutans Syncerus caffer* JN572693	17																
3	*T. mutans Syncerus caffer* FJ213586	23	26															
4	MT19	5	18	26														
5	MT20	5	18	26	0													
6	KT3	4	17	25	1	1												
7	MT18	4	17	25	1	1	0											
8	MT16	4	17	25	1	1	0	0										
9	MT14	4	17	25	1	1	0	0	0									
10	MT11	4	17	25	1	1	0	0	0	0								
11	MT17	4	17	25	1	1	0	0	0	0	0							
12	MT12	5	18	26	2	2	1	1	1	1	1	1						
13	MT13	5	18	26	2	2	1	1	1	1	1	1	0					
14	MT10	4	17	25	1	1	0	0	0	0	0	0	1	1				
15	MT15	4	17	25	1	1	0	0	0	0	0	0	1	1	0			
16	*Theileria* sp. strain MSD AF078816	16	26	28	21	21	20	20	20	20	20	20	21	21	20	20		
17	MT9	18	23	26	15	15	14	14	14	14	14	14	15	15	14	14	11	

The number of base differences from pairwise analyses of 17 sequences is shown. There were a total of 1582 positions in the dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Table 5.5: Estimates of evolutionary divergence between *Babesia bigemina* sequence variants and previously published sequences of *B. bigemina.* The sequences were identified in blood samples frm cattle in Karamoja Region, Uganda from November 2013 through January 2014

		1	2	3	4	5	6	7	8	9	10
1	*B. bigemina* BRC02 FJ426361										
2	*B. bigemina* JQ437261	6									
3	*B. bigemina* 563 HQ840960	7	6								
4	MT21	5	7	9							
5	MT22	5	6	9	2						
6	MT23	5	6	9	2	0					
7	MT24	5	6	9	2	0	0				
8	KT4	5	6	9	2	0	0	0			
9	MT25	5	6	9	2	0	0	0	0		
10	MT26	5	6	9	2	0	0	0	0	0	

The number of base differences from pairwise analyses of 10 sequences is shown. There were a total of 1581 positions in the dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Sequence alignment in the RLB probe region for *B. bigemina* showed three nucleotide differences between the *B. bigemina* sequences from this study and the probe sequence (Figure 5.2). The nucleotide differences most likely prevented hybridisation of the PCR amplicons to the RLB probe, resulting in failure of the RLB assay to identify *B. bigemina* in the samples.

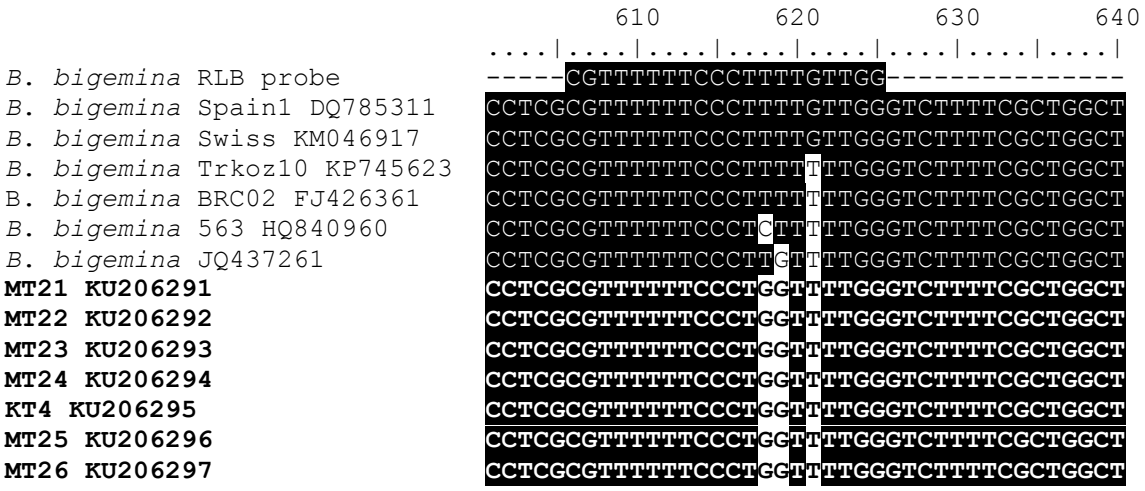

Figure 5.2: Nucleotide alignment of 18S rRNA gene sequences in the target region of the *Babesia bigemina* reverse line blot hybridisation probe. The alignment comprises the *B. bigemina* RLB probe sequence (on top), published sequences of *B. bigemina* and variants (highlighted in bold) identified from cattle samples from Karamoja Region, Uganda. The accession number of each sequence is indicated in the sequence name. Nucleotide differences are shown in black letters on a white background.

5.4.5 Phylogenetic analyses

Phylogenetic trees were used to demonstrate the relationships between the 18S rRNA gene variants identified in this study, and other related *Theileria* and *Babesia* species. The tree topologies were similar using different methods. Figures 5.3 and 5.4 show representative trees generated by maximum likelihood. Only representative sequences of the variants are shown. Three *T. mutans* sequences - KT3 (representing eight clones from three samples), MT12 and MT19 (each representing two clones from two samples) - grouped (clade 4) with *T. mutans* described from cattle in Kenya [AF078815] (Chae et al., 1999). It furthermore grouped separately from previously published *T. mutans* sequences from buffalo from South Africa [clades 1, 2, 3] (Chaisi et al., 2013a) [Figure 5.3]. Sequence MT9 grouped with *Theileria* sp. strain MSD (AF078816) from South Africa (Figure 5.3). The *T. velifera* sequence (MT1, representing 10 clones from two samples) formed a monophyletic group with previously published *T. velifera* sequences from African buffalo in South Africa [JN572702, JN572703, JN572705] (Chaisi et al., 2013a) and cattle [AF097993] in Tanzania (Gubbels et al., 1999), but more distantly within the same group from other sequences from African buffalo in South Africa [JN572701, JN572704] (Chaisi et al., 2013a) (shown in Figure 5.3). The two *B. bigemina*

sequences MT22 (representing six clones from two samples) and MT21 grouped with previously-published *B. bigemina* sequences, but they formed a separate group (clade 2) [Figure 5.4].

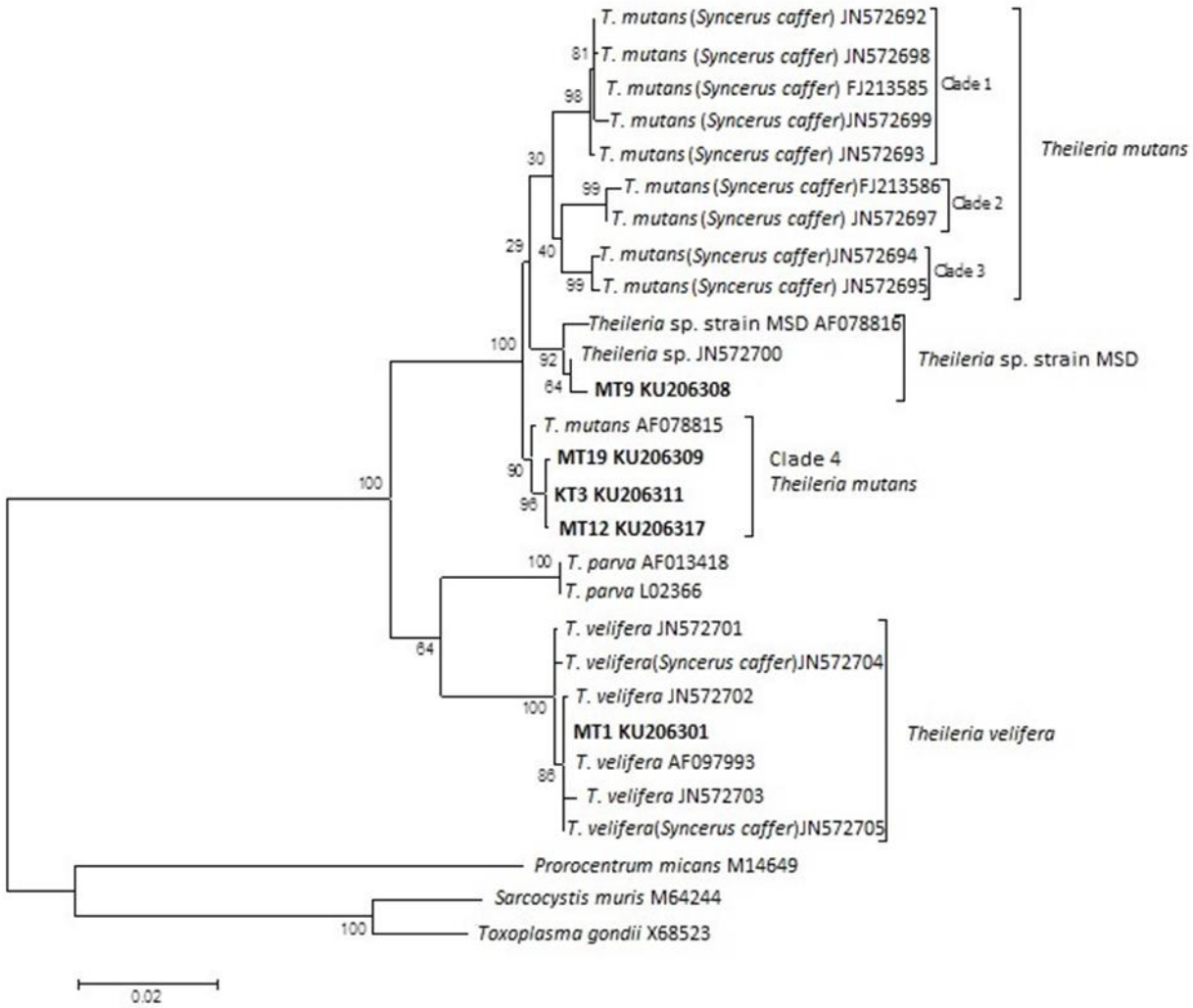

Figure 5.3: Maximum likelihood tree showing the phylogenetic relationship between the *T. mutans*, *Theileria* species (strain MSD) and *T. velifera* 18S rRNA sequence variants identified in blood samples from cattle in Karamoja Region, Uganda other *Theileria* species. Branch lengths are proportional to the estimated genetic distance (number of substitutions per site) between the species. The numbers at the internal nodes represent the percentage of 1,000 replicates (bootstrap) for which the same branching patterns were obtained. Some sequences obtained in this study were identical to each other; therefore, only representatives of variants are shown (in bold). Sequence KT3 represents eight clones (others are: MT10, MT11, MT14, MT15, MT16, MT17, MT18) from three samples, MT12 represents two clones (the other is MT13) from two samples, MT19 represents two clones (the other is MT20) from two samples. Sequence MT1 represents 10 clones from two samples. The tree was rooted using the 18S rRNA gene sequences of *Prorocentrum micans*, *Sarcocystis muris* and *Toxoplasma gondii*. The accession number of each sequence is indicated in the sequence name.

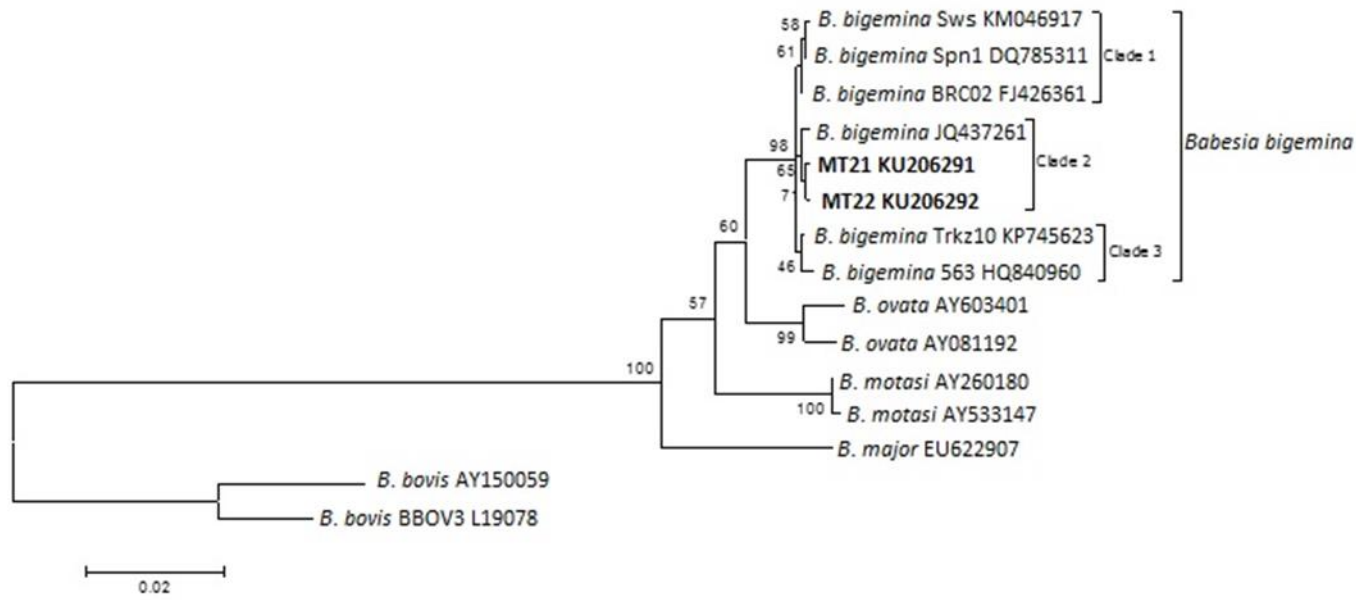

Figure 5.4: Maximum likelihood tree showing the phylogenetic relationship between the *Babesia bigemina* sequence variants identified in blood samples from cattle in Karamoja Region, Uganda and other *Babesia* species. Branch lengths are proportional to the estimated genetic distance (number of substitutions per site) between the taxa. The numbers represent the percentage of 1,000 replicates (bootstrap) for which the same branching patterns were obtained. The GenBank accession number of each sequence is indicated in the sequence name. Some sequences obtained in this study were identical to each other; therefore, only representatives of variants are shown (in bold). Sequence MT22 represents six clones (others are: MT23, MT24, MT25, MT26, KT4) from two samples. The tree was rooted using the 18S rRNA gene sequences of *B. bovis*.

5.5 Discussion

This study demonstrates a high diversity and prevalence of tick-borne haemoparasite infections among cattle in a transhumant pastoral system of Karamoja in north-eastern Uganda.

The proportion of cattle infected with *A. marginale* (73.8% [RLB] and 82.9% [qPCR]) was much higher than those reported in previous studies, using RLB hybridisation assay, in other parts of Uganda, i.e., 8.7% (Asiimwe et al., 2013), 3.7% (Muhanguzi et al., 2010a) and 18% (Oura et al., 2004), and no *A. marginale* in western Kenya (Njiiri et al., 2015). The poor tick control practices (e.g. irregular application of acaricides of less quantity and poor dilution) by the

Karamojong (Byaruhanga et al., 2015b) [Chapter 3 of this thesis], as compared with other areas, may lead to higher tick infestations among cattle in Karamoja. A recent study in Karamoja showed that most cattle were infested with *R*. *decoloratus* (53.4%), the tick vector for *A*. *marginale*. Moreover, haematophagous arthropods such as *Culicoides* midges, tsetse flies, and sheep keds (*Melophagus ovinus*) were abundant in the grazing areas (Byaruhanga et al., 2015a) [Chapter 4 of this thesis]. The pastoralists also used disposable needles on multiple animals without decontamination, which may mechanically transmit *Anaplasma* infections. Under these conditions, a high prevalence of infection with *A*. *marginale* is expected.

The prevalences of other pathogenic haemoparasites were low. The prevalence of *T*. *parva* (2.9% [RLB] and 3.3% [qPCR]) was similar to those observed on farms in central Uganda [7.0% by RLB] (Oura et al., 2004) and in Tororo District in eastern Uganda [5.3% by p104-based PCR] (Muhanguzi et al., 2014), but lower than those reported from western Uganda [24% by RLB] (Muhanguzi et al., 2010b), from a farm in central Uganda [34% by RLB] (Asiimwe et al., 2013), in some parts of Karamoja Region (18% by p104 nested PCR in < 30 cattle sampled) (Kabi et al., 2014), and in 15 districts of central and western Uganda [47.4% by Giemsa-stained blood smears, although this method may not truly differentiate between *Theileria* spp. (Kasozi et al., 2014)]. The low prevalence of *T*. *parva* may be attributed to low infection rates in ticks and consequently low challenge for the cattle, although we did not establish infection rates in ticks in this study; however, a high proportion of cattle infested with *R*. *appendiculatus* (77%) was observed in Karamoja Region (Byaruhanga et al., 2015a) [Chapter 4 of this thesis]. Furthermore, the piroplasms of *T*. *parva* undergo only limited replication (Conrad et al., 1986), and fluctuations and loss of carrier state have been documented (Mans et al., 2015), which may lead to low detectable infection. A further consideration is that cases of ECF were not effectively treated (Byaruhanga et al., 2015b) [Chapter 3 of this thesis], which resulted in high case fatality. Since ECF is often an acute disease (Norval et al., 1992), the proportion of cattle that are carriers declines with increased mortality rates among infected cattle (Dolan, 1986).

Only 1.7% of cattle sampled were positive for the bacterium *E. ruminantium* by qPCR, and none by RLB, consistent with previous studies, using RLB hybridisation, from other parts of Uganda, where the pathogen was detected in low proportions of cattle (4.5%, Muhanguzi et al., 2010a; 2.2%, Asiimwe et al., 2013). However, a previous study in Karamoja (Byaruhanga et al., 2015a) [Chapter 4 of this thesis] showed that high proportions of cattle were infested with *Amblyomma lepidum* (78.9%) and *Amblyomma variegatum* (49.1%), the tick vectors for *E. ruminantium* in Uganda. Ticks in the field in endemic areas exhibit low *E. ruminantium* infection levels, but present a highly virulent heartwater challenge (Allsopp, 2010). This may lead to a high case fatality rate among infected animals, and subsequently a low *E. ruminantium* infection prevalence in the cattle population. Furthermore, the qPCR can detect *E. ruminantium* parasites in the blood of experimentally infected animals only once the febrile stage of infection is reached (Allsopp et al., 2004; Steyn et al., 2008). This could be associated with the lifecycle of the pathogen. After an infected tick bite, the initial replication of the organism takes place in reticulo-endothelial cells and macrophages in the regional lymph nodes, after which the organisms are disseminated via the blood stream to invade the endothelial cells of blood vessels in various organs and tissues, including the brain (Allsopp, 2010). It has been postulated that the febrile stage coincides with the movement of organisms from the lymph to the blood stream, which probably explains why *E. ruminantium* cannot be detected in the circulating blood of experimentally infected sheep before the febrile stage [having fever] (Steyn et al., 2008). The organism has been detected using the qPCR assay in field samples obtained from healthy cattle, which were therefore considered to be in a putative carrier state (Steyn et al., 2008). However, little is known about the location of *E. ruminantium* in carrier animals. If the organisms are not present in the circulating blood of carrier animals, this could lead to low detection of the pathogen in blood samples from carrier cattle.

In this study, 5.0% of the sampled cattle were positive for *B. bigemina*, consistent with previous reports from elsewhere in Uganda, i.e., 4.0% (Asiimwe et al., 2013), 2.0% (Oura et al., 2004) and none (Muhanguzi et al., 2010b). Although *B. bigemina* infections result in high

parasitaemia, animals recovering from babesiosis remain infective for ticks for only 4 to 7 weeks and carriers for only a few months (Suarez and Noh, 2011).

About three quarters of the cattle sampled were positive for *T. mutans* and *T. velifera*, the causes of benign theileriosis. These findings are in agreement with those of Asiimwe et al. (2013) and Oura et al. (2004) from elsewhere in Uganda and Njiiri et al. (2015) in western Kenya, using RLB assay, but not consistent with other reports from Uganda: 28% [*T. mutans*] and 16% [*T. velifera*] (Oura et al., 2011), and 18.4% [*T. mutans*] and 13.7% [*T. velifera*] (Muhanguzi et al., 2010b) detected by RLB. One possibility for the high occurrence of *T. mutans* and *T. velifera* observed in the present study is the high and continuous challenge from the ticks *A. lepidum* and *A. variegatum* (Walker et al., 2013); however, an alternative explanation may be that these *Theileria* species are carried for a longer time at high levels after infection (Asiimwe et al., 2013). Unlike *T. parva*, *T. mutans* and *T. velifera* undergo limited lymphocytic merogony; their main mode of replication occurs in the erythrocytes, and this may cause high piroplasm parasitaemia (Sivakumar et al., 2014). Further studies on DNA from ticks in this study area may provide more information about these infections. A protective effect of benign *Theileria* co-infections against *T. parva* infection may occur. Recently, a study in western Kenya showed that concurrent infections with the less pathogenic theilerias at first infection with *T. parva* were associated with a reduction (89%) in mortality associated with *T. parva* in a population of East African short-horn zebu cattle (Woolhouse et al., 2015). However, pathogenic effects of *T. mutans* and *T. velifera* may also occur, leading to ECF-like clinical signs in cattle. A study on a farm in Uganda showed that 14 out of the 17 cattle that showed high levels of piroplasm parasitaemias in Giemsa-stained blood smears and showed signs of ECF, were positive for *T. mutans* and *T. velifera* but negative for *T. parva* on RLB; only two animals had *A. marginale* and no other tick-borne haemoparasites (Oura et al., 2004). Another study among Maasai zebu cattle in Kenya demonstrated clinical episodes (febrile responses, enlarged lymph nodes, anaemia and weight losses) of *T. mutans* infections (Moll et al., 1986), and in Zambia, *T. mutans* caused anaemia in calves following experimental infection (Musisi et al., 1984). These reports

emphasise the role that *T. mutans* and *T. velifera* may play in the epidemiology and control of ECF. In most laboratories in resource-poor countries, where only blood smears are used for diagnosis, misdiagnosis of ECF is most likely, because it is difficult to differentiate microscopically between *T. mutans* and *T. parva* piroplasms (Oura et al., 2004). There was no significant association between co-infections with *T. mutans*, or *T. velifera*, and *T. parva*. Therefore, there is less likelihood that benign *Theileria* co-infections may result in protective effects against *T. parva* amongst cattle in the Karamoja Region.

In this study, *Theileria taurotragi* was found in only 0.8% of cattle, in comparison with other studies in Uganda, using RLB hybridisation assay, that showed infection prevalence of 44% in crossbred cattle and 5% in indigenous cattle (Oura et al., 2004), 15% in *Bos indicus* calves (Asiimwe et al., 2013), and 14% in indigenous and crossbred cattle (Muhanguzi et al., 2010b). In another study in and around Lake Mburo National Park, 45-76% of indigenous *Bos indicus* calves were infected with *T. taurotragi* (Oura et al., 2011). Therefore, *T. taurotragi* circulates among cattle populations in Uganda. However, a lower prevalence of *T. taurotragi* in Karamoja may suggest low infection levels in the tick vectors (*R. appendiculatus* and *R. pulchellus*) and wildlife reservoirs (especially elands and bushbucks). In other reports from South Africa, *T. taurotragi* was associated with a case of bovine cerebral theileriosis (Tzaneen disease) and death in four cattle (De Vos et al., 1981), and infection that was characterised by a transient fever and small numbers of macroschizonts and piroplasms (De Vos and Roos, 1981).

Our study demonstrates a relatively high proportion of *A. centrale* positive cattle (20.4% [RLB] and 12.1% [qPCR]) in Karamoja as compared with previous studies, using RLB hybridisation, in central Uganda (0%, Oura et al., 2004; 4.3%, Asiimwe et al., 2013) and western Uganda (Muhanguzi et al., 2010a). Possibly, some of the tick species found in Karamoja Region and not reported in other parts of Uganda (Byaruhanga et al., 2015a) [Chapter 4 of this thesis], might transmit the field strains of *A. centrale* in the study area (no vaccination against anaplasmosis is done in Uganda). We observed a much higher proportion for *Anaplasma* sp.

(Omatjenne) (63.3%), an apparently non-pathogenic *Anaplasma* sp. (Allsopp et al., 1997), than was reported in western Uganda [1.9%] (Muhanguzi et al., 2010a).

There were few associations between haemoparasite infections and age group of cattle and none for district (from the mixed effect logistic regression analysis), which indicates a relatively uniform distribution of the infections across the study area. The fluctuations in the infection levels for *A*. *centrale* and *B*. *bigemina* with regard to age can be explained by acquired immunity as the animals grow or reduction in immunity as a result of physiological states (e.g. pregnancy and lactation) and stress (e.g. animal movements).

Although qPCR is reportedly more sensitive than RLB (Decaro et al., 2008), the assay detected fewer positive samples for *A*. *centrale* as compared with RLB. This may suggest nucleotide differences in the *groEL* gene region for *A*. *centrale* in Uganda, which may hinder detection by the *TaqMan* probe. The discordance between RLB and qPCR for *T*. *parva* may be explained by the possibility of sequence variations in the RLB probe, or qPCR anchor and sensor probe regions of the *T*. *parva* genotypes from Karamoja Region. Although previous studies have characterised the target sequences for the species-specific qPCR assays for *A*. *centrale* (*groEL* gene) from South Africa and Japan (Lew et al., 2003) and buffalo-derived *T*. *parva* (18S rRNA gene) from southern Africa (Chaisi et al., 2011; Mans et al., 2011; Chaisi et al., 2013b), there is no equivalent data for *A*. *centrale* and cattle-derived *T*. *parva* in Uganda. Further studies should investigate the *groEL* gene for *A*. *centrale* and the 18S rRNA gene for *T*. *parva* in Uganda.

This study demonstrated variants in the 18S rRNA gene of *T*. *mutans*, *Theileria* sp. strain MSD and *B*. *bigemina* among cattle in Uganda. Previous studies in southern Africa also showed variants of *T*. *mutans* (Chae et al., 1999; Mans et al., 2011; Chaisi et al., 2013a; Chaisi et al., 2013b), *Theileria* sp. strain MSD and *T*. *velifera* (Mans et al., 2011; Chaisi et al., 2013a; Chaisi et al., 2013b) in cattle and buffalo, indicating a wide genotypic diversity of these species. *Theileria* sp. strain MSD is most closely related to *T*. *mutans* and was first identified from a

naturally infected bovine in South Africa (Chae et al., 1999). The *B*. *bigemina* variant sequences identified in samples that tested negative by RLB hybridisation assay suggest that there is a higher occurrence of *B*. *bigemina* in cattle in the study area than demonstrated by the RLB assay. This is consistent with the observation of a high proportion of cattle infested with *R*. *decoloratus* (53.4%), the tick vector for *B*. *bigemina*, in the study area (Byaruhanga et al., 2015a). Future RLB probe designs should take into consideration the sequence variants identified in this study. A qPCR assay for *B*. *bigemina*, which might have detected the pathogen in the samples that were negative by RLB assay, was not used, and this was a limitation in this study. Alignment of the 18S rRNA gene sequences of variants from this study in the target region of the *B*. *bigemina* qPCR probe designed by Kim et al. (2007) showed no nucleotide differences.

5.6 Conclusions

This study demonstrated a high prevalence (nearly all cattle) and diversity of tick-borne haemoparasites among cattle in the pastoral region of Karamoja, Uganda. Taken together, the parasites are more prevalent in this region than previously reported in other parts of Uganda. The high prevalence of *T*. *mutans* and *T*. *velifera* infections among cattle may interfere with the diagnosis and control of theileriosis in cattle. There was variation over the full-length 18S rRNA gene for *B*. *bigemina* and *T*. *mutans*. These findings contribute to our understanding of the distribution and diversity of tick-borne parasites in Uganda, which is useful for control strategies.

5.7 References

ALLSOPP, B.A., 2010. Natural history of *Ehrlichia ruminantium*. Veterinary Parasitology 167, 123-135.

ALLSOPP, B.A., BEZUIDENHOUT, J.D., PROZESKY, L., 2004. Heartwater. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 507-535.

ALLSOPP, M.T.E.P., VISSER, E.S., DU PLESSIS, J.L., VOGEL, S.W., ALLSOPP, B.A., 1997. Different organisms associated with heartwater as shown by analysis of 16S ribosomal RNA gene sequences. Veterinary Parasitology 71, 283-300.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

ASIIMWE, B.B., WEIR, W., TAIT, A., LUBEGA, G.W., OURA, C.A.L., 2013. Haemoparasite infection kinetics and the population structure of *Theileria parva* on a single farm in Uganda. Veterinary Parasitology 193, 8-14.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

BEKKER, C.P.J., DE VOS, S., TAOUFIK, A., SPARAGANO, O.A.E., JONGEJAN, F., 2002. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Veterinary Microbiology 89, 223-238.

BYARUHANGA, C., COLLINS, N.E., KNOBEL, D., OOSTHUIZEN, M.C., 2015a. Endemic status of tick-borne infections and tick species diversity among transhumant zebu cattle in Karamoja Region, Uganda: support for control approaches. Veterinary Parasitology: Regional Studies and Reports, 1-2, 21-30.

BYARUHANGA, C., OOSTHUIZEN, M.C., COLLINS, N.E., KNOBEL, D., 2015b. 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, 287-297.

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, 107-114.

CHAE, J., ALLSOPP, B.A., WAGHELA, S.D., PARK, J., KAKUDA, T., SUGIMOTO, C., ALLSOPP, M.T.E.P., WAGNER, G.G., HOLMAN, P.J., 1999. A study of the systematics of *Theileria* spp. based upon small-subunit ribosomal RNA gene sequences. Parasitology Research 85, 877-883.

CHAISI, M.E., COLLINS, N.E., POTGIETER, F.T., OOSTHUIZEN, M.C., 2013a. Sequence variation identified in the 18S rRNA gene of *Theileria mutans* and *Theileria velifera* from the African buffalo (*Syncerus caffer*). Veterinary Parasitology 191, 132-137.

CHAISI, M.E., JANSSENS, M.E., VERMEIREN, L., OOSTHUIZEN, M.C., COLLINS, N.E., GEYSEN, D., 2013b. Evaluation of a real-time PCR test for the detection and discrimination of *Theileria* species in the African Buffalo (*Syncerus caffer*). PLoS ONE 8: e75827. doi:10.1371/journal.pone.0075827.

CHAISI, M.E., SIBEKO, K.P., COLLINS, N.E., POTGIETER, F.T., OOSTHUIZEN, M.C., 2011. Identification of *Theileria parva* and *Theileria* sp. (buffalo) 18S rRNA gene sequence variants in the African Buffalo (*Syncerus caffer*) in southern Africa. Veterinary Parasitology 182, 150-162.

CONRAD, P.A., DENHAM, D., BROWN, C.G.D., 1986. Intraerythrocytic multiplication of *Theileria parva in vitro*: An ultrastructural study. International Journal for Parasitology 16, 223-229.

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, 270-275.

CRIADO-FORNELIO, A., BULING, A., ASENZO, G., BENITEZ, D., FLORIN-CHRISTENSEN, M., GONZALEZ-OLIVA, A., HENRIQUES, G., SILVA,M.,ALONGI,A., AGNONE, A., TORINA, A., MADRUGA, C.R., 2009. Development of fluorogenic probe-based PCR assays for the detection and quantification of bovine piroplasmids. Veterinary Parasitology 162, 200-206.

DE VOS, A.J., BESSENGER, R., BANTING, L.F., 1981. *Theileria*? *taurotragi*: a probable agent of bovine cerebral theileriosis. Onderstepoort Journal of Veterinary Research 48, 177-178.

DE VOS, A.J., ROOS, J.A., 1981. The isolation of *Theileria*? Taurotragi in South Africa. Onderstepoort Journal of Veterinary Research 48, 149-153.

DECARO, N., CARELLI, G., LORUSSO, E., LUCENTE, M.S., GRECO, G., LORUSSO, A., RADOGNA, A., CECI, L., BUONAVOGLIA, C., 2008. Duplex real-time polymerase chain reaction for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale*. Journal of Veterinary Diagnostic Investigation 20, 606-611.

DOLAN, T.T., 1986. Chemotherapy of East Coast fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. Journal of Comparative Pathology 96, 137-146.

EGERU, A., WASONGA, O., KYAGULANYI, J., MAJALIWA, G.J.M., MACOPIYO, L., MBURU, J., 2014. Spatio-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda. Pastoralism: Research, Policy and Practice 4, 6.

FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the Bootstrap. Evolution 39, 783-791.

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, 208-223.

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, 1782-1789.

HALL, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95-98.

IBM SPSS, T., 2014. IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY.

IICD, 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities, Ruspantini, C. (Ed.), European Commission, Government of Uganda and Institute for International Co-operation and Development (IICD) Uganda, 67 pp. www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/C&DPublicationLivestockDiseaseSurveillance2.pdf. Accessed March 24, 2015.

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, 414.

KASOZI, K.I., MATOVU, E., TAYEBWA, D.S., NATUHWERA, J., MUGEZI, I., MAHERO, M., 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. Open Journal of Veterinary Medicine 4, 220-231.

KATOH, K., STANDLEY, D.M., 2013. MAFFT multiple sequence alignment software 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772-780.

KILLIP, S., MAHFOUD, Z., PEARCE, K., 2004. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers. The Annals of Family Medicine 2, 204-208.

KIM, C., ISEKI, H., HERBAS, M.S., YOKOYAMA, N., SUZUKI, H., XUAN, X., FUJISAKI, K., IGARASHI, I., 2007. Development of Taqman-based real-time PCR assays for diagnostic detection of *Babesia bovis* and *Babesia bigemina*. The American Journal of Tropical Medicine and Hygiene 77, 837-841.

KIVARIA, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production 38, 291-299.

LANDIS, J.R., KOCH, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

LEW, A.E., GALE, K.R., MINCHIN, C.M., SHKAP, V., DE WAAL, D.T., 2003. Phylogenetic analysis of the erythrocytic *Anaplasma* species based on 16S rDNA and GroEL (HSP60) sequences of *A. marginale*, *A. centrale*, and *A. ovis* and the specific detection of *A. centrale* vaccine strain. Veterinary Microbiology 92, 145-160.

MAGONA, J.W., WALUBENGO, J., KABI, F., 2011a. Response of Nkedi Zebu and Ankole cattle to tick infestation and natural tick-borne, helminth and trypanosome infections in Uganda. Tropical Animal Health and Production 43, 1019-1033.

MAGONA, J.W., WALUBENGO, J., OLAHO-MUKANI, W., JONSSON, N.N., WELBURN, S.W., EISLER, M.C., 2011b. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55, 203-213.

MANS, B.J., PIENAAR, R., LATIF, A.A., 2015. A review of *Theileria* diagnostics and epidemiology International Journal for Parasitology: Parasites and Wildlife 4, 104-118.

MANS, B.J., PIENAAR, R., LATIF, A.A., POTGIETER, F.T., 2011. Diversity in the 18S SSU rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitology 138, 766-779.

MOLL, G., LOHDING, A., YOUNG, A.S., LEITCH, B.L., 1986. Epidemiology of theileriosis in calves in an endemic area of Kenya. Veterinary Parasitology 19, 255-273.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010a. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

MUHANGUZI, D., MATOVU, E., WAISWA, C., 2010b. Prevalence and characterization of *Theileria* and *Babesia* species in cattle under different husbandry systems in Western Uganda. International Journal of Animal and Veterinary Advances 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, 91.

MUSISI, F.L., JONGEJAN, F., PEGRAM, R.G., 1984. Isolation and transmission of *Theileria mutans* (Chisamba) in Zambia. Research in Veterinary Science 36, 129-131.

NEI, M., KUMAR, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York, 352 pp.

NIJHOF, A.M., PENZHORN, B.L., LYNEN, G., MOLLEL, J.O., MORKEL, P., BEKKER, C.P.J., JONGEJAN, F., 2003. *Babesia bicornis* sp. nov. and *Theileria bicornis* sp. nov.: tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). Journal of Clinical Microbiology 41, 2249-2254.

NIJHOF, A.M., PILLAY, V., STEYL, J., PROZESKY, L., STOLTSZ, W.H., LAWRENCE, J.A., PENZHORN, B.L., JONGEJAN, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. Journal of Clinical Microbiology 43, 5907-5911.

NJIIRI, N.E., BRONSVOORT, B.M., COLLINS, N.E., STEYN, H.C., TROSKIE, M., VORSTER, I., THUMBI, S.M., SIBEKO, K.P., JENNINGS, A., VAN WYK, I.C., MBOLE-KARIUKI, M., KIARA, H., POOLE, E.J., HANOTTE, O., COETZER, K., OOSTHUIZEN, M.C., WOOLHOUSE, M., TOYE, P., 2015. The epidemiology of tick-borne haemoparasites as determined by the reverse line blot hybridization assay in an intensively studied cohort of calves in western Kenya. Veterinary Parasitology 210, 69-76.

NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S., LAWRENCE, J.A., MUKHEBI, A.W., BISHOP, R.P., MCKEEVER, D.J., SHAW, M.K., 1992. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

OCAIDO, M., MUWAZI, R.T., OPUDA, J.A., 2009a. Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. Tropical Animal Health and Production 41, 731-739.

OCAIDO, M., OTIM, C.P., KAKAIRE, D., 2009b. Impact of major diseases and vectors in smallholder cattle production systems in different agro-ecological zones and farming systems in Uganda. Livestock Research for Rural Development Volume 21 (9), Article #155. Retrieved November 29, 2013, from http://www.lrrd.org/lrrd21/9/ocai21155.htm.

OOSTHUIZEN, M.C., ZWEYGARTH, E., COLLINS, N.E., TROSKIE, M., PENZHORN, B.L., 2008. Identification of a novel *Babesia* sp. from a sable antelope (*Hippotragus niger* Harris, 1838). Journal of Clinical Microbiology 46, 2247-2251.

OURA, C.A.L., BISHOP, R.P., WAMPANDE, E.M., LUBEGA, G.W., TAIT, A., 2004a. Application of a reverse line blot assay to the study of haemoparasites in cattle in Uganda. International Journal for Parasitology 34, 603-613.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

R Console, 2015. "World-Famous Astronaut". Copyright © 2015. The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit).

RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

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, 8.

RUBAIRE-AKIIKI, C.M., OKELLO-ONEN, J., MUSUNGA, D., KABAGAMBE, E.K., VAARST, M., OKELLO, D., OPOLOT, C., BISAGAYA, A., OKORI, C., BISAGATI, C., ONGYERA, S., MWAYI, M.T., 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, 251-266.

SIBEKO, K.P., OOSTHUIZEN, M.C., COLLINS, N.E., GEYSEN, D., RAMBRITCH, N.E., LATIF, A.A., GROENEVELD, H.T., POTGIETER, F.T., COETZER, J.A.W., 2008. Development and evaluation of a real-time polymerase chain reaction test for the detection of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. Veterinary Parasitology 155, 37-48.

SIVAKUMAR, T., HAYASHIDA, K., SUGIMOTO, C., YOKOYAMA, N., 2014. Evolution and genetic diversity of *Theileria*. Infection, Genetics and Evolution 27, 250-263.

STEYN, H.C., PRETORIUS, A., MCCRINDLE, C.M.E., STEINMANN, C.M.L., VAN KLEEF, M., 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Veterinary Microbiology 131, 258-265.

SUAREZ, C.E., NOH, S., 2011. Emerging perspectives in the research of bovine babesiosis and anaplasmosis. Veterinary Parasitology 180, 109-125.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30, 2725-2729.

UILENBERG, G., 2006. *Babesia* - A historical overview. Veterinary Parasitology 138, 3-10.

WALKER, A.R., BOUATTOUR, A., CAMICAS, J.-L., ESTRADA-PEÑA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G., PRESTON, P.M., 2013. Ticks of domestic animals in Africa: a guide to identification of species. International Consortium on Ticks and Tick Borne Diseases (ICTTD-2), 221 pp.

WOOLHOUSE, M.E.J., THUMBI, S.M., JENNINGS, A., CHASE-TOPPING, M., CALLABY, R., KIARA, H., OOSTHUIZEN, M.C., MBOLE-KARIUKI, M.N., CONRADIE, I., HANDEL, I.G., POOLE, E.J., NJIIRI, E., COLLINS, N.E., MURRAY, G., TAPIO, M., AUGUET, O.T., WEIR, W., MORRISON, W.I., KRUUK, L.E.B., BRONSVOORT, B.M.C., HANOTTE, O., COETZER, K., TOYE, P.G., 2015. Co-infections determine patterns of mortality in a population exposed to parasite infection. Science Advances 1 (2), e1400026. DOI: 10.1126/sciadv.1400026.

CHAPTER 6

6. Phylogeny of *Anaplasma* species and sequence analysis of *Anaplasma marginale* among cattle from a pastoral area of Karamoja, Uganda

6.1 Abstract

Few data are available on the phylogeny of *Anaplasma* species and the genotypes of *Anaplasma marginale* in cattle in Uganda. In this study, we evaluated the prevalence of *A. marginale* and *Anaplasma centrale* and used 16S rRNA gene sequence analysis to characterise *Anaplasma* species from cattle in Karamoja Region, north-eastern Uganda. Sequences from the *msp1α* gene of *A. marginale* were also used to genotype the *A. marginale* strains found. A duplex quantitative real-time polymerase chain reaction (qPCR) assay was used for the detection of *A. marginale* (*msp1β* gene) and *A. centrale* (*groEL* gene) in blood samples collected from 240 cattle in 20 randomly-selected herds in two districts, from November 2013 through January 2014. The qPCR assay showed that most cattle (82.9%; 95% confidence interval [CI] 78.2-87.7%) were positive for *A. marginale*, while fewer cattle (12.1%; 95% CI 7.9-16.2%) were positive for *A. centrale*. The mixed effect logistic regression analysis showed that the prevalence of *A. marginale* infection varied significantly ($p < 0.05$) according to locality while that of *A. centrale* varied significantly according to age group ($p < 0.05$). The full-length 16S rRNA gene for *Anaplasma* species from selected samples was amplified with the universal primers fD1 and rP2, while the *mp1α* gene for *Anaplasma marginale* was amplified with 1733F and 2957R primers. The amplicons were cloned and sequenced. The 16S rRNA sequences were used to reconstruct phylogenetic trees. Most *A. marginale* sequences (16/19) were closely related to (99-100% identities, up to 5 bp difference) and clustered with *A. marginale* strain Veld from cattle in South Africa, with strong bootstrap support, while three sequences were 100% identical and clustered with *A. marginale* strain from Virginia, USA. The sequence variants of *A. marginale* differed from each other by up to 6 bp. Two *A. centrale* sequences were closely related (100% identity) and clustered with the Israel vaccine strain. We found

four different kinds of MSP1a tandem repeat sequences (UP39-F-M^2-3) from 14 sequences that correspond to one *A*. *marginale* strain unique to Uganda. One tandem repeat (UP39) is unique to Karamoja cattle. The study contributes to knowledge of the genetic variability of *A*. *marginale* and *A*. *centrale*, and provides a pivotal background for future epidemiological investigations and control strategies.

6.2 Introduction

The genus *Anaplasma* (phylum Proteobacteria, order Rickettsiales, family Anaplasmataceae) contains obligate intracellular tick-borne organisms that include pathogens of cattle, namely *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma phagocytophilum* and *Anaplasma bovis* (Dumler et al., 2001; Rar and Golovljova, 2011). The main cause of bovine anaplasmosis in cattle is *A*. *marginale*, which is an intra-erythrocytic bacterium, transmitted to cattle biologically by ticks, mechanically through biting flies and blood-contaminated fomites, and transplacentally from dams to their calves (Aubry and Geale, 2011). Anaplasmosis is characterised by severe anaemia and death in infected cattle, and is responsible for great economic losses due to high morbidity and mortality, reduced weight gains and milk production, abortions, and treatment costs among cattle worldwide (Kocan et al., 2010). Both male ticks and cattle become persistently infected with *A*. *marginale* and serve as reservoirs of infection (Kocan et al., 2010). *Anaplasma centrale*, an intra-erythrocytic species, is often considered as a sub-species of *A*. *marginale* and is distributed in many parts of the world (Dumler et al., 2001). *Anaplasma centrale* causes a milder form of anaplasmosis, and is used as a live vaccine against *A*. *marginale* in several African, South American and Middle Eastern countries including Israel (Bell-Sakyi et al., 2015). *Anaplasma phagocytophilum* infects a wide range of hosts including humans, rodents, birds, dogs, cats, donkeys, horses and ruminants, and has been detected in Europe, America (North and South), Asia and Africa (Atif, 2015). However, the pathogen seems to cause tick-borne fever (animals) and human granulocytic anaplasmosis in Europe and USA only (Atif, 2015). *Anaplasma bovis* causes bovine monocytic anaplasmosis and has been identified in Asia and Africa (Aubry and Geale, 2011). Previous

molecular studies have demonstrated the occurrence of *Anaplasma* spp. from cattle in various parts of Uganda, namely *A*. *marginale* and *A*. *bovis* (Oura et al., 2004; Muhanguzi et al., 2010; Oura et al., 2011; Asiimwe et al., 2013), *A*. *centrale* (Muhanguzi et al., 2010; Asiimwe et al., 2013) and *A*. *phagocytophilum* (Muhanguzi et al., 2010).

The comparison of 16S rRNA gene sequences has been recognised as a powerful and precise method for classification and to determine the phylogenetic relationships of the organisms in the order Rickettsiales, at family, genus and species levels (Dumler et al., 2001; Yu et al., 2001). However, there are few data (Ikwap et al., 2010) regarding the sequence and phylogenetic analysis of *Anaplasma* species in cattle in Uganda. Globally, strains of *A*. *marginale* which vary in genotype, antigenic composition, morphology and infectivity for ticks have been identified using the major surface protein 1a gene (*msp1α*) of the pathogen (de la Fuente et al., 2007; Cabezas-Cruz et al., 2013; Mutshembele et al., 2014; Silva et al., 2015). In the genome of *A*. *marginale*, the *msp1α* is a single copy gene that encodes a 70-100 kDa protein (MSP1a) containing variable numbers of tandem repeat sequences (Allred et al., 1990). Due to the diversity of the *msp1α* gene of *A*. *marginale*, and the fact that the number and sequence of tandem repeats remains the same in a given strain, the gene has been recognised as a stable marker to identify geographic strains of the pathogen (Bowie et al., 2002; Cabezas-Cruz and de la Fuente, 2015). Furthermore, MSP1a is an adhesin for bovine erythrocytes and tick cells, thus, tandem repeats provide information regarding tick transmissibility phenotypes of *A*. *marginale* strains (de la Fuente et al., 2003; Cabezas-Cruz and de la Fuente, 2015). In addition, this sequence contains T and B cell epitopes that are important in the protective immune response (Brown et al., 2001; Brown et al., 2002). Recombinant MSP1a has been used in immunisation trials in cattle against *A*. *marginale* and demonstrated promising results (Almazán et al., 2012; Torina et al., 2014). Although MSP1a tandem repeats have been assessed from various parts of the world, no data involving cattle in Uganda is available.

The objectives of this study were to determine the prevalence of *A*. *marginale* and *A*. *centrale* infections in cattle by quantitative real-time PCR (qPCR), to establish the 16S rRNA gene phylogeny of *Anaplasma* spp. from cattle in Karamoja Region, and to identify *A*. *marginale* genotypes in cattle based on the *msp1α* gene. This may contribute to the available molecular epidemiological data on *Anaplasma* spp., which is fundamental for the development of effective diagnosis and control strategies, including improved vaccines that are cross-protective among the diverse strains.

6.3 Materials and Methods

6.3.1 Ethics approval

The study was approved by the Animal Ethics Committee at the University of Pretoria (V026-14), and the National Agricultural Research Organisation of Uganda (no. 1416). Permission was obtained to do the research in terms of Section 20 of the Animal Diseases Act, 1984 (Department of Agriculture, Forestry and Fisheries [DAFF], Pretoria, South Africa; reference number 12/11/1/1). A veterinary import permit (permit number 13/1/1/30/2/0-201408003716, DAFF, Republic of South Africa) was obtained to transport blood samples from Uganda to South Africa. Standard techniques were followed during the collection of blood samples.

6.3.2 Study area

This cross-sectional study was conducted in Moroto and Kotido Districts of Karamoja Region, north-eastern Uganda from November 2013 through January 2014.

6.3.3 Blood samples and DNA extraction

Twenty herds, 10 from each district, were randomly selected from 20 purposively-selected superherds for the study (see Map in Chapter 3). The effective sample size of cattle that were bled was calculated by taking into account the design effect, which is the loss of effectiveness by the use of cluster sampling, instead of simple random sampling. The design effect is basically the ratio of the actual variance, under the sampling method actually used, to the

variance computed under the assumption of simple random sampling. The design effect was estimated using the formula below (Killip et al., 2004):

$$DE = 1 + \delta (n-1)$$

where DE is the design effect, δ is the intracluster correlation coefficient for being positive to tick-borne pathogens, and n is the average cluster (herd) size. No pilot study was conducted to calculate the intracluster correlation coefficient, therefore a δ value of 0.03 was assumed. A value of 0.03 is interpreted to mean that the cattle in a herd (cluster) are about only 3% more likely to have the same chance of exposure than if the two animals were chosen at random in the serosurvey. A relatively large within-cluster variance is expected within a herd in the study area because groups of herds (10 to 20) graze together over a large area, which is expected to greatly reduce the chance that animals from the same herd are equally exposed to infected ticks. The average herd size was 40 cattle and a DE value of 2.17 was calculated. Therefore, the sample variance is 2.17 times bigger than it would be if the survey were based on the same sample size but selected randomly. An effective sample size of 369 cattle, taking into consideration the design effect, was calculated using the formula below (Killip et al., 2004):

$$ESS = mk/DE$$

where ESS = effective sample size, m = average number of cattle in a herd, k = number of herds, and DE = design effect. Given the available laboratory logistics, a total of 240 cattle were sampled for blood collection. In each herd, 12 cattle of various age groups and both sexes were selected by systematic sampling to represent about 30% of a herd. In a herd, animals were grouped by age category, and then sub-grouped by sex. At first, one animal was selected at random in a sub-group, and then other animals were picked using pre-determined sampling intervals. Proportional allocation was used to determine the number of cattle sampled in each category.

An equal number of animals (120) was sampled in the Moroto and Kotido Districts. Each blood sample was spotted on FTA® Classic Card (Whatman®-Whatman International Ltd, Maidstone, England) and genomic DNA was extracted from the blood using the QIAamp® DNA Mini Kit (QIAGEN, Southern Cross Biotechnology Pty Ltd, Cape Town, South Africa). The extracted DNA was stored at -20°C until further analyses.

6.3.4 Duplex quantitative real-time polymerase chain reaction (qPCR)

A duplex qPCR assay was used for simultaneous detection and quantification of *A*. *marginale* and *A*. *centrale* DNA (Carelli et al., 2007; Decaro et al., 2008). The PCR was performed on a LightCycler® 2.0 (Roche Diagnostics, Mannheim, Germany) using a LightCycler® FastStart TaqMan® kit (Roche Diagnostics). The primers AM-For (5'-TTG GCA AGG CAG CAG CTT-3') and AM-Rev (5'-TTC CGC GAG CAT GTG CAT-3') were used to amplify a 95 bp fragment of the *msp1β* gene for *A*. *marginale*, while the AC-For (5'-CTA TAC ACG CTT GCA TCT C-3') and AC-Rev (5'-CGC TTT ATG ATG TTG ATG C-3') primers were used to amplify a 77 bp fragment of the *groEL* gene for *A*. *centrale*. A TaqMan probe labelled with the fluorescent reporter dye 6-carboxy-fluoroscein and with blackhole quencher 1 (5' 6-FAM-TCG GTC TAA CAT CTC CAG GCT TTC AT-BHQ1-3') was used for the detection of *A*. *marginale*, while a TaqMan probe labelled with the fluorescent reporter dye LightCycler® Red 610 and with blackhole quencher 2 (5'-LC610-ATC ATC ATT CTT CCC CTT TAC CTC GT-BHQ2-3') was used for *A*. *centrale*. Mastermix pure grade water was used as a negative control while known *A*. *marginale* (from cattle in Mnisi community area, Mpumalanga, South Africa) and *A*. *centrale* (*A*. *centrale* vaccine strain from Onderstepoort Biological Products, Pretoria, South Africa) positive samples (confirmed by qPCR and species-specific gene sequence analysis) were included to serve as positive controls. Each PCR reaction contained 0.4x of FastStart mix, 0.6 µM of each *A*. *marginale* primer, 0.9 µM of AC-For primer, 0.6 µM of AC-Rev primer, 0.2 µM of each probe, 1 U (final concentration 0.025x) of UDG, 2.5 µl test sample DNA or positive/negative control, and nuclease free water to a total volume of 20 µl. Thermal cycling started with UDG activation at 40°C for 10 min, followed by activation of iTaq DNA polymerase at 95°C for 10 min and 40 cycles of denaturing at 95°C for 1 min and annealing-extension at 60°C for 1 min. The cooling

step was at 40°C for 30 sec. Fluorescence data for all cycles was analysed using LightCycler® Software Version 4.1 (Roche Diagnostics).

6.3.5 PCR amplification of 16S rRNA and *msp1α* genes

The full-length 16S rRNA gene was amplified from positive samples to characterise *Anaplasma* spp., and the *msp1α* gene was amplified to characterise *A. marginale* strains. Each PCR reaction was duplicated four times. The 16S rRNA PCR was carried out using the oligonucleotide primers fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and rP2 (5'-ACG GCT ACC TTG TTA CGA CTT-3') as previously described (Weisburg et al., 1991). The reaction mixture contained 2 µl of DNA, 0.4 µM of each primer, 1x (final concentration) Phusion™ Flash High-Fidelity PCR Master Mix (Thermo Scientific™, LTC Tech South Africa [Pty] Ltd) and nuclease free water to a total volume of 25 µl. The amplification cycle had a denaturation stage at 98°C for 10 sec, followed by 30 cycles at 98°C for 1 sec, 55°C for 5 sec and 72°C for 15 sec, and a final extension of 72°C for 1 min. The *msp1α* PCR was performed using species-specific oligonucleotide primers 1733F (5'-TGT GCT TAT GGC AGA CAT TTC C-3') and 2957R (5'-AAA CCT TGT AGC CCC AAC TTA TCC-3') as previously described (Lew et al., 2002). PCR reactions were prepared using 1x (final concentration) Phusion™ Flash High-Fidelity PCR Master Mix (Thermo Scientific™), 0.5 µM of each primer, 2.5 µl of DNA and nuclease free water to a total volume of 25 µl. The amplification cycles, following an initial denaturation of 98°C for 10 sec, consisted of 40 cycles of 98°C for 1 sec, 63°C for 5 sec and 72°C for 18 sec, followed by a final extension step at 72°C for 1 min. The PCR products were analysed by electrophoresis on a 2.0% TAE agarose gel (stained with ethidium bromide) and visualised under UV light, using a 1 kb ladder as a DNA size marker (Thermo Scientific™). The positive control was a reaction with DNA prepared from blood of cattle known to be infected with *A. marginale* (confirmed by qPCR and species-specific gene sequence analysis) while the negative control was a reaction mixture without DNA.

6.3.6 Cloning and sequencing of PCR products

The PCR products from the four duplicate reactions were pooled, to obtain a total volume of 100 µl, in order to minimise the possibility of obtaining sequence errors originating early in any one of the reactions. The amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN), then ligated into the pJET vector (CloneJET® PCR Cloning Kit, Thermo Scientific™) and transformed into JM 109 High Efficiency Competent cells (Promega, Madison, USA), according to the manufacturers' instructions. At least 10 colonies per sample were picked and screened by colony PCR using primers pJET 1.2F (5'-CGA CTC ACT ATA GGG AGA GCG GC-3') and pJET 1.2R (5'-GAA GAA CAT CGA TTT TCC ATG GCA G-3'). The colony PCR mixture contained 0.2 µM of each primer, 0.95x (final concentration) DreamTaq Green PCR Master Mix (Thermo Fisher Scientific™), one colony as template and nuclease free water to a total volume of 21 µl. The amplification cycles, following an initial denaturation of 95°C for 3 min, consisted of 25 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min, followed by a final extension step cycle at 72°C for 7 min. The colony PCR products were analysed by gel electrophoresis to determine the plasmids with the desirable inserts. Colony PCR products were sequenced at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa).

6.3.7 Sequence and phylogenetic analyses

Sequence reads were processed and assembled using CLC Genomics Workbench version 7.5.1 (CLC Bio, Boston, MA, USA). Consensus sequences were obtained from 21 clones for the 16S rRNA gene (six samples) and 14 clones for the *msp1α* gene (one sample). The Basic Local Alignment Search Tool [BLAST] (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search for homologous sequences from GenBank using blastn program. The assembled sequences were aligned with published sequences of related *Anaplasma* species (16S rRNA gene) using the Multiple Alignment using Fast Fourier Transform (MAFFT) [version 7] program (Katoh and Standley, 2013). The alignment was then manually truncated to include only the sequences of similar length using BioEdit (version 7.2.5) program (Hall, 1999). The jModelTest 2.1.3 (Darriba et al., 2012) was used to select the best-fit nucleotide substitution model, TIM3 + I, for the 16S rRNA sequences under the Akaike Information Criterion (AIC). Phylogenetic trees

for the 16S rRNA gene were reconstructed using maximum likelihood as implemented in PhyML 3.1 (Guindon et al., 2010), parsimony in Phylogenetic Analysis Using Parsimony [PAUP] (Swofford, 2002) and neighbour-joining as implemented in MEGA version 6.0 (Tamura et al., 2013). The reliability for the internal branches was assessed using the bootstrapping method [1000 bootstrap replicates] (Felsenstein, 1985). MrBayes version 3.2 was used for Bayesian phylogenetic inference using Markov chain Monte Carlo (MCMC) methods by four independent runs of one million generations, sampled every 1000 generations. Post-run analyses were carried out to get the posterior probabilities on trees (sumt) and parameters (sump), each at 5000 burn-in (Ronquist et al., 2012). The MrModeltest2.3 (Nylander, 2004) was used to perform hierarchical likelihood ratio tests and calculate the approximate AIC values for selecting the DNA substitution model for the Bayesian inference for the 16S rRNA nucleotide sequences, implemented in PAUP* version 4.0 (Swofford, 2002) and MrBayes (Ronquist et al., 2012). The genetic distances between the sequences were estimated by determining the number of base differences between sequences using MEGA6 (Tamura et al., 2013). Graphical representation and editing for the phylogenetic tree from PhyML 3.1 were performed with MEGA6 and Paint Tool for Windows 10.0.

The *msp1α* nucleotide sequences were translated to amino acid sequences using CLC Genomics Workbench version 7.5.1 (CLC Bio, Boston, MA, USA). The presence of tandem repeats was determined using Tandem Repeats Finder (Benson, 1999). Annotation of the tandem repeat sequences was performed using an in-house MSP1a tandem repeat identification script following a nomenclature given by Cabezas-Cruz et al. (2013). Strain identification was done based on the sequences of the *msp1α* tandem repeats (de la Fuente et al., 2007; Cabezas-Cruz et al., 2013).

6.3.8 Nucleotide sequence accession numbers

Representative 16S rRNA sequences from this study have been deposited in GenBank under accession numbers: **KU686784** (*A. centrale*), **KU686774**, **KU686789**, **KU686790,** **KU686792** and **KU686793** (*A. marginale*).

6.3.9 Statistical analyses

The qPCR data for *A*. *marginale* and *A*. *centrale* were used to estimate the population prevalences, with 95% confidence intervals by performing descriptive statistics with 1000 bootstrap replications and sampling by clustering using the Statistical Package for the Social Sciences (SPSS) and using R Console and RStudio. Bootstrapping was used to estimate the sampling distribution of the prevalence by assigning a measure of accuracy, in this case confidence interval. Inference about the cattle population from the sample data was modelled by resampling from original sample data by using sampling with replacement. A conceptual framework was developed to select candidate variables identified as exposure factors for tick-borne infections for the production system of Karamoja Region. The exposure variables considered were age (5-12 months, 13-24 months and > 24 months), sex (male or female) and district (Moroto and Kotido). Intra-cluster correlations within the herds were tested for the qPCR test status for *A*. *marginale* and *A*. *centrale*, by performing log ratio tests between a model 'herd' as random effect and a null model. This was followed by a mixed effect logistic regression in which the herd was included as a random effect and the variables of age category, sex and district were fixed effects. The Generalised Linear Mixed Model based on the -2 log pseudo likelihood was used to compare models. The data were analysed using SPSS version 23.0 (IBM SPSS, 2014), and R Console version 3.2.1 (R Console, 2015) and RStudio version 3.2.1 (RStudio Team, 2015) at 5% level of significance.

6.4 Results

6.4.1 Prevalence of *A*. *marginale* and *A*. *centrale*

A total of 20 herds and 240 individual cattle (12 per herd) were sampled for blood collection in Moroto and Kotido Districts. The qPCR assay showed that most cattle were positive for *A*. *marginale* (82.9%; 95% confidence interval [CI] 78.2-87.7%), while fewer were positive for *A*. *centrale* (12.1%; 95% CI 7.9-16.2%). The log ratio test for herd effect showed that *A*. *marginale* and *A*. *centrale* qPCR test statuses were clustered within herds ($p < 0.05$). The mixed effect logistic regression analysis showed that age was significantly associated ($p=0.006$) with *A*.

centrale infection, but not for *A*. *marginale* among the three age groups. There was a significant difference in the proportions of cattle that were positive for *A*. *marginale* between Moroto (89.2%) and Kotido (76.7%) (p = 0.02), but not for *A*. *centrale* between the two districts ($p > 0.05$). Sex of cattle had no significant association (p>0.05) with *A*. *marginale* and *A*. *centrale* infections. The model fit was good (87.9% for *A*. *centrale* and 82.9% for *A*. *marginale*).

6.4.2 PCR amplification and cloning of 16S rRNA and *msp1α* genes

Eight DNA samples with strong positive reactions (threshold cycle 25-29) from the qPCR assay were amplified, targeting the full-length 16S rRNA gene to characterise *Anaplasma* spp., and targeting the *msp1α* gene to characterise *A*. *marginale* strains. PCR products were obtained from 16S rRNA gene amplification of six out of the eight samples. The PCR products of around 1,250 bp, for each sample, were cloned. For each cloned PCR product, ten clones were picked and colony PCR was done to identify recombinant clones. Figure 6.1 shows a representative example of colony PCR products from ten clones generated from a 1,250 bp 16S rRNA gene fragment amplified from sample RB 069. Fragments of the expected size (1,250 bp) were amplified from seven of the clones.

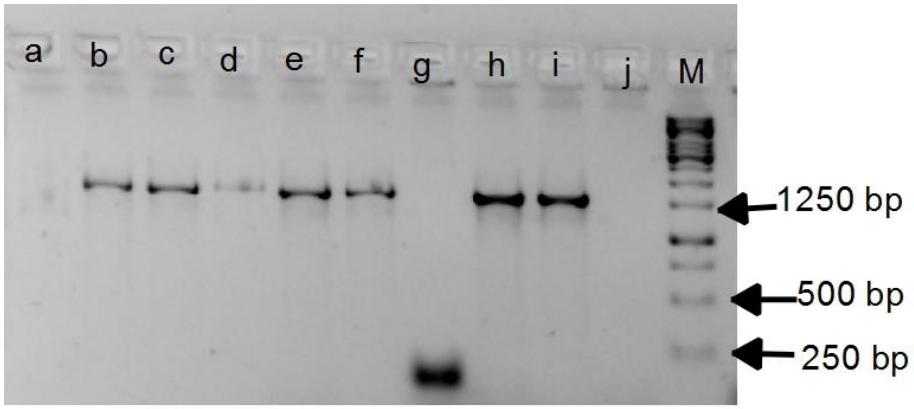

Figure 6.1: Gel electrophoresis in a 2.0% TAE agarose gel (stained with ethidium bromide) of colony PCR products amplified from clones of the full-length 16S rRNA gene from sample RB 069 that was positive for *Anaplasma marginale*. Colony PCR was done with the primers pJET 1.2F and pJET 1.2R. This representative DNA sample was obtained from blood collected from cattle in Karamoja Region, Uganda. Lanes a to j: amplification products from individual clones. Absence of bands in lanes may indicate colonies containing plasmids lacking inserts. Lane M: 1 kb bp DNA size marker (Thermo Scientific™, LTC Tech South Africa [Pty] Ltd).

The *msp1α* PCR for eight samples each yielded one or more amplicons of various sizes (300 to 1,000 bp). The presence of more than one *msp1α* PCR product in some samples indicates the presence of multiple *A*. *marginale* strains infecting individual animals. PCR products from all the eight selected samples were cloned and sequenced. Figure 6.2 illustrates amplicons from the *msp1α* PCR from five out of the eight selected DNA samples. Figure 6.3 shows the colony PCR products obtained after the cloning of the amplicons from two of the samples.

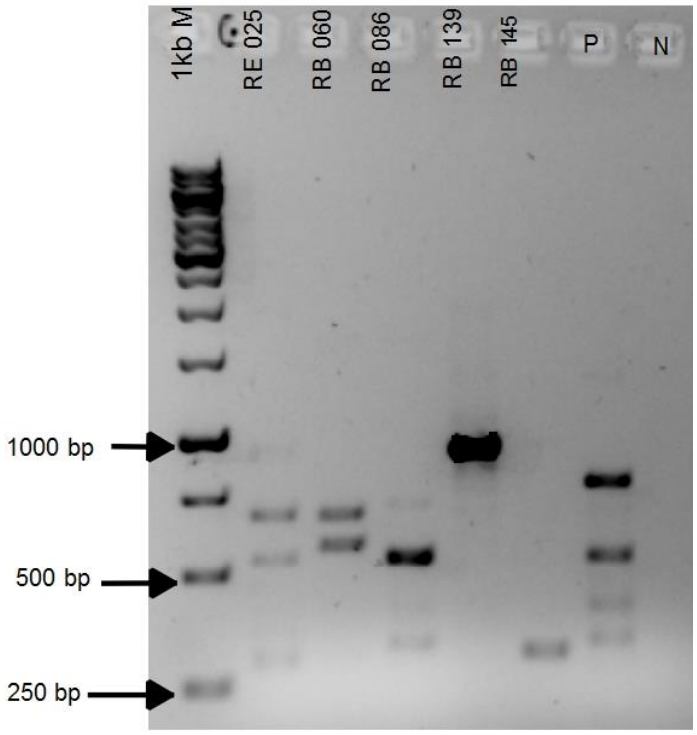

Figure 6.2: PCR products amplified from DNA extracted from blood samples using the *msp1α* gene primer set 1733F/2957R for genotyping *Anaplasma marginale* strains. The blood was taken from cattle in Karamoja Region in Uganda from November 2013 through January 2014. The PCR products were analysed by electrophoresis on a 2.0% TAE agarose gel (stained with ethidium bromide) and visualised under UV light. Lane M: 1 kb DNA size marker (Thermo Scientific™, LTC Tech South Africa [Pty] Ltd), lanes RE 025, RB 060, RB 086, RB 139 and RB 145: PCR products from five samples, lane P: positive control (*Anaplasma marginale* DNA from a known positive sample), lane N: negative control (no DNA).

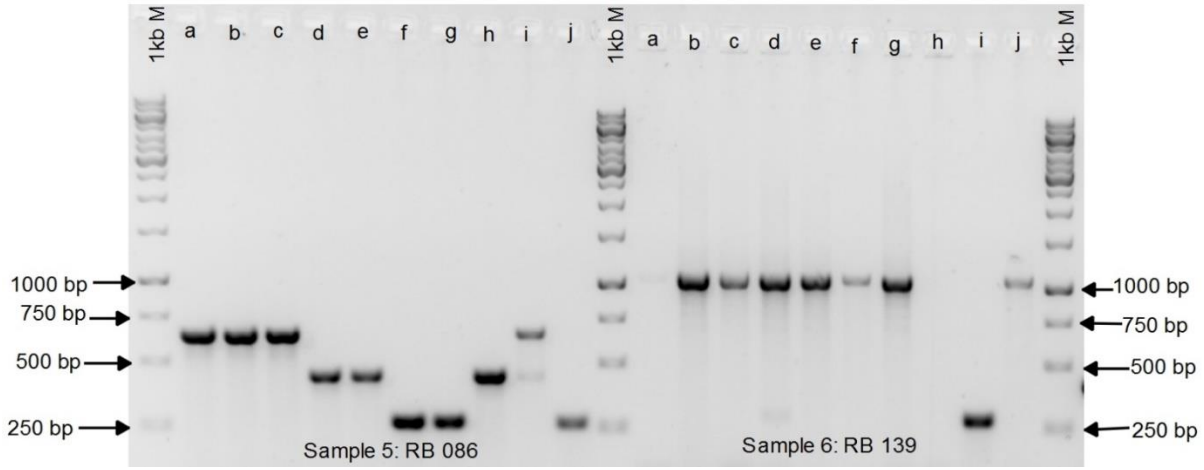

Figure 6.3: Gel electrophoresis in a 2.0% TAE agarose gel (stained with ethidium bromide) of colony PCR products of the *msp1α* gene (*Anaplasma marginale*) obtained using the primers pJET 1.2F and pJET 1.2R. The DNA (RB 086 and RB 139) was extracted from blood samples collected from cattle in Karamoja Region, Uganda. Lanes M: 1 kb DNA size marker (Thermo Scientific™, LTC Tech South Africa [Pty] Ltd), lanes a-j and through again a-j: amplification products from individual clones from two samples.

6.4.3 16S rRNA gene sequence analysis

Twenty-one near full-length 16S rRNA gene sequences were obtained from six cattle samples in this study. The BLASTn sequence homology search indicated that 16 sequences were closely related (99-100% identity, 99-100% query cover, up to 5 bp difference) to *A. marginale* from South Africa [Veld, AF414873] (Lew et al., 2003) and differed from each other by up to 6 bp. There was identity among 11 sequences (RE025b, RE028a, RB069a, RB069f, RB069g, RB083a, RB086a, RB123b, RB123c, RB123e, RB123f) from six samples, between two sequences (RE025c, RB069b) from two samples, and between two sequences (RB069c, RB069d) from one sample [Table 6.1]. One sequence, RB069e, from one sample differed from other *A. marginale* sequences by 2 to 3 bp. Three sequences (RE025a, RE025d, RE025e) from one sample showed 100% identity (100% query cover) with a sequence corresponding to *A. marginale* strain from Virginia, USA [AF311303] and were identical to each other (no nucleotide differences) [Table 6.1]. Two 16S rRNA sequences (RB123a, RB123d) of *A. centrale* were obtained from one sample, which was also positive for *A. marginale*. The *A. centrale* sequences were identical to each other and showed 100% identity (100% query cover) with a sequence corresponding to the *A. centrale* Israel vaccine strain from South Africa (accession

no. AF309869) [Rurangirwa, unpublished data). The two sequences were also 100% identical (95% cover query) to the Italian CC strain (EF520686) (Carelli et al., 2008). Sequence alignments of the 16S rRNA sequences of *A*. *marginale* and *A*. *centrale* with the respective RLB hybridisation probes showed that the V1 hypervariable region of the probe was identical in all sequences (data not shown).

Table 6.1: Estimates of evolutionary divergence between the 16S rRNA gene sequences of *Anaplasma marginale* and *A. centrale* identified in blood samples of cattle in Karamoja Region, Uganda, and previously published 16S rRNA sequences in GenBank. The number of base differences from pairwise analyses of 25 sequences is shown. There were a total of 1371 positions in the dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	*A. marginale*_Veld_South_Africa_AF414873																									
2	RB123b	0																								
3	RB069a	0	0																							
4	RE025c	1	1	1																						
5	RB069b	1	1	1	0																					
6	RB083a	0	0	0	1	1																				
7	RB069e	2	2	2	3	3	2																			
8	RB069c	5	5	5	6	6	5	3																		
9	RB069d	5	5	5	6	6	5	3	0																	
10	RB123c	0	0	0	1	1	0	2	5	5																
11	RB069f	0	0	0	1	1	0	2	5	5	0															
12	RE025b	0	0	0	1	1	0	2	5	5	0	0														
13	RB086a	0	0	0	1	1	0	2	5	5	0	0	0													
14	RB069g	0	0	0	1	1	0	2	5	5	0	0	0	0												
15	RB123e	0	0	0	1	1	0	2	5	5	0	0	0	0	0											
16	RB123f	0	0	0	1	1	0	2	5	5	0	0	0	0	0	0										
17	RE028a	0	0	0	1	1	0	2	5	5	0	0	0	0	0	0	0									
18	*A. marginale*_Virginia_AF311303	1	1	1	2	2	1	3	6	6	1	1	1	1	1	1	1	1								
19	RE025e	1	1	1	2	2	1	3	6	6	1	1	1	1	1	1	1	1	0							
20	RE025d	1	1	1	2	2	1	3	6	6	1	1	1	1	1	1	1	1	0	0						
21	RE025a	1	1	1	2	2	1	3	6	6	1	1	1	1	1	1	1	1	0	0	0					
22	*A. centrale* CC Italy EF520686	9	9	9	10	10	9	11	14	14	9	9	9	9	9	9	9	9	10	10	10	10				
23	*A. centrale* Israel vaccine AF309869	9	9	9	10	10	9	11	14	14	9	9	9	9	9	9	9	9	10	10	10	10	0			
24	RB123d	9	9	9	10	10	9	11	14	14	9	9	9	9	9	9	9	9	10	10	10	10	0	0		
25	RB123a	9	9	9	10	10	9	11	14	14	9	9	9	9	9	9	9	9	10	10	10	10	0	0	0	

6.4.4 16S rRNA phylogenetic analyses

Neighbour-joining, maximum likelihood and maximum parsimony were used to demonstrate the relationships between the 16S rRNA gene variants of *A. marginale* and *A. centrale* identified in this study and other related *Anaplasma* spp. The trees yielded similar topology and high bootstrap values. Figures 6.4 shows a representative tree generated by maximum likelihood. Only representative sequences of the variants are shown. The 16S rRNA sequences of *A. marginale* were grouped into two clusters, and they were separate from the *A. centrale* clade. Four *A. marginale* sequences - RB083a (representing 11 clones from six samples), RB069b (representing two clones from two samples), RB069c (representing two clones from one sample), and RB069e clustered with the published 16S rRNA sequences of *A. marginale* from cattle in South Africa and Zimbabwe [Clade 1] (Lew et al., 2003). The other cluster for *A. marginale* comprised sequence RE025a (representing three clones from one sample), from this study, and the 16S rRNA sequences of *A. marginale* from Uruguay, Australia, USA, and black wildebeest from South Africa (Clade 2). The *A. centrale* sequence RB123a (representing two clones from one sample) grouped with 16S rRNA sequences of *A. centrale* from South Africa, Israel, Italy and Australia.

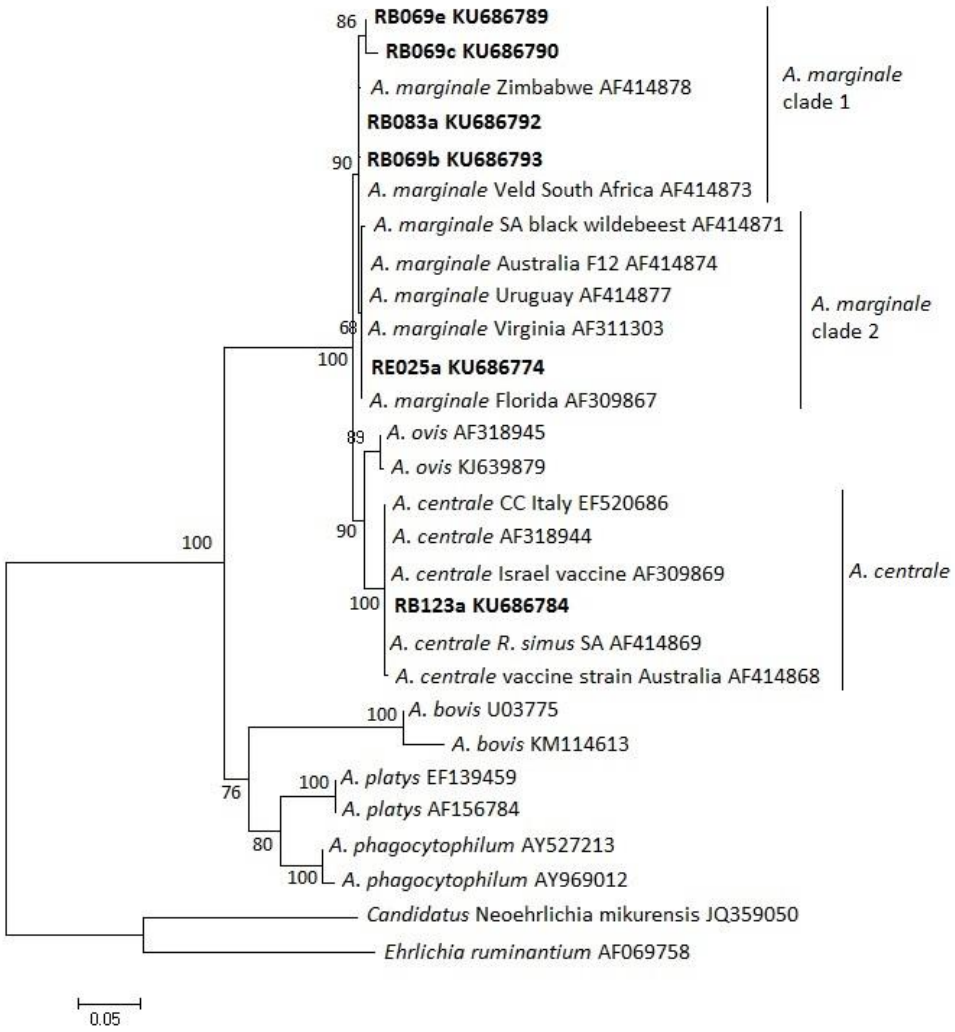

Figure 6.4: A maximum likelihood tree showing the phylogenetic relationship between the *A. marginale* and *A. centrale* 16S rRNA sequence variants, identified in blood samples from cattle in Karamoja Region, Uganda with other *Anaplasma* species. The numbers at the internal nodes represent the percentage of 1000 replicates (bootstrap) for which the same branching patterns were obtained. Some sequences obtained in this study are identical to each other; therefore, only representatives of variants are shown (in bold). Sequences: RB083a represents 11 clones (others are: RE025b, RE028a, RB069a, RB069f, RB069g, RB086a, RB123b, RB123c, RB123e, RB123f) from six samples, RB069c represents two clones (the other is RB069d) from one sample, RB069b represents two clones (the other is RE025c) from two samples, RE025a represents three clones (the others are RE025d, RE025e) from one sample. The *A. centrale* sequence RB123a represents two clones (the other is RB123d) from one sample. The tree was rooted using the 16S rRNA gene sequences of *Candidatus* Neoehrlichia mikurensis and *Ehrlichia ruminantium*. The accession number of each sequence is indicated in the sequence name. Branch lengths are proportional to the estimated genetic distance (number of substitutions per site over a length of 1378 bp of the 16S rRNA gene) between the taxa.

6.4.5 *Anaplasma marginale* msp1α tandem repeats

MSP1a tandem repeats were identified from 14 clones from one sample as shown in Table 6.2. The number and order of the tandem repeats were similar in the 14 sequences (828 to 949 bp) examined. There were four different kinds of tandem repeats (UP39-F-M^2-3), which correspond to one *A. marginale* strain in cattle in Karamoja Region, Uganda. One tandem repeat (UP39) was unique to this study.

Table 6.2: Amino acid sequences of MSP1a tandem repeats of *A. marginale* detected in infected cattle from Karamoja Region, Uganda (F, M, 3 and UP39) compared to the prototype *A. marginale* Florida strain MSP1a tandem repeat M32871 (A)

Repeat identity (ID)	Encoded sequence*
A**	DDSSSASGQQQESSVSSQSE-ASTSSQLG
F	T. . . . . GQ. . . . .
M	A. . . . . GQ. . . . .
M	A. . . . . GQ. . . . .
3	A. . . . . L. . . GQ. . . . .
UP39	EYE. . . . . L. . . GQ. . . . .

*The one-letter amino acid code was used to depict MSP1a repeat sequences. Dots indicate identical amino acids and gaps indicate deletions/insertions. **The prototype *A. marginale* Florida strain Msp1a tandem repeat M32871 (A) was used as a model for amino acid comparison. The identity (ID) of each repeat form, except the newly reported, was assigned as previously described in Cabezas-Cruz et al. (2013).

6.5 Discussion

In this study, we investigated the molecular prevalence and the 16S rRNA gene phylogeny of *Anaplasma* spp. and analysed the *msp1α* sequences of *A. marginale* from cattle in Karamoja Region, Uganda. The prevalence of *A. marginale* (82.9%) was 5-22 times higher than previously reported from cattle in other parts of Uganda [using reverse line blot hybridisation, RLB] (Oura et al., 2004; Muhanguzi et al., 2010; Asiimwe et al., 2013) and Tunisia [25.4% using qPCR, about thrice lower] (Belkahia et al., 2015), but similar to that reported in seven out of the nine provinces of South Africa [≥ 65% using *msp1α* PCR] (Mutshembele et al., 2014). The high prevalence of *A. marginale* in this study may be due to high infection challenge from

ticks. A recent study in Karamoja showed that most cattle (53.4%) were infested with *Rhipicephalus decoloratus* ticks (Byaruhanga et al., 2015) [Chapter 4 of this thesis]. Possibly, the ticks are persistently infected with *A. marginale*, with a likelihood of transmission of infection to cattle. Cattle in Moroto District were significantly more likely to be infected with *A. marginale* than those in Kotido. The variation in infection may be due to the higher tick infestation on cattle in Moroto than Kotido District (Byaruhanga et al., 2015) [Chapter 4 of this thesis]. The prevalence of *A. centrale* (12.1%) was thrice higher than that observed in other parts of Uganda (Muhanguzi et al., 2010; Asiimwe et al., 2013), and similar to that reported in Tunisia [15.1%] (Belkahia et al., 2015). Possibly the tick species found in Karamoja Region and not reported in other parts of Uganda (Byaruhanga et al., 2015) [Chapter 4 of this thesis] may be responsible for transmission of *A. centrale* resulting in a higher prevalence of the infection. The results of our study are more linked with the difference in the geographical situation rather than the methods used for detection of *Anaplasma* spp. Some of the differences in prevalence can be explained by the techniques used in the different studies, for example the qPCR assays can detect DNA of pathogens in samples previously diagnosed as negative by RLB hybridisation assay. However, big differences are most likely to be due to geographical differences.

The 16S rRNA gene sequence and phylogenetic analyses for *A. marginale* showed heterogeneity, suggesting phylo-geographical resolution, which is consistent with previous reports from Tunisia (Belkahia et al., 2015) and other parts of the world (Lew et al., 2003). The heterogeneity may be attributed to cattle movements, which increase the likelihood of infection with distinct *A. marginale* genotypes, and have been suggested as a main reason for increasing the number of genotypes identified in some geographic regions (de la Fuente et al., 2007; Kocan et al., 2010; Belkahia et al., 2015). The 16S rRNA sequences of *A. centrale* grouped in a single cluster that is separate from other *Anaplasma* spp. The *A. centrale* sequences showed a high genetic relatedness (100% identity) to the *A. centrale* Israel vaccine strain (Rurangirwa, unpublished data) and *A. centrale* strain CC which was recovered from an acute case of anaplasmosis, and which was associated an *A. centrale* infection in a dairy cow

in Italy (Carelli et al., 2008). Therefore, *A*. *centrale* infections among cattle in Karamoja may contribute to clinical cases of anaplasmosis. Since the attenuated *A*. *centrale* vaccine was never used in Uganda, and the neighbouring South Sudan and Kenya, it is unlikely that the strain of *A*. *centrale* in Karamoja that is identical to the Israel vaccine strain was introduced by immunisation. In a recent study, the Israel vaccine *msp1αS* genotype and one of the vaccine variant *msp1αS* genotypes were detected in unvaccinated cattle and buffalo in South Africa (Khumalo et al., 2016); therefore, it is possible that the vaccine strain could be circulating in wildlife in other parts of Africa, from which it could be introduced into cattle populations. It should be noted that the 16S rRNA gene, used for characterisation of *A*. *centrale* in this study, is often highly conserved within species and may not differentiate between strains of pathogens (Weisburg et al., 1991). The limited geographic specific differences among *A*. *centrale* strains from different geographical areas of the world (Lew et al., 2003) may have advantageous implications on the control of bovine anaplasmosis since *A*. *centrale* is widely used as a vaccine against *A*. *marginale* (Aubry and Geale, 2011).

We found four different kinds of *msp1α* tandem repeat sequences, following the nomenclature previously proposed (de la Fuente et al., 2007; Cabezas-Cruz et al., 2013). The tandem repeats correspond to one strain of *A*. *marginale* that is unique to Karamoja cattle. One of the tandem repeats is unique to this study, and has been named UP39 (Table 6.2). Tandem repeat M has been found in *A*. *marginale* strains in five continents of the world [countries: USA, Brazil, Argentina, Israel, Italy, Mexico, South Africa] (de la Fuente et al., 2007; Cabezas-Cruz et al., 2013; Mutshembele et al., 2014) while tandem repeat F was found in four continents [countries: Mexico, Argentina, Brazil, Israel, and South Africa] (de la Fuente et al., 2007). Tandem repeat 3 is widespread in *A*. *marginale* strains in South Africa (Mutshembele et al., 2014) and is also present in some strains in Israel (de la Fuente et al., 2007). However, a weak association between specific tandem repeat sequences and particular geographic regions was previously reported (de la Fuente et al., 2007) and may be attributed to worldwide cattle movement, among other factors (Cabezas-Cruz et al., 2013). The first glutamine (Q) and the last serine (S) in the neutralisation-sensitive epitope [Q/E (ASTSS)]

(Allred et al., 1990) were present in all four tandem repeats identified in this study, indicating a likelihood of the amino-acid oligopeptides of Ugandan *A*. *marginale* binding with neutralisation antibodies (Allred et al., 1990). However, the neutralisation sensitivity of *A*. *marginale* variants has not been defined (Cabezas-Cruz and de la Fuente, 2015). The sequence of amino acids in the immunodominant B-cell epitope [SSAGGQQQESS] (Garcia-Garcia et al., 2004) was also the same in the repeat structures identified in this study.

In this study, analysis of the *msp1α* gene PCR products from eight samples, by gel electrophoresis, revealed fragments of different lengths. However, attempts to clone and sequence the PCR products from most of the samples were not successful, possibly because there was inadequate DNA in the samples to generate sufficient amounts of PCR products to allow cloning. Multiple *msp1α* amplicons in individual cattle may represent different genotypes and may suggest that individual cattle harbour several genetically heterologous strains of *A*. *marginale*. Strain superinfection occurs when a second strain infects a host already infected with and having mounted an immune response to a primary strain (Palmer and Brayton, 2013). The prevalence of superinfection with *A*. *marginale* has been shown to be higher in areas with higher prevalence of infection (Castañeda-Ortiz et al., 2015). Further studies are needed to investigate *A*. *marginale* and *A*. *centrale* genotypes from cattle in Uganda, to provide more comprehensive data on their taxonomic position and diversity. Further studies are also needed to investigate the possibility of transmission and maintenance of *A*. *marginale* and *A*. *centrale* by various tick species in Ugandan cattle.

6.6 Conclusions

In this study, we evaluated the prevalence and 16S rRNA gene phylogeny of *Anaplasma* spp., and analysed the *msp1α* sequences for *A*. *marginale* from cattle in Karamoja Region, north-eastern Uganda. We demonstrated a high prevalence of *A*. *marginale* among cattle. There was 16S rRNA genetic heterogeneity within *A*. *marginale* in cattle in the study area. Four different kinds of *msp1α* tandem repeat sequences, which correspond to one genotype of *A*.

marginale were found. Our study contributes to knowledge of the genetic variability of *A*. *marginale* and *A*. *centrale* from different geographic areas, which is important in the development and implementation of control programmes.

6.7 References

ALLRED, D.R., MCGUIRE, T.C., PALMER, G.H., LEIB, S.R., HARKINS, T.M., MCELWAIN, T.F., BARBET, A.F., 1990. Molecular basis for surface antigen size polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. Proceedings of the National Academy of Sciences of the United States of America 87, 3220-3224.

ALMAZÁN, C., MORENO-CANTÚ, O., MORENO-CID, J.A., GALINDO, R.C., CANALES, M., VILLAR, M., DE LA FUENTE, J., 2012. Control of tick infestations in cattle vaccinated with bacterial membranes containing surface-exposed tick protective antigens. Vaccine 30, 265-272.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

ASIIMWE, B.B., WEIR, W., TAIT, A., LUBEGA, G.W., OURA, C.A.L., 2013. Haemoparasite infection kinetics and the population structure of *Theileria parva* on a single farm in Uganda. Veterinary Parasitology 193, 8-14.

ATIF, F.A., 2015. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. Parasitology Research 114, 3941-3957.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

BELKAHIA, H., SAID, M.B., ALBERTI, A., ABDI, K., ISSAOUI, Z., HATTAB, D., GHARBI, M., MESSADI, L., 2015. First molecular survey and novel genetic variants' identification of

Anaplasma marginale, *A*. *centrale* and *A*. *bovis* in cattle from Tunisia. Infection, Genetics and Evolution 34, 361-371.

BELL-SAKYI, L., PALOMAR, A.M., BRADFORD, E.L., SHKAP, V., 2015. Propagation of the Israeli vaccine strain of *Anaplasma centrale* in tick cell lines. Veterinary Microbiology 179, 270-276.

BENSON, G., 1999. Tandem repeats finder: a program to analyse DNA sequences. Nucleic Acids Research 27, 573-580.

BOWIE, M.V., DE LA FUENTE, J., KOCAN, K.M., BLOUIN, E.F., BARBET, A.F., 2002. Conservation of major surface protein 1 genes of *Anaplasma marginale* during cyclic transmission between ticks and cattle. Gene 282, 95-102.

BROWN, W.C., MCGUIRE, T.C., MWANGI, W., KEGERREIS, K.A., MACMILLAN, H., LEWIN, H.A., PALMER, G.H., 2002. Major histocompatibility complex class II DR-restricted memory $CD4^+$ T lymphocytes recognize conserved immunodominant epitopes of *Anaplasma marginale* major surface protein 1a. Infection and Immunity 70, 5521-5532.

BROWN, W.C., PALMER, G.H., LEWIN, H.A., MCGUIRE, T.C., 2001. $CD4^+$ T lymphocytes from calves immunized with *Anaplasma marginale* major surface protein 1 (MSP1), a heteromeric complex of MSP1a and MSP1b, preferentially recognize the MSP1a carboxyl terminus that is conserved among strains. Infection and Immunity 69, 6853-6862.

BYARUHANGA, C., COLLINS, N.E., KNOBEL, D., OOSTHUIZEN, M.C., 2015. Endemic status of tick-borne infections and tick species diversity among transhumant zebu cattle in Karamoja Region, Uganda: support for control approaches. Veterinary Parasitology: Regional Studies and Reports, 1-2, 21-30.

CABEZAS-CRUZ, A., DE LA FUENTE, J., 2015. *Anaplasma marginale* major surface protein 1a: A marker of strain diversity with implications for control of bovine anaplasmosis. Ticks and Tick-borne Diseases 6, 205-210.

CABEZAS-CRUZ, A., PASSOS, L.M.F., LIS, K., KENNEIL, R., VALDÉS, J.J., FERROLHO, J., TONK, M., POHL, A.E., GRUBHOFFER, L., ZWEYGARTH, E., SHKAP, V., RIBEIRO, M.F.B., ESTRADA-PEÑA, A., KOCAN, K.M., DE LA FUENTE, J., 2013. Functional and immunological relevance of *Anaplasma marginale* major surface protein 1a sequence and structural analysis. PLoS ONE 8 (6), e65243. doi: 10.1371/jounal.pone.0065243.

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, 107-114.

CARELLI, G., DECARO, N., LORUSSO, E., PARADIES, P., ELIA, G., MARTELLA, V., BUONAVOGLIA, C., CECI, L., 2008. First report of bovine anaplasmosis caused by *Anaplasma centrale* in Europe. Annals of the New York Academy of Sciences 1149, 107-110.

CASTAÑEDA-ORTIZ, E.J., UETI, M.W., CAMACHO-NUEZ, M., MOSQUEDA, J.J., MOUSEL, M.R., JOHNSON, W.C., PALMER, G.H., 2015. Association of *Anaplasma marginale* strain superinfection with infection prevalence within tropical regions. PLoS One 10 (3), e0120748. doi: 10.1371/journal.pone.0120748.

DARRIBA, D., TABOADA, G.L., DOALLO, R., POSADA, D., 2012. JModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

DE LA FUENTE, J., GARCIA-GARCIA, J.C., BLOUIN, E.F., KOCAN, K.M., 2003. Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia *Anaplasma marginale* to host cells. Veterinary Microbiology 91, 265-283.

DE LA FUENTE, J., RUYBAL, P., MTSHALI, M.S., NARANJO, V., SHUQING, L., MANGOLD, A.J., RODRÍGUEZ, S.D., JIMÉNEZ, R., VICENTE, J., MORETTA, R., TORINA, A., ALMAZÁN, C., MBATI, P.M., DE ECHAIDE, S.T., FARBER, M., ROSARIO-CRUZ, R., GORTAZAR, C., KOCAN, K.M., 2007. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. Veterinary Microbiology 119, 382-390.

DECARO, N., CARELLI, G., LORUSSO, E., LUCENTE, M.S., GRECO, G., LORUSSO, A., RADOGNA, A., CECI, L., BUONAVOGLIA, C., 2008. Duplex real-time polymerase chain reaction for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale*. Journal of Veterinary Diagnostic Investigation 20, 606-611.

DUMLER, J.S., BARBET, A.F., BEKKER, C.P.J., 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 combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. International Journal of Systematic and Evolutionary Microbiology 51, 2145-2165.

EGERU, A., WASONGA, O., KYAGULANYI, J., MAJALIWA, G.J.M., MACOPIYO, L., MBURU, J., 2014. Spatio-temporal dynamics of forage and land cover changes in Karamoja sub-region, Uganda. Pastoralism: Research, Policy and Practice 4, 6.

FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the Bootstrap. Evolution 39, 783-791.

GARCIA-GARCIA, J.C., DE LA FUENTE, J., KOCAN, K.M., BLOUIN, E.F., HALBUR, T., ONET, V.C., SALIKI, J.T., 2004. Mapping of B-cell epitopes in the N-terminal repeated peptides of *Anaplasma marginale* major surface protein 1a and characterization of the humoral immune response of cattle immunized with recombinant and whole organism antigens. Veterinary Immunology and Immunopathology 98, 137-151.

GUINDON, S., DUFAYARD, J.F., LEFORT, V., ANISIMOVA, M., HORDIJK, W., GASCUEL, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0 Systematic Biology 59, 307-321.

HALL, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95-98.

IBM SPSS, 2014. IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY.

IICD, 2010. Livestock disease surveillance in Karamoja. The importance of early detection of diseases and epidemics for livestock dependent communities, Ruspantini, C. (Ed.), European Commission, Government of Uganda and Institute for International Co-operation and Development (IICD) Uganda, 67 pp. www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/C&DPublicationLivestockDiseaseSurveillance2.pdf. Accessed March 24, 2015.

IKWAP, K., MUHANGUZI, R., MUWAZI, M.K., SAIMO, M.K., LUBEGA, G.W., 2010. Molecular detection of previously unknown *Anaplasma* genotype in cattle from Uganda. International Journal of Animal and Veterinary Advances 2, 97-103.

KATOH, K., STANDLEY, D.M., 2013. MAFFT multiple sequence alignment software 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772-780.

KHUMALO, Z.T.H., CATANESE, H.N., LIESCHING, N., HOVE, P., COLLINS, N.E., CHAISI, M.E., GEBREMEDHIN, A.H., OOSTHUIZEN, M.C., BRAYTON, K.A., 2016. Characterization of *Anaplasma marginale* subsp. *centrale* strains by use of msp1aS genotyping reveals a wildlife reservoir. Journal of Clinical Microbiology 54, 2503-2512.

KILLIP, S., MAHFOUD, Z., PEARCE, K., 2004. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers. The Annals of Family Medicine 2, 204-208.

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, 95-107.

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, 325-335.

LEW, A.E., GALE, K.R., MINCHIN, C.M., SHKAP, V., DE WAAL, D.T., 2003. Phylogenetic analysis of the erythrocytic *Anaplasma* species based on 16S rDNA and GroEL (HSP60) sequences of *A*. *marginale*, *A*. *centrale*, and *A*. *ovis* and the specific detection of *A*. *centrale* vaccine strain. Veterinary Microbiology 92, 145-160.

MAAIF, 2011. Statistical Abstract, Agricultural Planning Department, Ministry of Agriculture, Animal Industry and Fisheries. www.agriculture.go.ug/userfiles/Statistical Abstract 2011.pdf. Accessed June 7, 2013.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

MUTSHEMBELE, A.M., CABEZAS-CRUZ, A., MTSHALI, M.S., THEKISOE, O.M.M., GALINDO, R.C., DE LA FUENTE, J., 2014. Epidemiology and evolution of the genetic variability of *Anaplasma marginale* in South Africa. Ticks and Tick-borne Diseases 5, 624-631.

NYLANDER, J.A.A., 2004. MrModeltest2.3. C program for selecting DNA substitution models using PAUP*. Evolutionary Biology Centre, Uppsala University, Sweden.

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, 603-613.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

PALMER, G.H., BRAYTON, K.A., 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15, 1969-1975.

R Console, 2015. "World-Famous Astronaut". Copyright © 2015. The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit).

RAR, V., GOLOVLJOVA, I., 2011. *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia" bacteria: Pathogenicity, biodiversity, and molecular genetic characteristics, a review Infection, Genetics and Evolution 11, 1842-1861.

RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D.L., DARLING, A., SEBASTIAN, H., LARGET, B., LIU, L., SUCHARD, M.A., HUELSENBECK, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 1-4.

RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

SILVA, J.B., GONÇALVES, L.R., VARANI, A.M., ANDRÉ, M.R., MACHADO, R.Z., 2015. Genetic diversity and molecular phylogeny of *Anaplasma marginale* studied longitudinally under natural transmission conditions in Rio de Janeiro, Brazil. Ticks and Tick-borne diseases 6, 499-507.

SWOFFORD, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4.0b10. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, 142 pp.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., KUMAR, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30, 2725-2729.

TORINA, A., MORENO-CID, J.A., BLANDA, V., DE MERA, I.G.F., DE LA LASTRA, J.M.P., SCIMECA, S., BLANDA, M., SCARIANO, M.E., BRIGANÒ, S., DISCLAFANI, R., PIAZZA, A., VICENTE, J., GORTÁZAR, C., CARACAPPA, S., LELLI, R.C., DE LA FUENTE, J., 2014. Control of tick infestations and pathogen prevalence in cattle and sheep farms vaccinated with the recombinant Subolesin-Major Surface Protein 1a chimeric antigen. Parasites & Vectors 7, 10.

WEISBURG, W.G., BARNS, S.M., PELLETIER, D.A., LANE, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173, 697-703.

YU, X.J., ZHANG, X.F., MCBRIDE, J.W., ZHANG, Y., WALKER, D.H., 2001. Phylogenetic relationships of *Anaplasma marginale* and '*Ehrlichia platys*' to other *Ehrlichia* species determined by GroEL amino acid sequences. International Journal of Systematic and Evolutionary Microbiology 51, 1143-1146.

CHAPTER 7

7. General Discussion, Conclusions and Recommendations

7.1 Discussion

This study was conducted to understand the occurrence, diversity and epidemiology of tick-borne diseases (TBDs) among transhumant Zebu cattle in Karamoja Region, Uganda. This information may be useful to predict disease outbreak, develop effective control strategies, and increase livestock productivity in pastoral areas.

We used participatory epidemiology (PE) to define and prioritise cattle diseases, evaluate current control activities for ticks and TBDs, and identify constraints to the control of TBDs. Participants also proposed solutions to service delivery, disease control and surveillance. The informant data was gathered to determine the extent to which the herders' assessments of herd health are consistent with the findings from clinical examinations, sero-survey for antibodies and molecular analyses for tick-borne infections. As in other pastoral areas in eastern Africa, the herds in Karamoja are not regularly tested because conventional approaches to research and disease surveillance are constrained by the mobility of herds and limited resources (Anderson and Robinson, 2009; Shiferaw et al., 2010; Wesonga et al., 2010; Catley et al., 2014). The overall annual mortality estimates (44-53%) in cattle from all diseases are similar to that reported among livestock keepers in South Sudan (39%; Malak et al., 2012), but differ from those in Ethiopia (8% in Shiferaw et al., 2010; 6%-18% in Catley et al., 2014). The variations in the mortality may be due to differences in local epidemiologic conditions as well as control strategies and veterinary services. Our PE results indicated that Karamoja cattle keepers regard TBDs, in particular East Coast fever (ECF) and anaplasmosis, as the most important diseases of cattle due to high morbidity, high case fatality rates and treatment costs associated with the diseases. Our findings are consistent with previous studies in Machakos District, Kenya (Wesonga et al., 2010) and Kiruhura, Kayunga and Soroti Districts of Uganda

(Ocaido et al., 2009) that showed that TBDs cause major losses in cattle, mainly in terms of mortality and control costs. The estimated values for ECF incidence (18-21%) and case fatality rate (81.7-89.5%) are similar to 19.2% (incidence) and 70% (case fatality rate) reported in South Sudan (Malak et al., 2012). In the present study, inappropriate control practices and constraints to the control of ticks and TBDs may have contributed to the high morbidity and mortality. The pastoralists applied under-strength acaricides, often at irregular intervals, and they used very little or no acaricide wash. Veterinary services were inadequate, and this was blamed on the small number of local veterinarians (one or two per district) who could seldom reach the communities. The informants indicated that due to inadequate veterinary services, they lacked enough information to determine proper dosage and the correct treatment for diseases. Drug outlets were often lacking among communities; therefore, livestock keepers walked long distances in search of drugs. As a result, the cattle keepers could not control diseases in time. Furthermore, cases of TBDs were not effectively treated (using oxytetracycline for all diseases), which reduced the recovery rate of sick animals. The Karamojong livestock keepers identified solutions to the constraints in control of ticks and TBDs. The solutions include improved veterinary service delivery through provision of incentives, by government, to attract more veterinarians, and enhancing the capacity of community-based animal health workers (CAHWs) by continuous training, and subsidising and improving on the supply of drugs, vaccines and equipment. The cattle keepers also indicated that strengthening government-community partnership would facilitate the establishment and maintenance of dip tanks.

Ticks are among the most important vectors of pathogenic micro-organisms affecting livestock. In this study, we determined the diversity and abundance of ticks among cattle. The seroprevalences of antibodies to *Anaplasma marginale* and *Theileria parva* were also assessed using serological assays, and the risk factors for seropositivity in cattle were evaluated. Using participatory approaches, the livestock keepers estimated the proportion of annual ECF and anaplasmosis cases in different age groups of cattle. We also conducted clinical examinations of sick cattle. The parameters mentioned above are important indicators

of existence of endemic stability, and therefore scientific evidence to choose approaches for the control of TBDs (Norval et al., 1992; Perry and Young, 1995; Jonsson et al., 2012). Eight different species of ticks were found on cattle in the study area. Before this study, *Rhipicephalus pulchellus* (zebra tick) had not been reported from vegetation or animals in Uganda. *Rhipicephalus pulchellus* may be spreading into Karamoja Region from neighbouring countries (especially Kenya), possibly due to uncontrolled animal movements and sharing of grazing areas with cross-border communities. Although the distribution of the tick species *Amblyomma lepidum*, *A*. *gemma*, and *Hyalomma truncatum* includes Uganda (Walker et al., 2013), these ticks have not been reported in recent studies from other parts of the country (Rubaire-Akiiki et al., 2006; Chenyambuga et al., 2010; Magona et al., 2011a). Overall, the tick burden on cattle from the study area was twice to five times higher than previously reported from other parts of Uganda (Rubaire-Akiiki et al., 2006; Magona et al., 2011a). A high proportion of sampled cattle were infested with ticks that include the vectors of ECF, anaplasmosis, babesiosis and heartwater, indicating a high potential for transmission of TBDs (Magona et al., 2011b). Indigenous Zebu cattle are known to be relatively resistant to tick infestation (Latif, 1993) due to their ability to respond immunologically to tick infestation (Mattioli et al., 2000). The high tick-burden demonstrated amongst the Zebu cattle in Karamoja Region may be due to the extensive production system, which involves transhumance, sharing grazing with wild animals, which are reservoirs for ticks, and inappropriate tick control practices. Furthermore, stress factors such as inadequate feeding, concurrent disease and movement of cattle for long distances can result in a drop in resistance to ticks in Zebu cattle (Latif, 1993). The high tick infestation amongst cattle is likely to increase the risk of exposure to tick-borne pathogens.

There was a low true seroprevalence of antibodies to *T*. *parva* (16.5%), which may suggest a high likelihood of ECF in the animals at the time of peak exposure to *T*. *parva* and increased likelihood of disease following a single exposure (Jonsson et al., 2012). The high true seroprevalence for anaplasmosis (95.1%) may suggest that a high proportion of cattle were exposed, which led to high persistent infections.

Molecular biological techniques are more sensitive and specific in identifying DNA of tick-borne pathogens. We used reverse line blot (RLB) hybridisation, quantitative real-time polymerase chain reaction (qPCR), 18S rRNA gene and 16S rRNA gene sequence and phylogenetic analyses, and *msp1α* gene sequence analysis for the detection, identification and genetic characterisation of tick-borne infections. The results from RLB hybridisation assay demonstrated a high prevalence (99.6% of the sampled cattle) and diversity (10 species) of tick-borne haemoparasite infections among cattle in Karamoja Region. In general, the infections were more prevalent in Karamoja Region than previously reported from other parts of Uganda. The high prevalence of tick-borne pathogens in this study may be attributed to communal grazing and cross-border movement of cattle within and out of the country. These factors may increase the probability of exposure of cattle to infected ticks from grazing areas (Angwech et al., 2011). Furthermore, wildlife including African buffalo (*Syncerus caffer*) often share grazing areas with cattle in the study area (Chapter 3 of this thesis). African buffalo are reservoirs for many important tick-borne pathogens of cattle (Oura et al., 2011a; Oura et al., 2011b). Other factors that may contribute to the high prevalence of tick-borne infections in the study area are the limited availability of drugs and inadequate veterinary services, and poor tick control practices (e.g. irregular application of acaricides of less quantity and inappropriate dilution) by the Karamojong. Co-infections were high, and this may either lead to protective effects against infections or cause more harm to individual animals. A high proportion of cattle were infected with *Theileria mutans* (88.3%) and *Theileria velifera* (71.3%), which have occasionally been reported to cause pathogenic effects in cattle (Musisi et al., 1984; Moll et al., 1986; Oura et al., 2004a); however, these usually benign *Theileria* co-infections may also result in protective effects against *T. parva* in animals (Woolhouse et al., 2015). The benign infections may, therefore, be potential determinants both for the patterns of morbidity and mortality and of the impact of control measures against TBDs in cattle populations. However, in this study, there was no significant association found between co-infections with *T. mutans*, or *T. velifera*, and *T. parva*. Therefore, there is less likelihood that benign *Theileria* co-infections may result in protective effects against *T. parva* amongst cattle

in Karamoja Region. However, given the low exposure (seroprevalence, 16.5%) to *T. parva*, it might be difficult to conclude about the protective effects of the benign *Theileria* infections against *T. parva*.

Although the livestock keepers reported relatively high incidences, mortalities and case fatality rates due to TBDs during the PE activities, low prevalences of *T. parva* (2.9%, RLB; 3.3%, qPCR), *Babesia bigemina* (5.0%, RLB) and *Ehrlichia ruminantium* (0%, RLB; 1.7%, qPCR), but a high prevalence of *A. marginale* (73.7%, RLB; 82.9%, qPCR), were detected by molecular methods. The piroplasms of *T. parva* undergo only limited replication (Conrad et al., 1986), and fluctuations and loss of carrier state have been documented (Mans et al., 2015), which may lead to low detectable infection. A further consideration is that cases of ECF were not effectively treated which resulted in high case fatality. Since ECF is often an acute disease (Norval et al., 1992), the proportion of cattle that are carriers declines with increased mortality rates of infected cattle (Dolan, 1986). The low prevalence of *T. parva* as determined by the molecular methods is consistent with the low seroprevalence of antibodies to the pathogen (16.5%), as determined by indirect fluorescent antibody test (IFAT). Antibody responses against the schizont stage of *T. parva* are associated with exposure to the parasite (McKeever and Morrison, 1990). Low *T. parva* infection is usually accompanied by low antibody prevalence, which may contribute to increased susceptibility and case fatality of the infections (Perry and Young, 1995). *Ehrlichia ruminantium* infections often present highly virulent heartwater challenge which may lead to high case fatality rate among infected animals (Allsopp, 2010), and subsequently low *E. ruminantium* infection circulating in the cattle population. Furthermore, the qPCR for *E. ruminantium* only detects the parasite in the blood of experimentally infected animals at the febrile stage (having fever) of infection (Allsopp et al., 2004; Steyn et al., 2008). For *B. bigemina*, although infections result in high parasitaemia, animals recovering from babesiosis remain infective for ticks for only 4 to 7 weeks and carriers for only a few months (Suarez and Noh, 2011). The low proportions of cattle in which parasitaemia (*T. parva*, *E. ruminantium*, *B. bigemina*) was detected may indicate a likelihood of increased susceptibility in the cattle population to ECF, heartwater

and babesiosis. Sequence analyses of the 18S rRNA gene for *Theileria* and *Babesia* species demonstrated variants of *T*. *mutans*, *Theileria* sp. strain MSD and *B*. *bigemina* among cattle, indicating a wide genotypic diversity of these species. *Babesia bigemina* variant sequences were identified in samples which tested negative by RLB hybridisation assay, which may suggest a higher occurrence of *B*. *bigemina* in cattle from Karamoja Region than detected by the assay. A qPCR specific for *B*. *bovis* and *B*. *bigemina* could be used in future studies to better determine the occurrence of *Babesia* spp. amongst cattle, but unfortunately this test was not available when we did the study.

The high occurrence of *A*. *marginale* infections (73.7%, RLB; 82.9%, qPCR) is consistent with results from serological analysis, which showed a high seroprevalence of antibodies to the pathogen (86.6%). However, high seroprevalence may not truly be an indicator of high population immunity (or endemic stability), a status which may be difficult to achieve due to variation in climate and management practices which affect exposure of animals to infection (Jonsson et al., 2012). Moreover, seropositive animals may not all be solidly protected against immunologically diverse heterologous strains. This may explain the occurrence of clinical cases of anaplasmosis as observed during clinical examinations and as indicated by the livestock keepers during the PE activities. The clinical cases may be attributed to the few naïve animals and/or new infections as a result of superinfection. Although carrier status provides immunity to clinical disease, infection can be transmitted from infected to naïve cattle which results in manifestation of anaplasmosis (Aubry and Geale, 2011). At the host population level, high infection rates with a primary strain and consequent strain-specific immune responses would be expected to result in greater selective pressure for superinfection with antigenically distinct strains as there would be few naïve hosts and new infections would require superinfection (Palmer and Brayton, 2013; Castañeda-Ortiz et al., 2015). Furthermore, introduction of new animals into cattle herds, as a result of cattle rustling, is common in the study area. The introduced cattle may either be a source of new *A*. *marginale* strains in the herd or the new animals may be naïve to the existing strains. Moreover, the 16S rRNA gene sequence and phylogenetic analyses for *A. marginale* showed heterogeneity,

which indicates a variation in *A*. *marginale* strains in Karamoja. In this study, except for one sample, cloning and sequencing of the *msp1α* gene for *A*. *marginale* was not successful. However, the gel electrophoresis of *msp1α* gene PCR products revealed multiple amplicons of different lengths from individual samples. This indicates infection with more than one *A*. *marginale* strain. Further studies are therefore necessary to investigate *A*. *marginale* genotypes from cattle in Uganda to provide more comprehensive data of diversity of *A*. *marginale* strains and investigate the possibility of superinfection in cattle. Nevertheless, we found four different kinds of *msp1α* tandem repeat sequences (UP39-F-M^2-3) from amplicons of around 1,000 bp, which correspond to one strain of *A*. *marginale* that is unique to Karamoja cattle. One of the tandem repeats (UP39) was reported for the first time in this study. The prevalence of *A*. *centrale* in this study was relatively high (20.4% by RLB; 12.1% by qPCR) compared with previous studies elsewhere in Uganda, using RLB hybridisation (0%, Oura et al., 2004; 4.5%, Muhanguzi et al., 2010; 4.3%, Asiimwe et al., 2013). The 16S rRNA sequences of *A*. *centrale* from this study were identical (100%) to *A*. *centrale* strain CC, which was recovered from an acute case of anaplasmosis that was attributed to *A*. *centrale* infection (no *A*. *marginale*) in Italy (Carelli et al., 2008). It is possible that *A*. *centrale* infections, which were identified in the cattle from the study area, may lead to clinical cases of anaplasmosis.

A state of endemic stability implies an ecological balance between cattle, tick, parasite, and the environment where there is a high level of challenge of calves by infected ticks, absence of clinical disease in calves despite infection, and a high level of immunity in adult cattle with consequent low incidence of clinical disease or no morbidity and mortality in the herds (Norval et al., 1992; Perry and Young, 1995; Bock et al., 2004). Endemic stability to TBDs is determined by factors that include breed of cattle, intensity of tick control, management of clinical cases, grazing management, tick infestation, infection rate of ticks with pathogens, antigenic/genetic diversity of the pathogens and climatic factors. Endemic stability is more likely to occur in production systems that involve keeping of indigenous breeds of cattle than in systems where exotic breeds of cattle and their crosses with indigenous breeds are kept. Indigenous cattle have innate resistance to ticks and TBDs compared to the exotic cattle and

their crosses, and this is due to the evolutionary relationship between *Bos indicus* cattle, the tick vectors, and the pathogens (Bock et al., 1997; Jonsson et al., 2006). Animals that are subjected to intensive tick control are less exposed to infected ticks when they are still young, and the adults will not acquire immunity to TBDs. Cattle under extensive management systems are continually exposed to ticks, which may be infected, and therefore more likely to acquire immunity to TBDs, as compared to animals kept under zero grazing. In a zero grazing system, calves do not acquire sufficient levels of antibodies from colostrum due to inadequate exposure of cows to infected ticks. A higher incidence of TBDs, due to low exposure from infected ticks and lack of acquired immunity, is expected in areas/farms/individual animals where ticks have low infection with tick-borne pathogens, than in areas where ticks are sufficiently infected. Areas/farms with a higher genetic/antigenic diversity of tick-borne pathogens are likely to experience a higher incidence of TBDs due to limited or lack of cross-protection amongst pathogenic strains. Therefore, immunity to one strain is not protective against another strain in animals or populations with diverse strains. The concept of endemic stability for ECF is more complex than that for anaplasmosis, babesiosis and heartwater, and is influenced by variations in management practices and climate. For anaplasmosis and babesiosis, high seroprevalence of antibodies is associated with endemic stability. Inverse age immunity is clearly a feature of infection with *B*. *bovis*, *B*. *bigemina* and *A*. *marginale*. Innate immunity occurs in calves of up nine months of age for babesiosis, and up to one year of age for anaplasmosis. East Coast fever due to *T*. *parva* infection causes most mortality in young calves of 5-9 months, and therefore it would not appear to have inverse age immunity as per babesiosis and anaplasmosis (Jonsson et al., 2012). Passive immunity from colostrum might provide protection against infection with *T*. *parva* in calves of up to 4 months of age.

In this study, we found a lower seroprevalence of antibodies to *T*. *parva* and relatively high ECF-related mortality in all age groups of cattle than in other parts of Uganda and other areas in eastern Africa. Although the cattle in Karamoja Region are of the short-horned East African Zebu type, and are known to be more resistant to TBDs than the *Bos taurus* breeds, there seems to be a higher level of endemic instability to ECF amongst cattle in Karamoja Region

compared to other areas in Uganda. However, high numbers of *R*. *appendiculatus* ticks were found on cattle in Karamoja compared with other areas. Therefore, we would expect a relatively high exposure of cattle to *T*. *parva* and consequently establishment of acquired immunity to ECF amongst cattle in Karamoja Region. The most likely explanation for low seroprevalence of antibodies to *T*. *parva* and high incidence of ECF in Karamoja Region, than other areas in Uganda and elsewhere in eastern Africa, is low infection challenge of ticks with *T. parva*. Therefore, the calves are not sufficiently exposed to *T*. *parva* from ticks and do not receive sufficient antibodies in colostrum from the cows during the critical early period. Therefore, there is both low passive immunity in calves and low acquired immunity amongst adult cattle. The perceived lower infection challenge in ticks in Karamoja, compared to other areas, can be attributed to differences in climatic conditions. In Karamoja Region, cycles of drought, which occur after every 3-5 years, disrupt the life cycle of *R*. *appendiculatus*, which is a three-host tick, and *T*. *parva*, which is transstadially transmitted. Three-host ticks spend most of the life cycle in the environment, which makes them sensitive to changes in environmental and climatic conditions, as compared to one-host and two-host ticks. It is possible that in situations of severe drought, the life stages of *R*. *appendiculatus* in the environment are destroyed or severely reduced, and the lifecycle of *T*. *parva* is disrupted before transstadial transmission to the next host takes place. A small proportion of ticks and eggs of ticks may undergo diapause (Walker et al., 2013), but overall the *T*. *parva* challenge circulating in the ecosystem is reduced. This scenario is exacerbated by the fact that the tick loses its theilerial infection after having transmitted it. The infection does not persist to the post-transmission stage, let alone the next generation (Uilenberg, 2006). There is need to assess the annual dynamics of tick infestations and infection rate of ticks with *T. parva* in the Karamoja Region so as to establish the effect of climate on infestations and infections. The annual incidence and mortality due to ECF reported in Karamoja Region is higher than those reported in Ethiopia, but similar to that in South Sudan. All these areas experience drought conditions and the predominant cattle breeds kept by the communities are of the indigenous type. Differences in management practices may explain the variation in mortality due to ECF in these areas. Anectodally, inadequate availability and limited access to drugs to control ticks

and treat ECF is a bigger challenge in Karamoja Region and South Sudan than in Ethiopia. Therefore, cases of ECF are not effectively treated in in Karamoja, leading to a reduction in carrier state in the cattle population, and a reduction in the rate of acquired immunity amongst cattle. This leads to higher mortality amongst cattle in Karamoja.

Differences in antigenic/genetic diversities of *T. parva* may also explain the variation in incidence of ECF in different geographical areas. A higher incidence of ECF is expected in areas with high genetic diversity due to limited cross-protection amongst strains. Recently a higher genetic diversity of *T. parva* was reported for northern Uganda compared to eastern, central and western parts of the country (Muwanika et al., 2016), which may explain the generally lower mortality due to ECF in other regions of Uganda than in Karamoja. Furthermore, higher seroprevalences of antibodies to *T. parva* have been reported and lower mortality rates have been experienced in other parts of Uganda (field veterinarians, personal communication) than Karamoja Region. The climatic conditions in other parts of Uganda, especially in central Uganda, are mostly humid and wet, and may not therefore interrupt the life cycle of ticks and *T. parva*. Another factor that may explain higher mortality in Karamoja is that control of ticks in other parts of Uganda is more regular and the treatment of cases of ECF is more effective than in Karamoja. This is due to the ease of availability of drugs and better distribution of veterinarians in other parts of Uganda. Furthermore, communal grazing, which predisposes animals to high tick infestation, is not practiced in central and eastern Uganda, and is less common in western Uganda as compared to Karamoja Region. Due to the closed grazing system, which reduces exposure to ticks, and the relatively regular control of ticks in other parts of Uganda, cattle are exposed to fewer ticks throughout the year. The lower numbers of ticks in other parts of Uganda might have infection challenge that is just sufficient to expose cattle and establish antibodies in a high proportion of cattle. However, outbreaks or cases of ECF occur in some farms in other parts of Uganda, probably because management practices are not homogenous across farms. Moreover, a number of livestock keepers keep exotic breeds of cattle and their crosses with indigenous cattle, which are more susceptible to ECF than the indigenous breeds (e.g. Zebu cattle). A higher level of endemic instability (low

seroprevalence of antibodies to *T*. *parva* and high ECF mortality) is expected on farms with intensive tick control practices and where exotic breeds of cattle are kept under zerograzing. On the other hand, endemic stability is more likely to occur on farms where the cattle are continually exposed to infected ticks (extensive grazing and less tick control) and where indigenous breeds of cattle are kept. The relatively high incidence of TBDs in Karamoja compared to other areas in Uganda can also be explained by introduction of new animals into cattle herds, as a result of cattle rustling. The introduced cattle may either be a source of ticks infected with new pathogenic strains or the new animals are susceptible to the existing strains.

Given the high seroprevalence and high infection prevalence of *A*. *marginale* that was demonstrated in this study, endemic stability for anaplasmosis is expected in Karamoja Region compared to other areas of Uganda and eastern Africa, where lower proportions of cattle were reported to be infected with *A. marginale* or previously exposed to infection. However, a high prevalence of infection with *A*. *marginale* in a cattle population can be a driver for superinfection, a situation where animals with immunity to a primary strain are infected with secondary strains. The animals are not protected against the secondary strains. This leads to anaplasmosis in a cattle population, as demonstrated by clinical examinations and laboratory confirmation of clinical cases and reports from the pastoralists in Karamoja Region.

Although natural endemic stability is in principle an ideal condition where no control strategies are needed, this situation is rare and, when it does exist, it can be easily broken by varitaions in climate, host genotypes and management practices (Florin-Christensen et al., 2014). Therefore, natural endemic stability is unrealiable as the sole control strategy. Importantly, it has been argued that the threshold of 75% exposed cattle as a means to predict the appearance of clinical cases should be taken with caution when extrapolated to different regions and host-tick-pathogen systems from those on which the model was based (Jonsson et al., 2012). A number of studies have evaluated and compared seroprevalence and infection

with tick-borne pathogens in farms/areas with different cattle breeds, different grazing systems, and different control practices against ticks and TBDs. However, there is scanty data from a comprehensive study that evaluates the interaction of predictive factors for endemic stability to TBDS, including cattle breed, pathogen diversity, control practices, grazing system, climatic changes, infection rates in ticks, as well as tick species diversity and burden. In sum, it may not be appropriate to comment on the probability of endemic stability on the basis of single cross-sectional serological studies. There should be empirical data from longitudinal studies of clinical disease occurrence, host genotype, tick species and pathogen, taking into account wide variation in pathogenicity of various pathogen isolates.

However, the molecular and epidemiological information from this study provides useful information on TBDs amongst cattle in the transhumant pastoral area of Karamoja Region, Uganda. The information may be used as a basis for planning future strategies for the control of TBDs in the study area and other pastoral areas in eastern Africa. In Uganda, control of TBDs among livestock keepers is mainly in the form of spraying with acaricides and chemotherapy (Mugisha et al., 2005; Mugabi et al., 2010). Livestock keepers often use understrength acaricides and of less quantity than recommended by manufacturers (Mugisha et al., 2005; Mugisha et al., 2008). Efforts to improve livestock production and health in Karamoja Region have been spearheaded by the Food and Agriculture Organisation (FAO) of the United Nations by establishing a response mechanism comprising government agencies, non-governmental organisations (NGOs), and also training and equipping CAHWs, whose role is vital in mobilising and training pastoral communities and providing basic animal health support services (FAO, 2013). However, inadequate veterinary services and limited supply of drugs still constrain the control of TBDs in the region. An effective control of ticks and TBDs can be achieved by integrating strategic tick control using acaricides, treatment of clinical cases and immunisation (Norval et al., 1992; Minjauw and McLeod, 2003; Gachohi et al., 2013).

Immunisation can be a viable option for control of the most common TBDs in cattle. Currently in eastern Africa, immunisation is done only against ECF, using the Muguga cocktail infection and treatment method (ITM), coordinated by the Global Alliance for Livestock Veterinary Medicines [GALVmed] (Di Giulio et al., 2009). Widespread use of ITM has been affected by the high costs and poor access to the vaccine and liquid nitrogen for its storage, poor infrastructure, and inadequate expertise for ITM in pastoral areas, and *T. parva* diversity (Oura et al., 2011b; Kasozi et al., 2014; GALVmed, 2015). However, there is prospect for ITM in pastoral areas. Livestock keepers are willing to adopt and commit themselves to a product that works, which demonstrates a future for ITM in livestock production (Lynen et al., 2006). The ITM against ECF was shown to be highly effective (95% success rate) and was successfully adopted by cross-border pastoral communities in northern Tanzania, and the approach reduced ECF incidence, improved live weight gains, increased market value, and reduced calf deaths (Lynen et al., 2006). The ITM also resulted in reduced acaricide use, without compromising survival in the face of other TBDs (Lynen et al., 2012). The vaccination comprises a single injection and protects the animal against ECF for life. However, controlled spraying or dipping against ticks is necessary to maintain immunity and control other TBDs (GALVmed, 2015). In Uganda, ITM has been used in 43 districts, but mostly on exotic breeds of cattle and their crosses, conferring immunity in about 85% of vaccinated animals (SNV, 2013). Owing to the high distribution of tick vectors amongst cattle in Karamoja Region, immunisation may contribute to the attainment of endemic stability to ECF. This is because exposure to infected ticks is required to sustain immunity (Gachohi et al., 2013). Currently, the Centre for Ticks and Tick-Borne Diseases (CTTBD) in Malawi is producing the ECF vaccine, and GALVmed is providing support to reduce the time it takes to manufacture the vaccine, finding an approach to conserve sporozoite viability without the need for liquid nitrogen storage, and promote collaboration between governments, scientific think-tanks and private business innovators (GALVmed, 2015). Access to the ITM vaccine can be improved by encouraging cooperative associations of livestock keepers to facilitate the purchase of the vaccines, which individual herd owners cannot afford, as demonstrated in Simanjiro, Tanzania (Homewood et al., 2006). Government promotion of immunisation technology and assistance

in remote areas is also necessary to allow for fast adoption once the vaccine becomes available. The development of subunit vaccines from native antigens of *T. parva* parasites or as recombinant proteins from cloned DNA may offer an attractive alternative to virulent or attenuated parasites because they are less costly to produce, are relatively stable, and do not require a cold chain, making the logistics of access and delivery easier. Moreover, there is flexibility to incorporate only those antigens that elicit protective immune responses (Jenkins, 2001). However, even though research in this field has been going on for a long time, sub-unit vaccines are far from production. Vaccination against anaplasmosis has been used in other parts of the world to avert severe clinical disease in cattle; however, there is no vaccine universally accepted as safe, efficacious and practical for large scale production (Aubry and Geale, 2011). Moreover, the vaccines do not prevent cattle from becoming persistently infected with *A. marginale* or becoming reservoirs of infections. Another challenge is the increasing numbers of *A. marginale* field strains in a given geographical area that complicates effective vaccination (Aubry and Geale, 2011). The findings from this study, and further investigations, may assist in establishing the potential for the use of *A. centrale* vaccines against bovine anaplasmosis in Uganda; the vaccine has been used in Israel, South Africa, Australia and South America to provide a certain degree of protection (Aubry and Geale, 2011). Vaccines against heartwater (Marcelino et al., 2015) and babesiosis (Suarez and Noh, 2011) are used elsewhere in the world.

In this study, daily hand picking of ticks was done by all pastoralists to reduce tick numbers on the animals. However, this method is cumbersome and is not sustainable. Not all ticks can be picked from the animal, therefore the animals are still exposed to tick-borne pathogens. Given the high tick infestation amongst cattle in Karamoja Region, strategic tick control, during periods of high tick numbers, is required to allow the infected ticks to naturally sustain endemic stability through continuous challenge. However, a successful tick control programme depends on the knowledge of the biology and ecology of the targeted tick. Without this information, weak points in the life cycle cannot be determined and exploited (Barre and Garris, 1990). Pastoralists should be sensitised to improve their ecological

knowledge of the seasonal activity of ticks if strategic treatment with acaricides is to be successful. Treatment before the natural peak of adults will suppress their numbers, reduce greatly the number of eggs, and result in fewer adults and nymphs for the rest of the year. This will reduce acaricide use and other treatment expenses. The remaining ticks are likely to continue transmitting tick-borne pathogens at a low level. Endemic stability is more likely to develop and therefore less mortality in the cattle population due to TBDs. Given the fact that *A*. *variegatum* and *R*. *appendiculatus*, which are three-host ticks, were among the most abundant ticks in the study area, intensive treatment is required to have constant availability of residual acaricide on the livestock sufficient to kill all ticks present or attempting to attach. The three-host ticks spend less time on the host feeding, about 15 days for each stage of development. Acaricides may be applied once per week or longer, depending on the residual effect of the acaricide used. However, this treatment is likely to reduce tick infestations to such low levels that immunity to TBDs is lost in the livestock, resulting in endemic instability (Latif and Walker, 2004). Footbaths containing acaricides have been used to control *A*. *variegatum* with some success, as the ticks will often attach between the hooves (Prine et al., 2013).

In Karamoja Region, there is abundant vegetation during the wet season, which harbours more ticks than in the dry season (pastoralists, personal communication). Pasture rotation should be promoted to reduce tick densities on a large scale. The livestock keepers mentioned bush burning as one of the methods used to control ticks in the dry season, although the method was less effective. Tick eggs that are hatching can miss the effect of the fire while hiding in the surface soil, suggesting that pasture burning alone may not be an effective control method (Mapholi et al., 2014). However, a study in Oklahoma in the United States of America (USA) showed that patch burning can reduce tick populations (Polito et al., 2013). There were significant reductions in tick burden on cattle in pastures that were treated by patch burning, indicating that patch burning can be used to help control ticks in pastures (Polito et al., 2013). Patch burning uses frequent, spatially discrete fires to create variation in the composition and structure of the plant community. The complex vegetation changes

incurred from this type of burning regimen, in addition to the focal grazing of cattle induced by patch burning, has a potential to reduce tick populations by creating less favourable microhabitats (Polito et al., 2013). In another study in southwestern Georgia and northwestern Florida, USA, long-term prescribed burning significantly reduced tick counts in the vegetation (Gleim et al., 2014).

Approaches for the control of TBDs in livestock keeping areas should also involve adaptive trials and integration of appropriate anti-tick vaccines in control programmes. Control of ticks by vaccination has the advantages of being cost-effective, reducing environmental contamination and controlling both tick numbers and disrupting the tick vector-pathogen interface. The vacines prevent the selection of drug-resistant ticks that result from repeated acaricide application (Marcelino et al., 2012). The ultimate goal of tick vaccines is control of tick infestations while reducing pathogen infection and transmission, to protect against diseases caused by tick-borne pathogens. The feasibility of controlling tick infestations through immunisation of hosts with antigens has previously been demonstrated using recombinant antigens (Hajdusek et al., 2010; Almazán et al., 2012). Tick vaccines reduce the number of engorging female ticks, their weight and reproductive capacity, meaning that the greatest vaccination effect is seen as a reduced larval infestation in the subsequent generation (Willadsen, 2006). In one study, larval moulting to nymphs was significantly reduced by 14-48% in subolesin vaccinated mice when compared to controls (Mereno-Cid et al., 2013). In another study, Merino et al. (2011) demonstrated that infection levels for *A. marginale* and *B. bigemina* were over 87% lower in *R. microplus* fed on subolesin-vaccinated cattle and after gene knockdown by RNAi when compared to controls, suggesting that subolesin vaccines could be used for the dual control of tick infestations and pathogen infection. It was also shown that using the tick protective antigen, subolesin reduced *A. marginale* and *A. phagocytophilum* infection levels in ticks (de la Fuente et al., 2006). However, a recent anti-tick vaccination study showed that a multivalent cocktail vaccine containing three *R. appendiculatus* histamine binding proteins, two different *R. appendiculatus* cement cone protein antigens TRP64 and TRP truncate variants, the *R.*

appendiculatus subolesin homologue, and *T. parva* sporozoite antigen p67C did not appear to affect tick feeding success or reduce transmission of *T. parva* (Olds et al., 2016). At the moment, two vaccines based on the Bm86 antigen are commercially available, namely Tick-GARD (in Australia) and GAVAC (in Cuba and parts of South America), but they are not fully efficacious (Marcelino et al., 2012). The major disadvantage of some of the tick vaccines in current use is that they may not offer protection against multiple tick species. However, controlled immunisation trials conducted indicated that the *R. microplus* Bm86 antigen vaccine had good efficacy against *R. microplus*, *R. decoloratus*, *Hyalomma anatolicum anatolicum* and *Hyalomma dromedarii*, reducing the numbers of engorged female adult ticks, their weight and egg-laying capacity, leading to a reduction in reproductive capacity (de Vos et al., 2001). Due to the delay in the 'knock down' effect of tick vaccines, vaccine use should be coupled with limited acaricide application for short-term control of unacceptable tick burdens (Mapholi et al., 2014).

In the present study, the Karamojong pastoralists used hand sprayers to control ticks on cattle. The acaricides were applied in small quantities, at irregular intervals and at inaccurate dilutions. Acaricides were not applied on the whole body of the animals leaving tick infestation on a large portion of the animals' bodies. Given the relatively large number of animals kept by the pastoralists, hand spraying is not an effective method of tick control. Furthermore, hand spraying enhances environmental contamination because the animals are sprayed either on pastures or within the homesteads. There is also body contact with and inhalation of acaricides by the person spraying, which is detrimental to human health. Dip tanks are an efficient, practical and convenient means of applying acaricide to livestock herds. However, in most rural pastoral systems of East Africa, government veterinary services are often constrained by poor or inadequate veterinary infrastructure (Perry and Young, 1995). Dipping facilities are frequently not operational because of limited finances for refurbishment of dip tanks and provision of acaricides (Mugisha et al., 2005; Mbassa et al., 2009). Furthermore, dipping in the East African region is not compulsory, and is consequently inconsistent (Mugisha et al., 2005; Kivaria, 2006; Mbassa et al., 2009). This constrains the

control of TBDs (Kasozi et al., 2014). In the past, the dip tanks in Karamoja Region have been destroyed or vandalised. Water supply is also a challenge. The decentralisation and privatisation of veterinary services in eastern Africa emphasises a private sector demand-driven control of TBDs (Minjauw and McLeod, 2003; SNV, 2013; Kasozi et al., 2014), and yet livestock keepers have limited capacity to control TBDs. However, a study among pastoralists in Kambala, Tanzania, demonstrated an improvement in the organisational skills of livestock keepers, through cooperative societies, which addressed the problem of funding; thus providing a sustainable community-based model using cattle dips to reduce losses due to ticks and TBDs and increase cattle productivity (Mbassa et al., 2009). This approach can be beneficial if adopted elsewhere in eastern Africa.

The use of acaricides has a significant impact on the environmental tick load and is effective at preventing most cattle from being exposed to tick-borne pathogens (Walker et al., 2014). However, while interventions are largely targeted at domesticated animals for logistical reasons, pathogen dynamics are also driven by their relationship with wild animals. Tick-borne pathogens are multi-host pathogens as they can infect both domesticated and wild animals. Like most pastoral areas of sub-Saharan Africa, free-ranging wildlife and cattle share grazing grounds in Karamoja. The wildlife-livestock interface facilitates transmission of tick-borne parasites between wild animals and cattle (Oura et al., 2011a; Oura et al., 2011b; Kabuusu et al., 2013; Walker et al., 2014). Furthermore, various tick vectors feed on wild animals, increasing the risk for occurrence, abundance and distribution of ticks amongst cattle at the interface (Fyumagwa et al., 2013; Walker et al., 2013). African buffalo (*Syncerus caffer*), in particular, are considered to be an important reservoir for various tick-borne haemoparasites (Oura et al., 2011a; Oura et al., 2011b; Gachohi et al., 2012; Fyumagwa et al., 2013; Walker et al., 2014). Therefore a high prevalence of carrier buffalo means that buffalo are likely to be the dominant species in transmission of tick-borne pathogens to cattle at the wildlife-livestock interface. The elimination of tick-borne infections from cattle is unlikely to be accomplished solely by frequent acaricide use on cattle when grazing land is shared with buffalo. A recent study showed that acaricide use on domestic cattle did not prevent

transmission of *T. parva* to cattle at the wildlife-livestock interface in Kenya (Walker et al., 2014). Under the current control strategies in pastoral areas of East Africa, which target ticks on cattle only, pathogenic species like *T. parva* are likely to remain a significant problem in the region, and control will require continuous use of acaricides, which has significant economic and ecological consequences.

In this study, the pastoralists indicated lack of money as one of the constraints to the control of ticks and TBDs. Furthermore, there was limited access to acaricides and conventional therapeutic drugs. Various studies in East Africa have shown that the use of ethnoveterinary medicine by pastoralists to control livestock diseases and vectors is common (Gradé et al., 2009; Nabukenya et al., 2014; Byaruhanga et al., 2015). The use of ethnoveterinary botanicals is sustainable and ecologically sound because the plants are locally available and the acaricidal compounds are potentially easy to produce. The plants are, therefore, a great potential for cheaper alternative to expensive conventional medicine. A further advantage from the use of the compounds from the plants is that resistance develops slowly because there is usually a mixture of different active agents with different mechanisms of action (Habeeb, 2010). Ethnoveterinary plants have been evaluated and found to have acaricidal activities. A study in South Africa showed that ethanol extracts of the plant species *Calpurnia aurea* (leaves, flowers) and *Cissus quadrangularis* (stems) had a good acaricidal activity (>80% mortality) against larvae of *R. decoloratus* in an *in vitro* experiment (Fouche et al., 2016). In another *in vitro* study in the Democratic Republic of Congo, Kalume et al. (2012) demonstrated a 100% acaricidal effect of ethanol extracts of *Tephrosia vogelii* against adults of *R. appendiculatus*. In the present study, the livestock keepers interviewed mentioned that ethnoveterinary plants were less effective to control ticks, and were used as a desperate measure when conventional drugs were not available. However, from our observation, the plant products were not processed appropriately, and possibly the active compounds, which have acaricidal activity, were not effectively extracted.

A strategy to mitigate the impact of TBDs in pastoral areas should also include early management of clinical cases. However, cattle often become long-term asymptomatic carriers of *T*. *parva* following treatment or spontaneous recovery, thereby maintaining the parasite population (Kariuki et al., 1995; Kabi et al., 2014). For anaplasmosis, imidocarb is effective in treating the disease and also eliminates *A*. *marginale* from carrier animals (Potgieter and Stoltsz, 2004). Chemosterilisation for *A*. *marginale* was also demonstrated in cattle following oral administration of chlortetracycline for up 80 days (Reinbold et al., 2010). However, the recommendation of this practice should be scrutinised because of the potential to inadvertently disrupt endemic stability by chemosterilising infected cattle; furthermore, chemosterilised cattle are susceptible to re-infection (Reinbold et al., 2010). Chemoprophylaxis with imidocarb can protect animals from clinical babesiosis while allowing the development of immunity. However, there are concerns about residues in milk and meat, and this drug is expensive for most livestock keepers (CFSPH, 2008). Veterinary services are also essential as a source of knowledge for appropriate use of drugs and proper husbandry practices, and monitoring immunisation. Given the scarcity of veterinarians in rural pastoral areas, the activities of CAHWs can be strengthened through training, improving access to supply of drugs and equipment, and putting in place appropriate policies (Swai et al., 2014).

7.2 Conclusions

We investigated the epidemiology and diversity of tick-borne haemoparasite infections amongst transhumant Zebu cattle in Karamoja Region, Uganda, using PE methods, serological techniques, tick vector assessment and molecular techniques. Our PE results indicated that Karamoja cattle keepers regard TBDs, in particular ECF and anaplasmosis, as the most important diseases of cattle due to high morbidity, high case fatality rates and treatment costs associated with the diseases. Control of ticks was done mostly by daily hand picking of ticks and irregular hand spraying with acaricides. The main constraints to the control activities were inadequate knowledge to manage the diseases, inadequate veterinary services and

limited availability of drugs. Inappropriate control practices and constraints to the control of ticks and TBDs may have contributed to the high morbidity and mortality.

The low seroprevalence for *T. parva* in this study may be attributed to low *T. parva* infection challenge from ticks. This suggests a high likelihood of ECF in the animals at the time of peak exposure to *T. parva* and increased likelihood of disease following a single exposure. There seems to be a high level of endemic instability for ECF (*T. parva* infections) in the Karamoja Region that may be resultimg in high levels of mortality in all age groups of cattle in the region. High seroprevalence for *A. marginale* may be attributed to biological and mechanical transmission leading to high persistent infections, and subsequent seropositivity. Tick species that have not been reported in recent studies in Uganda were found in Karamoja Region. This may suggest that the ticks are spreading into Karamoja Region from neighbouring countries (especially Kenya), possibly due to uncontrolled animal movements and sharing of grazing areas with cross-border communities. The high tick infestation amongst cattle is likely to increase the risk of exposure of cattle to tick-borne pathogens. In general, this study demonstrated a high prevalence (nearly all cattle) and diversity of tick-borne haemoparasites amongst cattle in Karamoja Region. The prevalence was higher than previously reported from other parts of Uganda. The high prevalence of *T. mutans* and *T. velifera* infections amongst cattle may interfere with the diagnosis and control of theileriosis in cattle.

There was variation over the full-length 18S rRNA gene for *B. bigemina* and *T. mutans*, indicating a wide genotypic diversity of these species. The low proportions of cattle in which parasitaemia for the more pathogenic parasites, *T. parva*, *E. ruminantium*, *B. bigemina*, was detected may indicate a likelihood of increased susceptibility of the cattle population to ECF, heartwater and babesiosis. The 16S rRNA gene sequence and phylogenetic analyses for *A. marginale* showed heterogeneity, indicating a variation in *A. marginale* strains in Karamoja. Amplification of the *msp1α* gene revealed multiple amplicons of different lengths from individual samples, which indicates infection with more than one *A. marginale* strain. We found four different kinds of *msp1α* tandem repeat sequences (UP39-F-M^2-3), which

correspond to one strain of *A. marginale* that is unique to Karamoja cattle. One of the tandem repeats (UP39) was reported for the first time in this study. Our findings suggest significant effects of TBDs on cattle and this impacts cattle productivity and the livelihoods of the pastoralists. The information from this study can be used as a basis for planning future strategies for the control of TBDs in the study area and other pastoral areas in eastern Africa.

7.3 Recommendations

More effective control and prevention measures against TBDs should urgently be implemented in Karamoja Region. Given the relatively high incidence and case fatality rates of ECF, there is need to introduce and promote ITM vaccination to control ECF amongst cattle. Calves of 5 to 9 months, that are free from ECF, should be vaccinated so that acquired immunity is attained in the adults. The local governments and non-governmental organisations in the region should facilitate the immunisation initiative by providing financial and logistical support for delivery, storage and application of the vaccine. The immunisation technology can be promoted by facilitating the formation of cooperative associations of livestock keepers to facilitate the purchase of the vaccine, which individual herd owners cannot afford, since the vaccines comes in doses of 40. This will assist fast adoption once the vaccine becomes available. Owing to the high distribution of tick vectors amongst cattle in Karamoja Region, immunisation may contribute to the attainment of endemic stability to ECF. However, it will still be necessary to control ticks as ticks carry diseases besides ECF. Mild exposure to ticks is important to keep animals immune for life. The findings from this study will be presented to GALVmed and other stakeholders involved in the production and deployment of the ECF vaccine for possible financial support of vaccination of cattle in Karamoja Region.

High tick infestation and high diversity of tick species were encountered amongst cattle in this study. Pastoralists should be sensitised to improve their ecological knowledge of the seasonal activity of ticks if strategic treatment with acaricides is to be successful. Strategic tick control,

during periods of high tick numbers, is required to allow the infected ticks to naturally sustain endemic stability through continuous challenge. Treatment before the natural peak of adults will suppress their numbers, reduce greatly the number of eggs, and result in many fewer adults and nymphs for the rest of the year. This will reduce acaricide use and other treatment expenses. A rational strategy for the control of ticks must also feature appropriate policies on drug usage and management. Extension services in Karamoja Region need to be improved. The government of Uganda should provide incentives to motivate veterinarians to work in the Karamoja Region. Veterinary services are essential as a source of knowledge for appropriate use of drugs and proper husbandry practices, and monitoring immunisation. Furthermore, a more creative and 'pastoralist-friendly' information network is needed to advise the livestock keepers. There is need to increase farmer awareness about the biology and ecology of tick species in the grazing areas and the clinical picture of TBDs, so as to optimise both the use of acaricides, by applying the most appropriate frequency, and early management of infection. Given the scarcity of veterinarians in rural pastoral areas, the activities of CAHWs can be strengthened through continuous training, improving access to supply of drugs and equipment, and putting appropriate policies in place. The local governments in Karamoja Region should create a favourable business environment so as to motivate private companies to extend and scale-up veterinary drug supplies, at modest prices, to the region.

Long term approaches to the control of TBDs in Karamoja Region could target control of ticks by use of anti-tick vaccines, establishment of dip tanks and control transmission at the wildlife-livestock interface. There is also need for further research to generate more knowledge of TBDs and facilitate control strategies. Approaches for the control of TBDs in livestock keeping areas should involve adaptive trials and integration of appropriate anti-tick vaccines, especially those that can offer protection against multiple tick species, in control programmes. Control of ticks by vaccination has the advantages of being cost-effective, reducing environmental contamination and controlling both tick numbers and disrupting the tick vector-pathogen interface. Dip tanks are an efficient, practical and convenient means of

applying acaricide to livestock herds. The government of Uganda should facilitate the rehabilitation of the dilapidated dip tanks in Karamoja Region and construction of new ones. The livestock extension workers in the region can assist to improve the organisational skills of livestock keepers, through cooperative societies, to mitigate the problem of funding; thus providing a sustainable community-based model using cattle dips to reduce losses due to ticks and TBDs and increase cattle productivity.

Lack of money to buy acaricides was mentioned by the pastoralists as one of the constraints to the control of TBDs in this study. We suggest that plant species especially those that have shown the highest consensus amongst Karamojong pastoralists (Byaruhanga et al., 2015), with regard to tick control, should be subjected to pharmacological studies for their anti-tick activity, and then processed and promoted for use in pastoral areas. The elimination of tick-borne infections from cattle is unlikely to be accomplished solely by frequent acaricide use on cattle when grazing land is shared with buffalo. Long-term strategies to control transmission of TBDs at the wildlife-livestock interface should include the creation of grazing barriers between national parks or game reserves and the grazing areas for livestock. This can be done by electronic fencing and creation of grazing buffer zones so as to limit contact and transmission of diseases between wildlife and domestic animals. Further studies on the most important tick-borne infections in the area, including characterisation of multiple strains of tick-borne pathogens and their potential use as vaccines for cattle may enhance the control of TBDs. There is a need to investigate *A*. *marginale* genotypes from cattle in Uganda to provide more comprehensive data of diversity of *A*. *marginale* strains and investigate the possibility of superinfection in cattle.

With regard to the endemic status of TBDs, more work would be required to generate data related to infection rates of ticks by pathogens, serial seroprevalence of antibodies in cattle, disease incidence for animals with diverse genotypes, and the dynamics of tick species diversity and burden over different years or seasons. There is need to investigate and predict the incidence of TBDs and seroprevalence to tick-borne infections in areas/farms/individual

animals with differing combinations of predictive factors including host genotype, genetic/antigenic diversity of pathogen, climatic variations, grazing system, tick burden, tick and TBD control approaches, and infection challenge in ticks. This will provide more comprehensive information on the endemic status of TBDs amongst cattle. Future studies should also evaluate the efficacy of other potential control methods on the tick species present, including the use of acaricides (various compositions, spraying intervals and application methods), which may contribute to the development of cost-effective methods for control of ticks and TBDs. Research should also target the contribution of multiple host species, including wildlife. The potential of the ticks as vectors of diseases of livestock, wildlife and humans should be further investigated, by testing the ticks for pathogens and conducting transmissibility studies.

7.4 References

ALLSOPP, B.A., 2010. Natural history of *Ehrlichia ruminantium*. Veterinary Parasitology 167, 123-135.

ALLSOPP, B.A., BEZUIDENHOUT, J.D., PROZESKY, L., 2004. Heartwater. In: Infectious Diseases of Livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 507-535.

ALMAZÁN, C., MORENO-CANTÚ, O., MORENO-CID, J.A., GALINDO, R.C., CANALES, M., VILLAR, M., DE LA FUENTE, J., 2012. Control of tick infestations in cattle vaccinated with bacterial membranes containing surface-exposed tick protective antigens. Vaccine 30, 265-272.

ANDERSON, I.M., ROBINSON, W.I., 2009. Tenth EDF Karamoja Livelihoods Programme (KALIP) Technical Reference Guide. European Union Delegation to the Republic of Uganda, The Republic of Uganda, and Food and Agriculture Organization of the United Nations. 74 pp. http://www.opm.nulep.org/partners/kalip-partners/item/download/436_f6ec429c540744f00d64b5ce83770ca2. Accessed October 20, 2015.

ANGWECH, H., KADDU, J.B., NYEKO, J.H.P., 2011. Tick-borne parasites of domestic ruminants in Gulu district, Uganda: prevalence varied with the intensity of management. Veterinary Research 4, 28-33.

ASIIMWE, B.B., WEIR, W., TAIT, A., LUBEGA, G.W., OURA, C.A.L., 2013. Haemoparasite infection kinetics and the population structure of *Theileria parva* on a single farm in Uganda. Veterinary Parasitology 193, 8-14.

AUBRY, P., GEALE, D.W., 2011. A review of bovine anaplasmosis. Transboundary and Emerging Diseases 58, 1-30.

BARRE, N., GARRIS, G.I., 1990. Biology and ecology of *Amblyomma variegatum* (Acari: Ixodidae) in the Caribbean: Implications for a regional eradication program. Journal of Agricultural Entomology 7, 1-9.

BOCK, R.E., DE VOS, A.J., KINGSTON, T.G., MCLELLAN, D.J., 1997. Effect of breed of cattle on innate resistance to infection with *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*. Australian Veterinary Journal 75, 337-340.

BOCK, R., JACKSON, L., DE VOS, A., JORGENSEN, W., 2004. Babesiosis of cattle. Parasitology 129, S247-S269.

BYARUHANGA, C., NDUKUI, J.N., OLINGA, S., EGAYU, G., BOMA, P., ALEPER, D., 2015. Ethnoveterinary practices in the control of helminthosis and ticks of livestock amongst pastoralists in Karamoja Region, Uganda. Livestock Research for Rural Development Volume 27 (8), Article #160. http://www.lrrd.org/lrrd27/8/byar27160.html. Accessed November 2, 2015.

CARELLI, G., DECARO, N., LORUSSO, E., PARADIES, P., ELIA, G., MARTELLA, V., BUONAVOGLIA, C., CECI, L., 2008. First report of bovine anaplasmosis caused by *Anaplasma centrale* in Europe. Annals of the New York Academy of Sciences 1149, 107-110.

CASTAÑEDA-ORTIZ, E.J., UETI, M.W., CAMACHO-NUEZ, M., MOSQUEDA, J.J., MOUSEL, M.R., JOHNSON, W.C., PALMER, G.H., 2015. Association of *Anaplasma marginale* strain superinfection with infection prevalence within tropical regions. PLoS One 10 (3), e0120748. doi: 10.1371/journal.pone.0120748.

CATLEY, A., ADMASSU, B., BEKELE, G., ABEBE, D., 2014. Livestock mortality in pastoralist herds in Ethiopia and implications for drought response. Disasters 38, 500-516.

CFSPH, 2008. The Center for Food Security & Public Health. Institute for International Cooperation in Animal Biologics, Iowa State University. Accessed on January 6, 2017 from http://www.cfsph.iastate.edu/Factsheets/pdfs/bovine_babesiosis.pdf.

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. Livestock Research for Rural Development Volume 22 (7), Article # 135. Retrieved May 2, 2013, from http://www.lrrd.org/lrrd22/7/chen22135.htm.

CONRAD, P.A., DENHAM, D., BROWN, C.G.D., 1986. Intraerythrocytic multiplication of *Theileria parva in vitro*: An ultrastructural study. International Journal for Parasitology 16, 223-229.

DE LA FUENTE, J., ALMAZÁN, C., BLOUIN, E.F., NARANJO, V., KOCAN, K.M., 2006. Reduction of tick infections with *Anaplasma marginale* and *A. phagocytophilum* by targeting the tick protective antigen subolesin. Parasitology Research 100, 85-91.

DE VOS, S., ZEINSTRA, L., TAOUFIK, A., WILLADSEN, P., JONGEJAN, F., 2001. Evidence for the utility of the Bm86 antigen from *Boophilus microplus* in vaccination against other tick species. Experimental & Applied Acarology 25, 245-261.

DI GIULIO, G., LYNEN, G., MORZARIA, S., OURA, C., BISHOP, R., 2009. Live immunization against East Coast fever - current status. Trends in Parasitology 25, 85-92.

DOLAN, T.T., 1986. Chemotherapy of East Coast fever: The long term weight changes, carrier state and disease manifestations of parvaquone treated cattle. Journal of Comparative Pathology 96, 137-146.

FAO, 2013. FAO at work in Karamoja. Food and Agriculture Organization of the United nations (FAO) Representation in Uganda.

http://www.fao.org/emergencies/resources/documents/resources-detail/en/c/212585/. Accessed October 23, 2015.

FOUCHE, G., RAMAFUTHULA, M., MASELELA, V., MOKOENA, M., SENABE, J., LEBOHO, T., SAKONG, B.M., ADENUBI, O.T., ELOFF, J.N., WELLINGTON, K.W., 2016. Acaricidal activity of the organic extracts of thirteen South African plants against *Rhipicephalus* (*Boophilus*) *decoloratus* (Acari: Ixodidae). Veterinary Parasitology 224, 39-43.

FLORIN-CHRISTENSEN, M., SUAREZ, C.E., RODRIGUEZ, A.E., FLORES, D.A., SCHNITTGER, L., 2014. Vaccines against bovine babesiosis: where we are now and possible roads ahead. Parasitology 141, 1563-1592.

FYUMAGWA, R.D., MDAKI, M.L., SOOMBE, C., NYAKI, A., 2013. African buffalo is an important reservior of *Anaplasma bovis* in the Ngorongoro Crater, Tanzania. Research Opinions in Animal and Veterinary Sciences 3, 105-110.

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, 194.

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, 103-113.

GALVMED, 2015. The CTTBD ECF Vaccine: Greater opportunity to control East Coast fever and other tick-borne diseases in Africa. Global Alliance for Livestock Veterinary Medicines, Pentlands SciencePark, Bush Loan, Edinburgh EH26 OPZ, UK.

GLEIM, E.R., CONNER, L.M., BERGHAUS, R.D., LEVIN, M.L., ZEMTSOVA, G.E., YABSLEY, M.J., 2014. The phenology of ticks and the effects of long-term prescribed burning on tick

population dynamics in southwestern Georgia and northwestern Florida. PLoS ONE 9 (11), e112174. doi: 10.1371/journal.pone.0112174.

GRADÉ, J.T., TABUTI, J.R.S., VAN DAMME, P., 2009. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. Journal of Ethnopharmacology 122, 273-293.

HABEEB, S.M., 2010. Ethno-veterinary and medical knowledge of crude plant extracts and its methods of application (traditional and modern) for tick control. World Applied Sciences Journal 11, 1047-1054.

HAJDUSEK, O., ALMAZÁN, C., LOOSOVA, G., VILLAR, M., CANALES, M., GRUBHOFFER, L., KOPACEK, P., DE LA FUENTE, J., 2010. Characterization of ferritin 2 for the control of tick infestations. Vaccine 28, 2993-2998.

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, 248-271.

JENKINS, M.C., 2001. Advances and prospects for subunit vaccines against protozoa of veterinary importance. Veterinary Parasitology 101, 291-310.

JONSSON, N.N., 2006. The productivity effects of cattle tick (*Boophilus microplus*) infestation on cattle, with particular reference to *Bos indicus* cattle and their crosses. Veterinary Parasitology 137, 1-10.

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, 85-89.

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, 414.

KABUUSU, R.M., ALEXANDER, R., KABUUSU, A.M., MUWANGA, S.N., ATIMNEDI, P., MACPHERSON, C., 2013. Effect of wildlife-livestock interface on the prevalence of intra-erythrocytic haemoparasites in cattle. Open Journal of Veterinary Medicine 3, 315-318.

KALUME, M.K., LOSSON, B., ANGENOT, L., TITS, M., WAUTERS, J.N., FRÉDÉRICH, M., SAEGERMAN, C., 2012. Rotenoid content and *in vitro* acaricidal activity of *Tephrosia vogelii* leaf extract on the tick *Rhipicephalus appendiculatus*. Veterinary Parasitology 190, 204-209.

KARIUKI, D.P., YOUNG, A.S., MORZARIA, S.P., LESAN, A.C., MINING, S.K., OMWOYO, P., WAFULA, J.L.M., MOLYNEUX, D.H., 1995. *Theileria parva* carrier state in naturally infected and artificially immunised cattle. Tropical Animal Health and Production 27, 15-25.

KASOZI, K.I., MATOVU, E., TAYEBWA, D.S., NATUHWERA, J., MUGEZI, I., MAHERO, M., 2014. Epidemiology of increasing hemo-parasite burden in Ugandan cattle. Open Journal of Veterinary Medicine 4, 220-231.

KIVARIA, F.M., 2006. Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Tropical Animal Health and Production 38, 291-299.

LATIF, A.A., 1993. Sustainable control methods of ticks and tick-borne diseases in Africa. In: Future of livestock industries in East and Southern Africa, KATEGILE, J.A., MUBI, S. (Eds.), Proceedings of the Workshop held at Kadoma Ranch Hotel, Zimbabwe 20-23 July 1992. lLCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia, 227 pp.

LATIF, A.A., WALKER, A.R., 2004. An introduction to the biology and control of ticks in Africa, 29 pp. Accessed October 22, 2016, from www.alanrwalker.com/assets/PDF/tick-biology-africa.pdf.

LYNEN, G., DI GIULIO, G., HOMEWOOD, K., REID, R., MWILAWA, A., 2006. Deployment of a live ECF vaccine in pastoral areas: lessons learned from Tanzania. In: The role of biotechnology in animal agriculture to address poverty in Africa: opportunities and challenges. Proceedings of the 4th All African Conference on Animal Agriculture and the 31st Annual Meeting of the Tanzania Society for Animal Production (TSAP), 20-24 September, 2005, REGE, J.E.O., NYAMU, A.M., SENDALO, D. (Eds.), Arusha, Tanzania, All Africa Society for Animal Production (AASAP), Nairobi, Kenya, 193-201.

LYNEN, G., YRJÖ-KOSKINEN, A.E., BAKUNAME, C., DI GIULIO, G., MLINGA, N., KHAMA, I., HANKS, J., TAYLOR, N.M., JAMES, A.D., MCKEEVER, D., PETERS, A.R., RUSHTON, J., 2012. East Coast fever immunisation field trail in crossbred dairy cattle in Hanang and Handeni districts in northern Tanzania. Tropical Animal Health and Production 44, 567-572.

MAGONA, J.W., WALUBENGO, J., KABI, F., 2011a. Response of Nkedi Zebu and Ankole cattle to tick infestation and natural tick-borne, helminth and trypanosome infections in Uganda. Tropical Animal Health and Production 43, 1019-1033.

MAGONA, J.W., WALUBENGO, J., OLAHO-MUKANI, W., JONSSON, N.N., WELBURN, S.W., EISLER, M.C., 2011b. Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale*, *Babesia bigemina* and *Theileria parva* infections in Uganda. Experimental and Applied Acarology 55, 203-213.

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, 216-223.

MANS, B.J., PIENAAR, R., LATIF, A.A., 2015. A review of *Theileria* diagnostics and epidemiology International Journal for Parasitology: Parasites and Wildlife 4, 104-118.

MARCELINO, I., DE ALMEIDA, A.M., VENTOSA, M., PRUNEAU, L., MEYER, D.F., MARTINEZ, D., LEFRANÇOIS, T., VACHIÉRY, N., COELHO, A.V., 2012. Tick-borne diseases in cattle: Applications of proteomics to develop new generation vaccines. Journal of Proteomics 75, 4232-4250.

MARCELINO, I., LEFRANÇOIS, T., MARTINEZ, D., GIRAUD-GIRARD, K., APRELON, R., MANDONNET, N., GAUCHERON, J., BERTRAND, F., VACHIÉRY, N., 2015. A user-friendly and scalable process to prepare a ready-to-use inactivated vaccine: The example of heartwater in ruminants under tropical conditions. Vaccine 33, 678-685.

MATTIOLI, R.C., PANDEY, V.S., MURRAY, M., FITZPATRICK, J.L., 2000. Immunogenetic influences on tick resistance in African cattle with particular reference to trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Gobra zebu (*Bos indicus*) cattle. Acta Tropica 75, 263-277.

MBASSA, G.K., MGONGO, F.O.K., MELAU, L.S.B., MLANGWA, J.E.D., SILAYO, R.S., BIMBITA, E.N., HAYGHAIMO, A.A., MBIHA, E.R., 2009. A financing system for the control of tick-borne diseases in pastoral herds: The Kambala (Tanzania) model. Livestock Research for Rural Development 21 (3), Article #44. http://www.lrrd.org/lrrd21/3/mbas21044.htm. Accessed May 20, 2015.

MCKEEVER, D.J., MORRISON, W.I., 1990. *Theileria parva*: the nature of the immune response and its significance for immunoprophylaxis. Scientific and Technical Review of the Office International des Epizooties (Paris) 9, 405-421.

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, 8575-8579.

MINJAUW, B., MCLEOD, A., 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, 116 pp.

MOLL, G., LOHDING, A., YOUNG, A.S., LEITCH, B.L., 1986. Epidemiology of theileriosis in calves in an endemic area of Kenya. Veterinary Parasitology 19, 255-273.

MORENO-CID, J.A., DE LA LASTRA, J.M.P., VILLAR, M., JIMÉNEZ, M., PINAL, R., ESTRADA-PEÑA, A., MOLINA, R., LUCIENTES, J., GORTÁZAR, C., DE LA FUENTE, J., 2013. Control of multiple arthropod vector infestations wih subolesin/akirin vaccines. Vaccine 31, 1187-1196.

MUGABI, K.N., MUGISHA, A., OCAIDO, M., 2010. Socio-economic factors influencing the use of acaricides on livestock: a case study of the pastoralist communities of Nakasongola District, Central Uganda. Tropical Animal Health and Production 42, 131-136.

MUGISHA, A., MCLEOD, A., PERCY, R., KYEWALABYE, E., 2005. Strategies, effectiveness and rationale of vector-borne disease control in the pastoralist system of south-western Uganda. Tropical Animal Health and Production 37, 479-489.

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.

MUHANGUZI, D., IKWAP, K., PICOZZI, K., WAISWA, C., 2010. Molecular characterization of *Anaplasma* and *Ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). International Journal of Animal and Veterinary Advances 2, 76-88.

MUSISI, F.L., JONGEJAN, F., PEGRAM, R.G., 1984. Isolation and transmission of *Theileria mutans* (Chisamba) in Zambia. Research in Veterinary Science 36, 129-131.

MUWANIKA, V., KABI, F., MASEMBE, C., 2016. Population genetic structure of *Theileria parva* field isolates from indigenous cattle populations of Uganda. Ticks and Tick-borne Diseases 7, 291-297.

NABUKENYA, I., RUBAIRE-AKIIKI, C., OLILA, D., IKWAP, K., HÖGLUND, J., 2014. Ethnopharmacological practices by livestock farmers in Uganda: Survey experiences from Mpigi and Gulu districts. Journal of Ethnobiology and Ethnomedicine 10, 9.

NORVAL, R.A.I., PERRY, B.D., YOUNG, A.S., LAWRENCE, J.A., MUKHEBI, A.W., BISHOP, R.P., MCKEEVER, D.J., SHAW, M.K., 1992. The epidemiology of theileriosis in Africa. Academic Press Limited, London NWI 7DX, UK, 481 pp.

OCAIDO, M., OTIM, C.P., KAKAIRE, D., 2009. Impact of major diseases and vectors in smallholder cattle production systems in different agro-ecological zones and farming systems in Uganda. Livestock Research for Rural Development Volume 21 (9), Article #155. Retrieved November 29, 2013, from http://www.lrrd.org/lrrd21/9/ocai21155.htm.

OLDS, C.L., MWAURA, S., ODONGO, D.O., SCOLES, G.A., BISHOP, R., DAUBENBERGER, C., 2016. Induction of humoral immune response to multiple recombinant *Rhipicephalus appendiculatus* antigens and their effect on tick feeding success and pathogen transmission. Parasites & Vectors 9, 484.

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, 603-613.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011a. Haemoparasite prevalence and *Theileria parva* strain diversity in Cape buffalo (*Syncerus caffer*) in Uganda. Veterinary Parasitology 175, 212-219.

OURA, C.A.L., TAIT, A., ASIIMWE, B., LUBEGA, G.W., WEIR, W., 2011b. *Theileria parva* genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. Parasitology Research 108, 1365-1374.

PALMER, G.H., BRAYTON, K.A., 2013. Antigenic variation and transmission fitness as drivers of bacterial strain structure. Cellular Microbiology 15, 1969-1975.

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, 107-120.

POLITO, V.J., BAUM, K.A., PAYTON, M.E., LITTLE, S.E., FUHLENDORF, S.D., REICHARD, M.V., 2013. Tick abundance and levels of infestation on cattle in response to patch burning. Rangeland Ecology & Management 66, 545-552.

POTGIETER, F.T., STOLTSZ, W.H., 2004. Bovine anaplasmosis. In: Infectious diseases of livestock, COETZER, J.A.W., TUSTIN, R.C. (Eds.), 2nd edn, Oxford University Press Southern Africa, Cape Town, South Africa, pp. 594-616.

PRINE, K.C., HODGES, A.C., 2013. Entomology and Nematology: Featured Creatures. University of Florida. Accessed October 19, 2016, from www.entnemdept.ufl.edu/creatures/livestock/ticks/tropical_bont_tick.htm.

REINBOLD, J.B., COETZEE, J.F., HOLLIS, L.C., NICKELL, J.S., RIEGEL, C., OLSON, K.C., GANTA, R.R., 2010. The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. Veterinary Microbiology 145, 69-75.

RUBAIRE-AKIIKI, C.M., OKELLO-ONEN, J., MUSUNGA, D., KABAGAMBE, E.K., VAARST, M., OKELLO, D., OPOLOT, C., BISAGAYA, A., OKORI, C., BISAGATI, C., ONGYERA, S., MWAYI, M.T., 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, 251-266.

SHIFERAW, T.J., MOSES, K., MANYAHILISHAL, K.E., 2010. Participatory appraisal of foot and mouth disease in the Afar pastoral area, northeast Ethiopia: implications for understanding disease ecology and control strategy. Tropical Animal Health and Production 42, 193-201.

SNV, 2013. Policy Brief on Management of Ticks and East Coast fever. SNV Netherlands Development Organisation, 6 pp. Retrieved December 20, 2015, from http://www.m.snvworld.org/en/countries/Uganda/publications/policy-brief-on-management-of-ticks-and-east-coast-fever.

STEYN, H.C., PRETORIUS, A., MCCRINDLE, C.M.E., STEINMANN, C.M.L., VAN KLEEF, M., 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Veterinary Microbiology 131, 258-265.

SUAREZ, C.E., NOH, S., 2011. Emerging perspectives in the research of bovine babesiosis and anaplasmosis. Veterinary Parasitology 180, 109-125.

SWAI, E.S., MASAAZA, S., DABORN, C.J., 2014. Evaluation of community animal health delivery systems in Simanjiro, Tanzania. Livestock Research for Rural Development Volume 26 (8), Article # 138. Retrieved September 20, 2014, from http://www.irrd.org/Irrd26/8/swai26138.htm.

UILENBERG, G., 2006. *Babesia* - A historical overview. Veterinary Parasitology 138, 3-10.

WALKER, A.R., BOUATTOUR, A., CAMICAS, J.-L., ESTRADA-PEÑA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G., PRESTON, P.M., 2013. Ticks of domestic animals in Africa: a guide to identification of species. International Consortium on Ticks and Tick Borne Diseases (ICTTD-2), 221 pp.

WALKER, J.G., KLEIN, E.Y., LEVIN, S.A., 2014. Disease at the wildlife-livestock interface: Acaricide use on domestic cattle does not prevent transmission of a tick-borne pathogen with multiple hosts. Veterinary Parasitology 199, 206-214.

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. Livestock Research for Rural Development Volume 22 (6), Article #111. Retrieved December 13, 2013, from http://www.irrd.org/Irrd22/6/weso22111.htm.

WILLADSEN, P., 2006. Vaccination against ectoparasites. Parasitology 133, S9-S25.

WOOLHOUSE, M.E.J., THUMBI, S.M., JENNINGS, A., CHASE-TOPPING, M., CALLABY, R., KIARA, H., OOSTHUIZEN, M.C., MBOLE-KARIUKI, M.N., CONRADIE, I., HANDEL, I.G., POOLE, E.J., NJIIRI, E., COLLINS, N.E., MURRAY, G., TAPIO, M., AUGUET, O.T., WEIR, W., MORRISON, W.I., KRUUK, L.E.B., BRONSVOORT, B.M.C., HANOTTE, O., COETZER, K., TOYE, P.G., 2015. Co-infections determine patterns of mortality in a population exposed to parasite infection. Science Advances 1 (2), e1400026. DOI: 10.1126/sciadv.1400026.

APPENDICES

Appendix 1: Table showing the detection of antibodies in serum samples of 397 cattle using competitive enzyme-linked immunosorbent assay [cELISA] (*Anaplasma marginale*) and indirect fluorescent antibody test [IFAT] (*Theileria parva*). The samples were collected from 20 herds in two districts of Karamoja Region, Uganda, from November 2013 through January 2014. The results are indicated as '+' for positive and '-' for negative.

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
1	Moroto	1	MT1-1	F	12	+	+
2	Moroto	1	MT1-2	F	12	-	-
3	Moroto	1	MT1-3	F	12	+	-
4	Moroto	1	MT1-4	M	12	+	-
5	Moroto	1	MT1-5	F	12	+	-
6	Moroto	1	MT1-6	F	8	+	-
7	Moroto	1	MT1-7	F	9	+	+
8	Moroto	1	MT1-8	F	7	+	+
9	Moroto	1	MT1-9	F	18	+	-
10	Moroto	1	MT1-11	F	15	+	-
11	Moroto	1	MT1-12	F	18	+	-
12	Moroto	1	MT1-14	F	14	+	+
13	Moroto	1	MT1-15	F	9	+	+
14	Moroto	1	MT1-16	F	17	+	+
15	Moroto	1	MT1-17	F	18	+	-
16	Moroto	2	MT2-1	M	10	+	+
17	Moroto	2	MT2-2	F	18	+	+
18	Moroto	2	MT2-3	M	8	+	-
19	Moroto	2	MT2-4	F	36	+	+
20	Moroto	2	MT2-7	F	24	+	-
21	Moroto	2	MT2-9	F	12	+	+
22	Moroto	2	MT2-11	F	24	+	-
23	Moroto	2	MT2-12	M	18	+	-
24	Moroto	2	MT2-13	F	12	+	-
25	Moroto	2	MT2-14	F	24	+	-
26	Moroto	2	MT2-15	F	7	+	-
27	Moroto	2	MT2-21	F	36	+	-
28	Moroto	2	MT2-24	F	6	+	-
29	Moroto	2	MT2-25	M	5	+	+

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S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
30	Moroto	2	MT2-26	F	6	+	-
31	Moroto	3	MT3-1	F	>48	+	-
32	Moroto	3	MT3-2	F	36	+	-
33	Moroto	3	MT3-3	F	24	+	-
34	Moroto	3	MT3-4	F	14	+	-
35	Moroto	3	MT3-5	M	12	+	-
36	Moroto	3	MT3-6	M	10	+	-
37	Moroto	3	MT3-7	M	9	+	-
38	Moroto	3	MT3-8	M	11	+	-
39	Moroto	3	MT3-9	F	48	+	-
40	Moroto	3	MT3-10	F	12	+	-
41	Moroto	3	MT3-11	F	9	+	+
42	Moroto	3	MT3-12	F	>48	+	-
43	Moroto	3	MT3-13	M	15	+	+
44	Moroto	3	MT3-14	F	10	+	+
45	Moroto	3	MT3-15	F	11	+	-
46	Moroto	4	MT4-1	F	8	+	-
47	Moroto	4	MT4-2	M	8	+	-
48	Moroto	4	MT4-3	F	24	+	+
49	Moroto	4	MT4-4	F	24	+	+
50	Moroto	4	MT4-6	F	18	+	-
51	Moroto	4	MT4-7	F	20	+	+
52	Moroto	4	MT4-8	F	24	+	-
53	Moroto	4	MT4-9	F	>48	+	+
54	Moroto	4	MT4-10	F	42	+	-
55	Moroto	4	MT4-12	F	30	+	-
56	Moroto	4	MT4-13	M	8	+	+
57	Moroto	4	MT4-14	F	12	+	+
58	Moroto	4	MT4-15	M	15	+	+
59	Moroto	4	MT4-16	F	>48	+	+
60	Moroto	4	MT4-17	M	24	+	+
61	Moroto	5	MT5-1	F	12	+	+
62	Moroto	5	MT5-2	F	13	+	+
63	Moroto	5	MT5-3	F	13	+	-
64	Moroto	5	MT5-4	F	15	+	-
65	Moroto	5	MT5-5	F	8	+	+
66	Moroto	5	MT5-6	F	11	+	-
67	Moroto	5	MT5-7	F	12	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
68	Moroto	5	MT5-8	F	18	+	+
69	Moroto	5	MT5-9	F	9	+	-
70	Moroto	5	MT5-10	F	10	+	+
71	Moroto	5	MT5-11	F	18	+	+
72	Moroto	5	MT5-12	F	19	+	+
73	Moroto	5	MT5-13	F	16	+	+
74	Moroto	5	MT5-14	F	9	+	-
75	Moroto	5	MT5-15	F	15	+	+
76	Moroto	17	MR1-1	M	8	+	-
77	Moroto	17	MR1-2	M	12	-	-
78	Moroto	17	MR1-3	M	8	+	-
79	Moroto	17	MR1-4	F	8	-	-
80	Moroto	17	MR1-5	F	>48	+	-
81	Moroto	17	MR1-6	F	42	+	-
82	Moroto	17	MR1-7	M	10	+	-
83	Moroto	17	MR1-8	M	14	+	-
84	Moroto	17	MR1-9	F	>48	+	-
85	Moroto	17	MR1-10	F	7	+	-
86	Moroto	17	MR1-12	F	42	+	-
87	Moroto	17	MR1-13	F	42	+	-
88	Moroto	17	MR1-14	F	42	+	-
89	Moroto	17	MR1-15	F	36	+	-
90	Moroto	17	MR1-16	M	42	+	-
91	Moroto	18	MR2-1	F	>48	+	-
92	Moroto	18	MR2-2	M	36	+	-
93	Moroto	18	MR2-3	F	24	+	-
94	Moroto	18	MR2-4	F	>48	+	-
95	Moroto	18	MR2-5	F	>48	+	-
96	Moroto	18	MR2-6	M	>48	+	-
97	Moroto	18	MR2-7	M	30	+	-
98	Moroto	18	MR2-8	M	>48	+	-
99	Moroto	18	MR2-9	F	36	+	-
100	Moroto	18	MR2-10	M	36	+	-
101	Moroto	18	MR2-11	F	30	+	-
102	Moroto	18	MR2-17	F	7	+	-
103	Moroto	18	MR2-18	F	12	+	-
104	Moroto	18	MR2-19	F	24	+	-
105	Moroto	18	MR2-20	M	12	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
106	Moroto	19	MR3-1	M	18	+	-
107	Moroto	19	MR3-2	F	10	+	-
108	Moroto	19	MR3-3	F	42	+	-
109	Moroto	19	MR3-4	F	30	+	-
110	Moroto	19	MR3-5	F	10	+	-
111	Moroto	19	MR3-6	F	10	+	-
112	Moroto	19	MR3-7	F	10	+	-
113	Moroto	19	MR3-8	F	11	+	-
114	Moroto	19	MR3-9	F	24	+	-
115	Moroto	19	MR3-10	F	24	+	-
116	Moroto	19	MR3-11	M	24	+	-
117	Moroto	19	MR3-13	F	42	+	-
118	Moroto	19	MR3-14	F	18	+	-
119	Moroto	19	MR3-15	M	6	+	-
120	Moroto	19	MR3-16	F	7	+	-
121	Moroto	20	MR4-1	M	>48	+	-
122	Moroto	20	MR4-2	F	30	+	-
123	Moroto	20	MR4-3	M	>48	+	-
124	Moroto	20	MR4-4	F	>48	+	-
125	Moroto	20	MR4-5	M	30	+	-
126	Moroto	20	MR4-6	M	36	+	-
127	Moroto	20	MR4-7	F	>48	+	-
128	Moroto	20	MR4-8	F	30	+	-
129	Moroto	20	MR4-9	F	36	+	-
130	Moroto	20	MR4-10	M	30	+	-
131	Moroto	20	MR4-11	F	36	+	-
132	Moroto	20	MR4-12	F	36	+	-
133	Moroto	20	MR4-20	F	5	-	-
134	Moroto	20	MR4-21	F	6	-	-
135	Moroto	20	MR4-22	M	12	-	-
136	Moroto	21	MR5-1	M	24	-	-
137	Moroto	21	MR5-2	M	9	-	-
138	Moroto	21	MR5-3	F	8	-	-
139	Moroto	21	MR5-4	F	9	-	-
140	Moroto	21	MR5-5	F	42	-	-
141	Moroto	21	MR5-6	M	7	-	+
142	Moroto	21	MR5-7	F	12	-	-
143	Moroto	21	MR5-8	F	36	-	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
144	Moroto	21	MR5-10	F	8	+	-
145	Moroto	21	MR5-11	M	8	+	-
146	Moroto	21	MR5-12	M	6	+	-
147	Moroto	21	MR5-13	M	5	+	-
148	Moroto	21	MR5-14	M	5	+	-
149	Moroto	21	MR5-18	F	42	+	+
150	Moroto	21	MR5-19	M	12	+	-
151	Kotido	6	KN1-1	F	>48	+	-
152	Kotido	6	KN1-2	F	>48	+	-
153	Kotido	6	KN1-3	M	9	+	-
154	Kotido	6	KN1-4	M	9	+	-
155	Kotido	6	KN1-5	F	24	+	-
156	Kotido	6	KN1-6	F	>48	+	-
157	Kotido	6	KN1-7	F	36	+	-
158	Kotido	6	KN1-8	M	>48	+	-
159	Kotido	6	KN1-9	F	>48	+	-
160	Kotido	6	KN1-10	F	>48	+	-
161	Kotido	6	KN1-11	F	30	+	-
162	Kotido	6	KN1-12	F	>48	+	-
163	Kotido	6	KN1-13	F	>48	+	-
164	Kotido	6	KN4-1	M	>48	+	-
165	Kotido	6	KN4-2	F	>48	+	-
166	Kotido	7	KN2-1	F	36	+	-
167	Kotido	7	KN2-2	F	30	+	-
168	Kotido	7	KN2-3	F	30	+	-
169	Kotido	7	KN2-4	F	36	+	-
170	Kotido	7	KN2-5	F	>48	+	-
171	Kotido	7	KN2-6	F	>48	+	-
172	Kotido	7	KN2-7	F	12	+	-
173	Kotido	7	KN2-8	F	8	+	-
174	Kotido	7	KN2-9	F	>48	-	-
175	Kotido	7	KN2-10	F	>48	+	-
176	Kotido	7	KN2-11	F	36	+	-
177	Kotido	7	KN2-12	F	42	+	-
178	Kotido	7	KN2-13	F	42	+	-
179	Kotido	7	KN2-14	F	42	+	-
180	Kotido	7	KN2-15	F	36	+	-
181	Kotido	8	KN3-1	F	24	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
182	Kotido	8	KN3-2	M	12	+	-
183	Kotido	8	KN3-3	M	9	+	-
184	Kotido	8	KN3-4	M	>48	+	-
185	Kotido	8	KN3-5	M	36	+	-
186	Kotido	8	KN3-6	F	>48	+	-
187	Kotido	8	KN3-7	F	>48	+	-
188	Kotido	8	KN3-8	F	48	+	-
189	Kotido	8	KN3-9	F	42	+	-
190	Kotido	8	KN3-10	M	7	+	-
191	Kotido	8	KN3-11	M	9	+	-
192	Kotido	8	KN3-12	F	42	+	-
193	Kotido	8	KN3-13	M	36	+	-
194	Kotido	8	KN3-14	F	30	-	-
195	Kotido	8	KN3-15	M	12	+	-
196	Kotido	11	KN5-1	F	>48	+	-
197	Kotido	11	KN5-2	F	>48	+	-
198	Kotido	11	KN5-3	F	24	+	-
199	Kotido	11	KN5-4	F	30	+	-
200	Kotido	11	KN5-5	F	>48	+	-
201	Kotido	11	KN5-6	F	8	+	-
202	Kotido	11	KN5-7	F	7	+	-
203	Kotido	11	KN5-8	F	8	+	-
204	Kotido	11	KN5-9	M	9	+	-
205	Kotido	11	KN5-10	F	18	+	-
206	Kotido	11	KN5-11	M	8	-	-
207	Kotido	11	KN5-12	F	9	+	-
208	Kotido	11	KN5-13	F	42	+	-
209	Kotido	11	KN5-14	F	9	-	-
210	Kotido	11	KN5-15	F	12	+	-
211	Kotido	10	KN6-1	F	>48	+	-
212	Kotido	10	KN6-2	F	36	+	-
213	Kotido	10	KN6-3	F	>48	+	-
214	Kotido	10	KN6-4	F	36	+	-
215	Kotido	10	KN6-5	F	42	+	-
216	Kotido	10	KN6-6	F	>48	+	-
217	Kotido	10	KN6-7	F	30	+	-
218	Kotido	10	KN6-8	F	9	+	-
219	Kotido	10	KN6-9	F	36	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
220	Kotido	10	KN6-10	F	>48	+	-
221	Kotido	10	KN6-18	F	7	+	-
222	Kotido	10	KN6-19	M	7	+	-
223	Kotido	10	KN6-20	F	6	-	-
224	Kotido	10	KN6-24	M	12	+	-
225	Kotido	10	KN6-27	M	6	+	-
226	Kotido	12	KR1-1	M	8	+	-
227	Kotido	12	KR1-2	M	12	+	-
228	Kotido	12	KR1-3	F	7	+	-
229	Kotido	12	KR1-4	F	9	+	-
230	Kotido	12	KR1-5	F	24	+	-
231	Kotido	12	KR1-6	F	18	-	-
232	Kotido	12	KR1-7	F	36	-	-
233	Kotido	12	KR1-8	F	24	-	-
234	Kotido	12	KR1-9	F	48	-	-
235	Kotido	12	KR1-10	F	36	+	-
236	Kotido	12	KR1-11	M	7	+	-
237	Kotido	12	KR1-12	M	8	+	-
238	Kotido	12	KR1-13	F	36	+	-
239	Kotido	12	KR1-14	M	8	+	-
240	Kotido	12	KR1-15	F	24	-	-
241	Kotido	13	KR2-1	F	30	+	-
242	Kotido	13	KR2-2	F	48	+	-
243	Kotido	13	KR2-3	F	36	+	-
244	Kotido	13	KR2-4	F	24	+	-
245	Kotido	13	KR2-5	F	>48	+	-
246	Kotido	13	KR2-6	F	48	+	-
247	Kotido	13	KR2-7	F	36	+	-
248	Kotido	13	KR2-8	F	>48	+	-
249	Kotido	13	KR2-9	F	36	+	-
250	Kotido	13	KR2-10	F	36	+	-
251	Kotido	13	KR2-11	F	18	+	-
252	Kotido	13	KR2-27	F	6	+	-
253	Kotido	13	KR2-28	F	6	+	-
254	Kotido	13	KR2-29	M	6	+	-
255	Kotido	13	KR2-30	M	6	+	-
256	Kotido	14	KR3-1	F	30	+	-
257	Kotido	14	KR3-2	F	18	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
258	Kotido	14	KR3-3	F	36	+	-
259	Kotido	14	KR3-4	F	36	+	-
260	Kotido	14	KR3-5	F	>48	+	-
261	Kotido	14	KR3-6	F	42	+	-
262	Kotido	14	KR3-7	F	36	+	-
263	Kotido	14	KR3-8	F	24	+	-
264	Kotido	14	KR3-9	F	30	+	-
265	Kotido	14	KR3-10	F	30	+	-
266	Kotido	14	KR3-11	F	30	-	-
267	Kotido	14	KR3-12	F	36	+	-
268	Kotido	14	KR3-13	F	36	+	-
269	Kotido	14	KR3-17	F	6	+	-
270	Kotido	14	KR3-18	M	5	+	-
271	Kotido	15	KR4-2	M	9	_	-
272	Kotido	15	KR4-3	M	9	+	-
273	Kotido	15	KR4-4	F	8	-	-
274	Kotido	15	KR4-5	F	36	+	-
275	Kotido	15	KR4-6	F	>48	+	-
276	Kotido	15	KR4-7	F	12	+	-
277	Kotido	15	KR4-8	F	36	+	-
278	Kotido	15	KR4-9	F	6	+	-
279	Kotido	15	KR4-10	F	36	-	-
280	Kotido	15	KR4-11	F	24	-	-
281	Kotido	15	KR4-12	F	>48	+	-
282	Kotido	15	KR4-13	F	48	+	-
283	Kotido	15	KR4-14	F	>48	+	-
284	Kotido	15	KR4-15	F	5	+	-
285	Kotido	15	KR4-16	F	5	+	-
286	Kotido	16	KR5-1	F	42	+	-
287	Kotido	16	KR5-2	M	48	+	-
288	Kotido	16	KR5-3	F	36	+	-
289	Kotido	16	KR5-4	F	8	+	-
290	Kotido	16	KR5-5	F	48	+	-
291	Kotido	16	KR5-6	F	30	+	-
292	Kotido	16	KR5-7	F	24	+	-
293	Kotido	16	KR5-8	F	48	+	-
294	Kotido	16	KR5-9	F	36	+	-
295	Kotido	16	KR5-10	F	24	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
296	Kotido	16	KR5-11	F	36	-	-
297	Kotido	16	KR5-27	M	7	-	-
298	Kotido	16	KR5-28	M	9	+	-
299	Kotido	16	KR5-29	F	7	+	-
300	Kotido	16	KR5-30	F	7	+	-
301	Moroto	17	MR1-17	F	7	+	-
302	Moroto	17	MR1-18	M	6	+	-
303	Moroto	17	MR1-21	F	8	+	-
304	Moroto	17	MR1-22	F	7	-	+
305	Moroto	17	MR1-24	M	10	+	-
306	Moroto	18	MR2-21	F	6	+	-
307	Moroto	18	MR2-22	F	6	-	-
308	Moroto	18	MR2-23	F	12	-	-
309	Moroto	18	MR2-24	F	8	+	+
310	Moroto	18	MR2-30	F	6	+	-
311	Moroto	19	MR3-12	M	11	+	-
312	Moroto	19	MR3-17	M	8	+	-
313	Moroto	19	MR3-18	M	7	-	+
314	Moroto	19	MR3-19	M	7	-	-
315	Moroto	19	MR3-20	F	6	+	-
316	Moroto	20	MR4-18	M	8	+	-
317	Moroto	20	MR4-19	M	6	+	-
318	Moroto	20	MR4-23	F	8	+	-
319	Moroto	20	MR4-24	M	12	+	-
320	Moroto	20	MR4-25	M	8	+	-
321	Moroto	20	MR4-26	F	9	-	+
322	Moroto	20	MR4-27	F	8	+	+
323	Moroto	20	MR4-28	M	6	-	-
324	Moroto	21	MR5-15	F	6	-	-
325	Moroto	21	MR5-16	M	7	+	-
326	Moroto	21	MR5-17	M	6	+	-
327	Moroto	21	MR5-20	M	10	+	-
328	Moroto	21	MR5-21	F	9	-	-
329	Moroto	1	MT1-25	F	24	+	-
330	Moroto	1	MT1-26	F	12	+	+
331	Moroto	1	MT1-27	F	>48	+	-
332	Moroto	1	MT1-28	F	>48	+	+
333	Moroto	2	MT2-8	F	36	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
334	Moroto	2	MT2-18	M	7	+	+
335	Moroto	2	MT2-19	F	15	+	+
336	Moroto	2	MT2-20	F	24	+	+
337	Moroto	2	MT2-22	F	6	+	-
338	Moroto	2	MT2-23	F	12	+	-
339	Moroto	3	MT3-16	F	12	+	+
340	Moroto	3	MT3-19	F	12	+	+
341	Moroto	3	MT3-23	M	10	+	-
342	Moroto	3	MT3-24	F	10	+	-
343	Moroto	3	MT3-26	M	12	+	+
344	Moroto	3	MT3-29	F	9	-	-
345	Moroto	3	MT3-30	F	12	+	-
346	Moroto	3	MT3-31	F	12	+	+
347	Moroto	3	MT3-32	F	9	+	+
348	Moroto	4	MT4-5	F	>48	+	+
349	Moroto	4	MT4-20	M	24	+	+
350	Moroto	4	MT4-23	F	7	+	-
351	Moroto	4	MT4-25	M	7	+	-
352	Moroto	4	MT4-27	F	9	+	-
353	Moroto	5	MT5-18	F	19	+	-
354	Moroto	5	MT5-19	F	18	+	-
355	Moroto	5	MT5-20	F	24	+	-
356	Moroto	5	MT5-30	F	10	+	-
357	Moroto	5	MT5-32	F	20	+	-
358	Kotido	12	KR1-16	F	7	+	-
359	Kotido	12	KR1-17	F	18	+	+
360	Kotido	12	KR1-22	M	7	+	+
361	Kotido	12	KR1-26	M	8	+	+
362	Kotido	12	KR1-31	M	6	+	-
363	Kotido	13	KR2-12	F	>48	+	-
364	Kotido	13	KR2-13	F	>48	-	-
365	Kotido	13	KR2-14	F	36	+	-
366	Kotido	13	KR2-15	F	36	+	-
367	Kotido	13	KR2-24	F	18	+	-
368	Kotido	14	KR3-14	F	30	+	-
369	Kotido	14	KR3-15	F	42	+	-
370	Kotido	14	KR3-16	F	>48	-	-
371	Kotido	15	KR4-17	M	5	+	-

APPENDICES

S/No.	District	Identity of herd	Identity of cattle	Sex of cattle	Age of cattle (months)	cELISA for *Anaplasma marginale*	IFAT for *Theileria parva*
372	Kotido	15	KR4-19	M	6	+	-
373	Kotido	15	KR4-20	F	9	-	+
374	Kotido	15	KR4-21	F	7	-	-
375	Kotido	15	KR4-22	F	36	+	-
376	Kotido	16	KR5-18	F	11	+	-
377	Kotido	16	KR5-24	M	9	+	+
378	Kotido	16	KR5-25	F	18	+	-
379	Kotido	6	KN4-3	F	12	+	+
380	Kotido	6	KN4-4	F	9	+	-
381	Kotido	7	KN2-16	F	48	-	-
382	Kotido	7	KN2-17	F	48	+	-
383	Kotido	7	KN2-18	F	48	+	-
384	Kotido	7	KN2-19	F	48	-	-
385	Kotido	8	KN3-16	F	>48	+	+
386	Kotido	8	KN3-17	F	>48	+	+
387	Kotido	8	KN3-18	F	30	+	-
388	Kotido	8	KN3-19	F	36	-	-
389	Kotido	8	KN3-20	M	18	+	+
390	Kotido	11	KN5-16	F	36	-	-
391	Kotido	11	KN5-17	M	>48	-	-
392	Kotido	10	KN6-14	M	9	-	-
393	Kotido	10	KN6-15	F	8	-	-
394	Kotido	10	KN6-17	F	12	+	-
395	Kotido	10	KN6-22	M	11	-	-
396	Kotido	10	KN6-23	F	36	+	-
397	Moroto	1	MT1-24	F	24	+	-

Appendix 2: Representative pictures of ticks, which were collected on cattle in Karamoja Region, from November 2013 through January 2014

Rhipicephalus pulchellus

Hyalomma truncatum

Amblyomma variegatum

Amblyomma lepidum

Appendix 3: Table showing the detection of *Anaplasma marginale*, *A. centrale*, *Theileria parva* and *Ehrlichia ruminantium* in blood samples of cattle using reverse line blot (RLB) hybridisation and quantitative real-time PCR (qPCR) assays. The samples were collected from 20 herds in two districts of Karamoja Region, Uganda, from November 2013 through January 2014. The results are indicated as '+' for positive and '-' for negative. Cells with no result correspond to samples that were not tested.

Cattle ID	**District**	***A. marginale***		***A. centrale***		***T. parva***		***E. ruminantium***	
		RLB	**qPCR**	**RLB**	**qPCR**	**RLB**	**qPCR**	**RLB**	**qPCR**
MR1-1	Moroto	+	+	+	-	-	-	-	-
MR1-2	Moroto	+	+	+	-	-	-	-	-
MR1-3	Moroto	+	+	-	-	-	-		
MR1-5	Moroto	+	+	+	+	-	-		
MR2-18	Moroto	+	+	+	-	-	-	-	-
MR2-19	Moroto	-	+	+	-	-	-	-	-
MR2-20	Moroto	+	+	-	-	-	-		
MR2-21	Moroto	+	+	+	+	-	-		
MR3-14	Moroto	+	+	-	-	-	-	-	-
MR3-15	Moroto	+	+	+	+	-	-	-	-
MR3-16	Moroto	+	+	+	+	-	-		
MR3-17	Moroto	+	+	+	+	-	-		
MR4-5	Moroto	+	+	-	-	-	-	-	-
MR4-6	Moroto	+	+	-	-	-	-	-	-
MR4-7	Moroto	-	+	-	-	-	-		
MR4-8	Moroto	+	+	-	-	-	-		
MR5-1	Moroto	+	+	+	-	-	-	-	-
MR5-2	Moroto	+	+	+	-	-	-	-	-
MR5-3	Moroto	+	+	+	+	-	-	-	-
MR5-4	Moroto	+	+	+	-	-	-	-	-
MR5-14	Moroto	-	+	-	-	-	-		
MR5-18	Moroto	-	-	-	-	-	-		
MR5-19	Moroto	+	+	-	-	-	-		
MR5-23	Moroto	-	+	+	+	-	-		
MT5-1	Moroto	+	+	-	-	-	-	-	-
MT5-2	Moroto	-	+	+	-	-	-	-	-
MT5-3	Moroto	+	+	+	-	-	-		
MT5-4	Moroto	-	+	+	+	-	-		
MT4-9	Moroto	+	+	-	-	-	-	-	-
MT4-10	Moroto	+	+	-	-	-	-	-	-
MT4-11	Moroto	+	+	-	-	-	-		
MT4-12	Moroto	+	+	-	-	-	-		

APPENDICES

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
MT3-17	Moroto	+	+	-	-	-	-	-	-
MT3-18	Moroto	+	+	-	-	-	-	-	-
MT3-19	Moroto	+	+	+	+	-	-		
MT3-20	Moroto	+	+	-	-	-	-		
MT2-6	Moroto	-	+	-	-	-	-	-	-
MT2-7	Moroto	+	+	-	-	-	-	-	-
MT2-8	Moroto	-	+	-	-	-	-		
MT2-9	Moroto	+	+	+	+	-	-		
MT1-27	Moroto	+	+	-	-	-	-	-	-
MT1-30	Moroto	-	-	-	-	-	-	-	+
MT1-29	Moroto	-	-	-	-	-	-	-	-
MT1-28	Moroto	+	+	-	-	-	-	-	-
MT1-5	Moroto	-	-	-	-	-	-		
MT1-6	Moroto	-	+	-	-	-	-		
MT1-7	Moroto	-	-	-	-	-	-		
MT1-8	Moroto	+	+	-	-	-	-		
MT5-17	Moroto	+	+	-	-	-	-	-	+
MT5-18	Moroto	+	+	-	-	-	-	-	-
MT5-19	Moroto	+	+	-	-	-	-		
MT5-20	Moroto	+	+	-	-	-	-		
KN6-23	Kotido	+	+	-	-	-	-	-	-
KN6-24	Kotido	+	+	-	-	-	-	-	-
KN6-25	Kotido	+	+	-	-	-	-		
KN6-27	Kotido	+	+	-	-	-	-		
KN2-1	Kotido	-	+	-	-	-	-	-	-
KN2-2	Kotido	-	+	-	-	-	-	-	-
KN2-3	Kotido	+	+	+	+	-	-		
KN2-4	Kotido	+	+	-	+	-	-		
KN5-4	Kotido	+	+	-	-	-	-	-	-
KN5-5	Kotido	+	+	-	-	-	-	-	-
KN5-6	Kotido	-	+	-	-	-	-		
KN5-7	Kotido	+	+	+	+	-	-		
KN1-9	Kotido	+	+	-	-	-	-	-	-
KN1-10	Kotido	+	+	-	-	-	-	-	-
KN1-11	Kotido	+	+	-	-	-	-		
KN1-12	Kotido	+	+	-	-	-	-		
KN3-1	Kotido	-	-	-	-	-	-	-	-
KN3-2	Kotido	+	+	-	-	-	-	-	-
KN3-3	Kotido	+	+	+	+	-	-		

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
KN3-4	Kotido	+	+	-	-	-	-		
KN1-1	Kotido	-	+	-	-	-	-	-	-
KN1-2	Kotido	+	-	-	-	-	-	-	-
KN1-3	Kotido	-	-	-	-	-	-		
KN1-4	Kotido	+	+	-	-	-	-		
KR5-1	Kotido	-	+	-	-	-	-	-	-
KR5-2	Kotido	+	+	+	+	-	-	-	-
KR5-3	Kotido	+	+	+	-	-	-	-	-
KR5-4	Kotido	+	+	-	-	-	-	-	-
KR5-27	Kotido	+	+	-	-	-	-		
KR5-28	Kotido	+	+	-	-	-	-		
KR5-30	Kotido	+	+	+	+	-	-		
KR5-29	Kotido	+	+	+	+	-	-		
KR4-10	Kotido	+	+	-	-	-	-	-	-
KR4-11	Kotido	+	+	-	-	-	-	-	-
KR4-12	Kotido	-	-	-	-	-	-		
KR4-13	Kotido	+	+	-	-	-	-		
KR3-7	Kotido	-	+	-	-	-	-	-	-
KR3-8	Kotido	+	-	-	-	-	-	-	-
KR3-9	Kotido	-	-	-	-	-	-		
KR3-10	Kotido	+	+	-	-	-	-		
KR2-5	Kotido	-	-	+	-	-	-	-	-
KR2-6	Kotido	-	-	-	-	-	-	-	-
KR2-7	Kotido	+	-	-	-	-	-		
KR2-8	Kotido	+	+	-	-	-	-		
KR1-13	Kotido	+	+	-	-	-	-	-	-
KR1-14	Kotido	-	-	-	-	-	-	-	-
KR1-15	Kotido	-	-	-	-	-	-		
KR1-16	Kotido	+	+	-	-	-	-		
MR1-6	Moroto	+	+	-	-	-	-	-	-
MR1-7	Moroto	-	-	+	+	-	-	-	-
MR1-8	Moroto	+	+	-	-	-	-	-	-
MR1-9	Moroto	+	+	-	-	-	-	-	-
MR1-12	Moroto	-	+	-	-	-	-		
MR1-13	Moroto	+	+	-	-	-	-		
MR1-14	Moroto	+	+	-	-	-	-		
MR1-15	Moroto	+	+	-	-	-	-		
MR2-1	Moroto	+	+	-	-	-	-	-	-
MR2-2	Moroto	+	+	-	-	-	-	-	-

APPENDICES

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
MR2-3	Moroto	+	+	-	-	-	-	-	-
MR2-4	Moroto	+	+	+	+	-	-	-	-
MR2-5	Moroto	+	-	-	-	-	-		
MR2-6	Moroto	+	+	+	-	-	-		
MR2-7	Moroto	+	+	+	-	-	-		
MR2-8	Moroto	+	+	-	-	-	-		
MR3-1	Moroto	+	+	+	+	-	-	-	-
MR3-2	Moroto	-	+	-	-	-	-	-	-
MR3-3	Moroto	-	-	-	-	-	-	-	-
MR3-4	Moroto	+	+	-	-	-	-	-	-
MR3-5	Moroto	+	+	-	-	-	-		
MR3-6	Moroto	+	+	+	-	-	-		
MR3-7	Moroto	-	-	-	-	-	-		
MR3-8	Moroto	+	+	-	-	-	-		
MR4-1	Moroto	+	+	+	+	-	-	-	-
MR4-2	Moroto	+	+	+	+	-	-	-	-
MR4-3	Moroto	+	+	-	-	-	-	-	-
MR4-4	Moroto	+	+	+	+	-	-	-	-
MR4-9	Moroto	+	+	-	-	-	+		
MR4-10	Moroto	+	+	-	-	-	-		
MR4-11	Moroto	+	+	-	-	-	-		
MR4-12	Moroto	+	+	-	-	-	-		
MR5-5	Moroto	+	+	+	-	-	-	-	-
MR5-6	Moroto	+	+	-	-	-	-	-	-
MR5-7	Moroto	+	+	+	-	-	-		
MR5-8	Moroto	+	+	-	-	-	-		
MT1-1	Moroto	-	+	-	-	-	-	-	-
MT1-2	Moroto	-	+	-	-	-	-	-	-
MT1-3	Moroto	-	-	-	-	-	-		
MT1-4	Moroto	+	+	+	+	-	-		
MT2-1	Moroto	+	+	-	-	+	+	-	-
MT2-2	Moroto	+	+	-	-	-	+	-	-
MT2-3	Moroto	+	+	-	-	+	-	-	-
MT2-4	Moroto	+	+	-	-	-	+	-	-
MT2-10	Moroto	+	+	-	-	-	-		
MT2-11	Moroto	+	+	-	-	-	-		
MT2-12	Moroto	+	+	-	-	-	-		
MT2-13	Moroto	+	+	-	-	-	-		
MT3-1	Moroto	+	+	-	-	-	-	-	-

APPENDICES

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
MT3-2	Moroto	+	+	-	-	-	-	-	-
MT3-3	Moroto	+	+	-	-	+	-	-	-
MT3-4	Moroto	+	+	-	-	+	-	-	-
MT3-5	Moroto	+	+	-	-	+	-		
MT3-6	Moroto	-	+	-	-	-	-		
MT3-7	Moroto	+	+	-	-	-	-		
MT3-8	Moroto	-	-	+	-	-	-		
MT4-1	Moroto	+	+	-	-	-	-	-	-
MT4-2	Moroto	-	-	-	-	-	+	-	-
MT4-3	Moroto	+	+	-	-	-	-	-	-
MT4-4	Moroto	+	+	-	-	-	-	-	-
MT4-5	Moroto	+	+	-	-	+	+		
MT4-6	Moroto	-	+	-	-	-	-		
MT4-7	Moroto	-	-	-	-	-	-		
MT4-8	Moroto	+	+	-	-	-	-		
MT5-5	Moroto	+	+	-	-	-	-	-	-
MT5-6	Moroto	+	+	-	-	-	-	-	-
MT5-7	Moroto	+	+	-	-	-	-		
MT5-8	Moroto	+	+	-	-	-	-		
KN1-5	Kotido	+	+	-	-	-	-	-	-
KN1-6	Kotido	-	-	-	-	-	-	-	-
KN1-7	Kotido	+	+	-	-	-	-		
KN1-8	Kotido	+	+	-	-	-	-		
KN2-5	Kotido	+	+	-	-	-	-	-	-
KN2-6	Kotido	-	+	-	-	-	-	-	-
KN2-7	Kotido	-	-	-	-	-	-	-	-
KN2-8	Kotido	+	+	-	-	-	-	-	-
KN2-9	Kotido	-	+	-	-	-	-		
KN2-10	Kotido	+	+	-	-	-	-		
KN2-11	Kotido	+	+	-	-	-	-		
KN2-12	Kotido	+	+	-	-	-	-		
KN3-5	Kotido	+	+	-	-	-	-	-	-
KN3-6	Kotido	+	+	-	-	-	-	-	-
KN3-7	Kotido	+	+	-	-	-	-	-	-
KN3-8	Kotido	-	+	-	-	-	-	-	-
KN3-9	Kotido	+	+	-	-	-	-		
KN3-10	Kotido	+	-	-	-	-	-		
KN3-11	Kotido	-	-	-	-	-	-		
KN3-12	Kotido	-	-	-	-	-	-		

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
KN5-8	Kotido	+	+	+	+	-	-	-	-
KN5-9	Kotido	+	+	-	-	-	+	-	-
KN5-10	Kotido	+	+	-	-	-	-	-	-
KN5-11	Kotido	+	+	+	+	-	-	-	-
KN5-12	Kotido	-	-	-	-	-	-		
KN5-13	Kotido	+	+	-	-	-	-		
KN5-14	Kotido	+	+	+	+	-	-		
KN5-15	Kotido	+	+	-	-	-	-		
KN6-3	Kotido	+	+	-	-	-	+	-	-
KN6-4	Kotido	+	+	-	-	-	-	-	-
KN6-5	Kotido	+	+	-	-	-	-	-	-
KN6-6	Kotido	-	-	-	-	-	-	-	-
KN6-7	Kotido	-	+	-	-	-	-		
KN6-8	Kotido	-	-	-	-	-	-		
KN6-9	Kotido	+	+	-	-	-	-		
KN6-10	Kotido	-	-	-	-	-	-		
KR1-1	Kotido	+	+	-	-	-	-	-	-
KR1-2	Kotido	+	+	-	-	-	-	-	-
KR1-3	Kotido	+	+	+	-	-	-	-	-
KR1-4	Kotido	+	+	+	+	-	-	-	-
KR1-5	Kotido	+	+	-	-	-	-		
KR1-6	Kotido	+	+	-	-	-	-		
KR1-7	Kotido	-	+	-	-	-	-		
KR1-8	Kotido	+	+	-	-	-	-		
KR2-1	Kotido	+	+	-	-	-	-	-	-
KR2-2	Kotido	+	+	-	-	-	-	-	-
KR2-3	Kotido	-	+	-	-	-	-	-	-
KR2-4	Kotido	-	-	-	-	-	-	-	-
KR2-9	Kotido	+	+	-	-	-	-		
KR2-10	Kotido	+	+	-	-	-	-		
KR2-11	Kotido	+	+	-	-	-	-		
KR2-12	Kotido	-	+	-	-	-	-		
KR3-3	Kotido	+	+	-	-	-	-	-	-
KR3-4	Kotido	+	+	-	-	-	-	-	-
KR3-5	Kotido	+	+	-	-	-	-	-	-
KR3-6	Kotido	-	-	-	-	-	-	-	-
KR3-11	Kotido	+	+	-	-	-	-		
KR3-12	Kotido	+	-	-	-	-	-		
KR3-13	Kotido	+	+	+	-	-	-		

Cattle ID	District	*A. marginale*		*A. centrale*		*T. parva*		*E. ruminantium*	
		RLB	qPCR	RLB	qPCR	RLB	qPCR	RLB	qPCR
KR3-14	Kotido	+	+	-	-	-	-		
KR4-2	Kotido	-	-	+	-	-	-	-	-
KR4-3	Kotido	+	+	+	+	-	-	-	-
KR4-4	Kotido	-	-	-	-	+	-	-	-
KR4-5	Kotido	+	+	-	-	-	-	-	-
KR4-6	Kotido	+	+	-	-	-	-		
KR4-7	Kotido	+	-	-	-	-	-		
KR4-8	Kotido	+	+	-	-	-	-		
KR4-9	Kotido	+	+	-	-	-	-		
KR5-5	Kotido	-	-	-	-	-	-	-	-
KR5-6	Kotido	+	+	+	-	-	-	-	-
KR5-7	Kotido	-	-	-	-	-	-		
KR5-8	Kotido	-	+	-	-	-	-		

Appendix 4: Table showing the detection of tick-borne pathogens in blood samples of 240 cattle using genus- and species-specific probes that were covalently linked to a Biodyne C blotting membrane in a reverse line blot hybridisation assay. The samples were collected from 20 herds in two districts of Karamoja Region, Uganda, from November 2013 through January 2014. The results are indicated as '+' for positive and '-' for negative. Genus-specific probes: E/A, *Ehrlichia*/*Anaplasma*; T/B, *Theileria*/*Babesia*; T, *Theileria*; B1, *Babesia* 1; B2, *Babesia* 2.
Species-specific probes: A cent, *Anaplasma centrale*; A. marg, *Anaplasma marginale*; A. phag, *Anaplasma phagocytophilum*; A. bov, *Anaplasma bovis*; A. sp. Om, *Anaplasma* species Omatjenne; B. big, *Babesia bigemina*; T. mut, *Theileria mutans*; T. pv, *Theileria parva*; T. tauro, *Theileria taurotragi*; T. vel: *Theileria velifera*.

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MR1-1	+	+	+	-	-	-	+	+	+	+	-	+	-	-	+
Moroto	MR1-2	+	+	+	+	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR1-3	+	-	+	-	-	+	+	+	+	+	+	+	-	-	-
Moroto	MR1-5	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR2-18	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR2-19	+	+	-	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MR2-20	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR2-21	+	+	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MR3-14	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR3-15	+	+	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MR3-16	+	+	+	-	-	+	+	+	+	+	-	+	-	-	-
Moroto	MR3-17	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+
Moroto	MR4-5	+	-	+	-	-	+	+	+	+	-	-	+	-	-	-
Moroto	MR4-6	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR4-7	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MR4-8	+	-	+	-	-	+	+	+	+	+	+	+	-	-	+
Moroto	MR5-1	+	+	+	-	-	-	+	+	+	-	-	+	-	-	+
Moroto	MR5-2	+	+	+	-	-	-	+	+	-	-	-	+	-	-	-
Moroto	MR5-3	+	+	+	-	-	+	+	+	+	-	+	+	-	-	+
Moroto	MR5-4	+	+	+	-	-	+	+	+	+	+	+	+	-	-	+
Moroto	MR5-14	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-
Moroto	MR5-18	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR5-19	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR5-23	+	+	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT5-1	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT5-2	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-
Moroto	MT5-3	+	+	+	-	-	+	+	+	+	+	-	+	-	-	-
Moroto	MT5-4	+	+	-	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT4-9	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MT4-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT4-11	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Moroto	MT4-12	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT3-17	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Moroto	MT3-18	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MT3-19	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT3-20	+	-	+	-	-	-	+	+	+	-	-	+	-	-	+
Moroto	MT2-6	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Moroto	MT2-7	+	-	+	-	-	+	+	+	-	-	-	-	-	-	+

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MT2-8	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+
Moroto	MT2-9	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-27	+	-	+	-	-	-	+	+	-	-	-	-	-	-	+
Moroto	MT1-30	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-29	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-28	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-5	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT1-6	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-7	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MT1-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MT5-17	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT5-18	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-
Moroto	MT5-19	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT5-20	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN6-23	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN6-24	+	-	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KN6-25	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN6-27	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN2-1	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KN2-2	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KN2-3	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KN2-4	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN5-4	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Kotido	KN5-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN5-6	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN5-7	+	+	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN1-9	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN1-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN1-11	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KN1-12	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN3-1	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN3-2	+	-	+	-	-	+	+	+	+	-	-	+	-	-	-
Kotido	KN3-3	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN3-4	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN1-1	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KN1-2	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN1-3	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN1-4	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR5-1	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR5-2	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR5-3	+	+	+	-	-	+	+	+	-	-	-	-	-	-	+
Kotido	KR5-4	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KR5-27	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Kotido	KR5-28	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
Kotido	KR5-30	+	+	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR5-29	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Kotido	KR4-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-11	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR4-12	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR4-13	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR3-7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kotido	KR3-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR3-9	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+
Kotido	KR3-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR2-5	+	+	-	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR2-6	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+
Kotido	KR2-7	+	-	+	-	-	-	+	+	+	-	-	+	-	-	+
Kotido	KR2-8	+	-	+	-	-	-	+	+	-	-	-	-	-	-	+
Kotido	KR1-13	+	-	+	-	-	-	+	+	+	-	-	+	-	-	+
Kotido	KR1-14	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KR1-15	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR1-16	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Moroto	MR1-6	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR1-7	+	+	-	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR1-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR1-9	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR1-12	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+
Moroto	MR1-13	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR1-14	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MR1-15	+	-	+	-	-	+	+	+	+	-	-	+	-	-	+
Moroto	MR2-1	+	-	+	-	-	+	+	+	-	-	-	-	-	-	+
Moroto	MR2-2	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Moroto	MR2-3	+	-	+	-	+	+	+	+	+	-	-	+	-	-	+
Moroto	MR2-4	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR2-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR2-6	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR2-7	+	+	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR2-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR3-1	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR3-2	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR3-3	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR3-4	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MR3-5	+	-	+	-	-	+	+	+	+	+	-	+	-	-	-
Moroto	MR3-6	+	+	+	-	-	+	+	+	+	-	+	+	-	-	-
Moroto	MR3-7	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+
Moroto	MR3-8	+	-	+	-	-	+	+	+	+	+	-	+	-	-	+
Moroto	MR4-1	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR4-2	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+
Moroto	MR4-3	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR4-4	+	+	+	-	-	+	+	+	+	-	+	+	-	-	+
Moroto	MR4-9	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR4-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MR4-11	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR4-12	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Moroto	MR5-5	+	+	+	-	-	+	+	+	+	-	-	+	-	-	-
Moroto	MR5-6	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MR5-7	+	+	+	-	-	-	+	+	+	-	-	+	-	-	+
Moroto	MR5-8	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-1	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Moroto	MT1-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moroto	MT1-3	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT1-4	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT2-1	+	-	+	-	-	-	+	+	-	-	-	-	+	-	-
Moroto	MT2-2	+	-	+	-	-	-	+	+	+	-	-	+	-	-	-
Moroto	MT2-3	+	-	+	-	-	+	+	+	+	-	+	+	+	-	+
Moroto	MT2-4	+	-	+	-	-	+	+	+	-	-	-	-	-	-	+
Moroto	MT2-10	+	-	+	-	+	+	+	+	-	-	-	+	-	-	-
Moroto	MT2-11	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT2-12	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT2-13	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+
Moroto	MT3-1	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT3-2	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+
Moroto	MT3-3	+	-	+	-	-	+	+	+	+	-	-	+	+	-	+
Moroto	MT3-4	+	-	+	-	+	+	+	+	-	-	-	+	+	-	+
Moroto	MT3-5	+	-	+	-	-	+	+	+	+	-	-	+	+	-	+

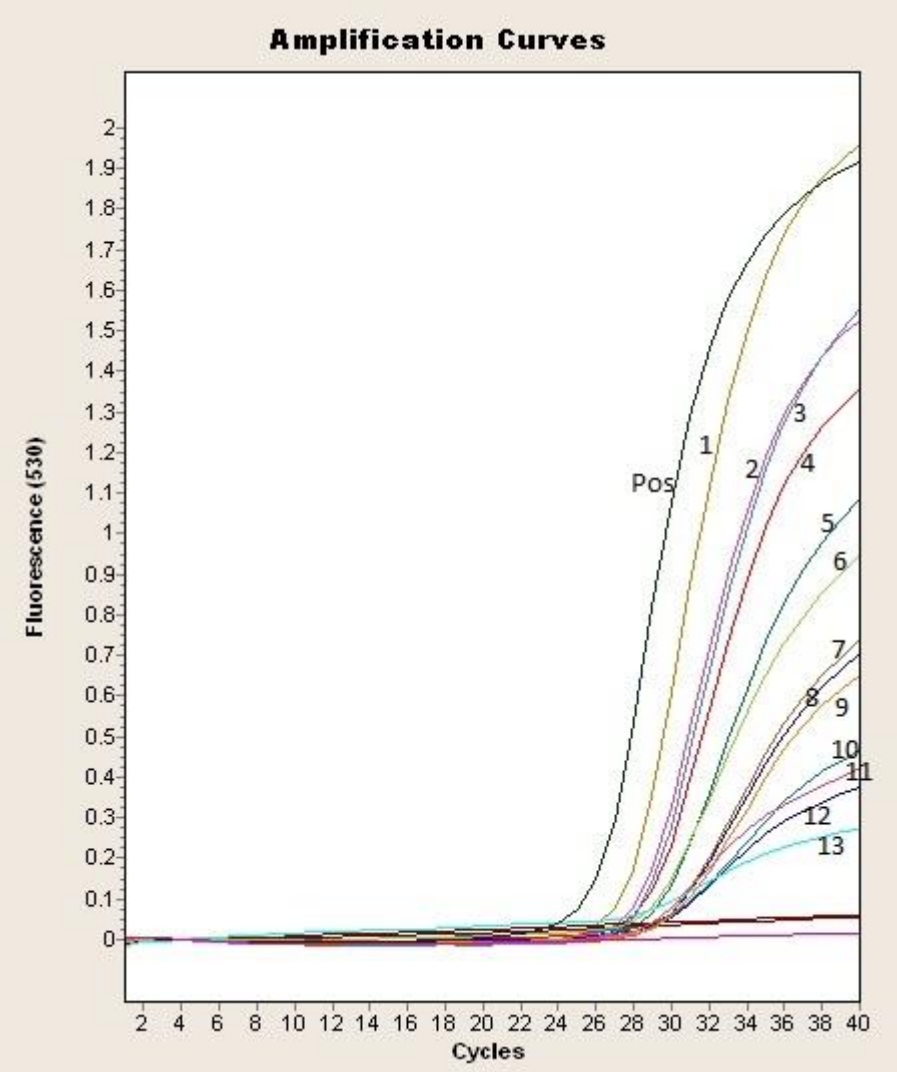
		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Moroto	MT3-6	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+
Moroto	MT3-7	+	-	+	-	-	-	+	+	+	-	-	+	-	-	-
Moroto	MT3-8	+	+	-	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT4-1	+	-	+	-	+	+	+	+	-	-	-	+	-	+	+
Moroto	MT4-2	+	-	-	-	+	+	+	+	+	-	-	+	-	-	+
Moroto	MT4-3	+	-	+	-	+	+	+	+	+	-	-	+	-	-	+
Moroto	MT4-4	+	-	+	-	+	+	+	+	-	-	-	+	-	-	+
Moroto	MT4-5	+	-	+	-	-	+	+	+	-	-	-	+	+	-	-
Moroto	MT4-6	+	-	-	-	-	+	+	+	-	-	-	-	-	+	-
Moroto	MT4-7	+	-	-	-	+	+	+	+	-	-	-	-	-	-	+
Moroto	MT4-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT5-5	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Moroto	MT5-6	+	-	+	-	+	-	+	+	-	-	-	+	-	-	+
Moroto	MT5-7	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Moroto	MT5-8	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN1-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN1-6	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Kotido	KN1-7	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN1-8	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN2-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN2-6	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN2-7	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN2-8	+	-	+	-	-	-	+	+	+	-	+	+	-	-	-

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Kotido	KN2-9	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN2-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN2-11	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN2-12	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN3-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN3-6	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN3-7	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN3-8	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN3-9	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN3-10	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN3-11	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KN3-12	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN5-8	+	+	+	-	-	-	+	+	+	-	-	+	-	-	+
Kotido	KN5-9	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN5-10	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN5-11	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN5-12	+	-	-	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KN5-13	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN5-14	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KN5-15	+	-	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KN6-3	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN6-4	+	-	+	-	-	-	+	+	+	-	-	+	-	-	+
Kotido	KN6-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-

		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Kotido	KN6-6	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KN6-7	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KN6-8	+	-	-	-	+	+	+	+	-	-	-	+	-	-	+
Kotido	KN6-9	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KN6-10	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR1-1	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+
Kotido	KR1-2	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR1-3	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
Kotido	KR1-4	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+
Kotido	KR1-5	+	-	+	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KR1-6	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR1-7	+	-	-	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR1-8	+	-	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KR2-1	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR2-2	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR2-3	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR2-4	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR2-9	+	-	+	-	-	+	+	+	+	-	-	+	-	-	+
Kotido	KR2-10	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR2-11	+	-	+	-	-	-	+	+	+	-	-	-	-	-	+
Kotido	KR2-12	+	-	-	-	+	+	+	+	-	-	-	+	-	-	+
Kotido	KR3-3	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR3-4	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+

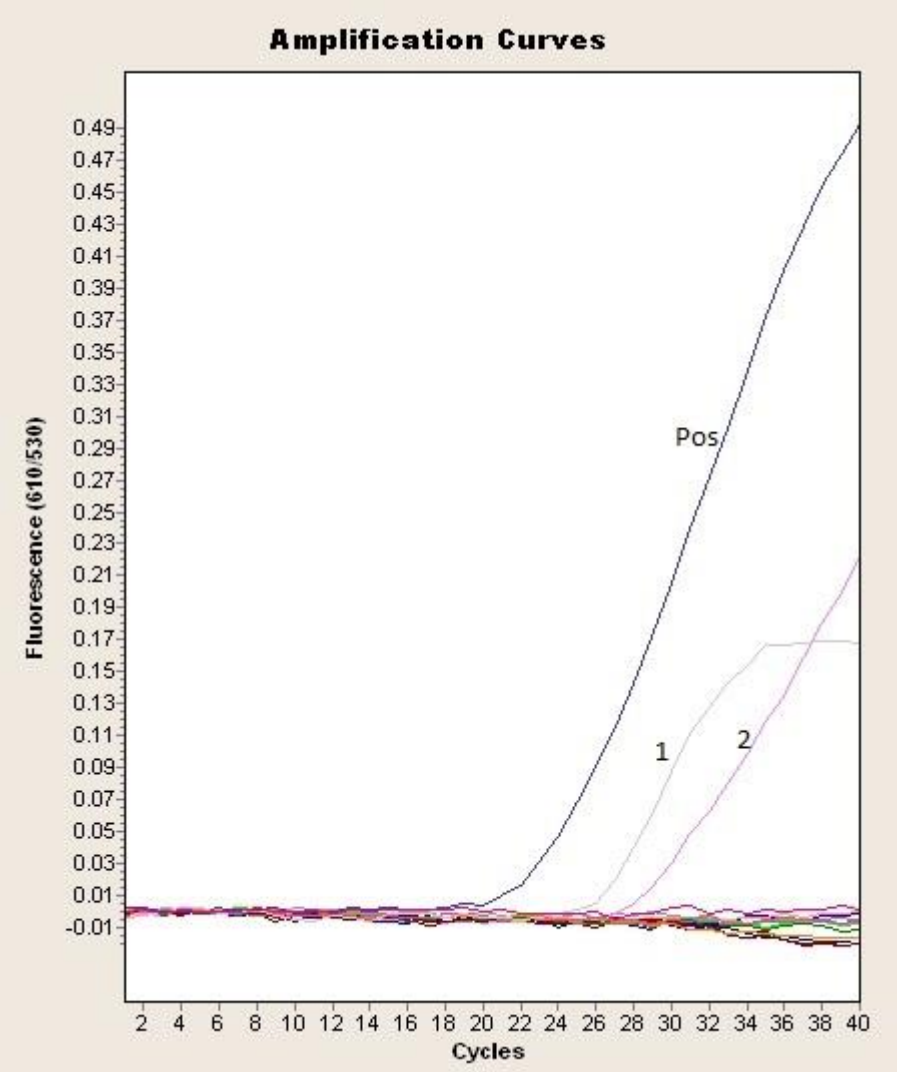
		Reverse line blot hybridisation assay genus- and species-specific probes														
District	**Cattle ID**	**E/A**	**A. cent**	**A. marg**	**A. phag**	**A. bov**	**A. sp. Om**	**T/B**	**T**	**B1**	**B2**	**B. big**	**T. mut**	**T. pv**	**T. tauro**	**T. vel**
Kotido	KR3-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR3-6	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+
Kotido	KR3-11	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR3-12	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR3-13	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR3-14	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-2	+	+	-	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-3	+	+	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-4	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+
Kotido	KR4-5	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-6	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+
Kotido	KR4-7	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
Kotido	KR4-8	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR4-9	+	-	+	-	-	-	+	+	-	-	-	+	-	-	+
Kotido	KR5-5	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-
Kotido	KR5-6	+	+	+	-	-	+	+	+	+	-	+	+	-	-	-
Kotido	KR5-7	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-
Kotido	KR5-8	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+

Appendix 5: An amplification plot of fluorescence signal versus cycle number during the detection of *Anaplasma marginale* in blood samples collected from cattle in Karamoja Region of Uganda, using TaqMan hydrolysis probes in a duplex quantitative real-time PCR specific for *A*. *marginale* (*msp1β* gene) and *A*. *centrale* (*groEL* gene) as previously described [Decaro et al., 2008].



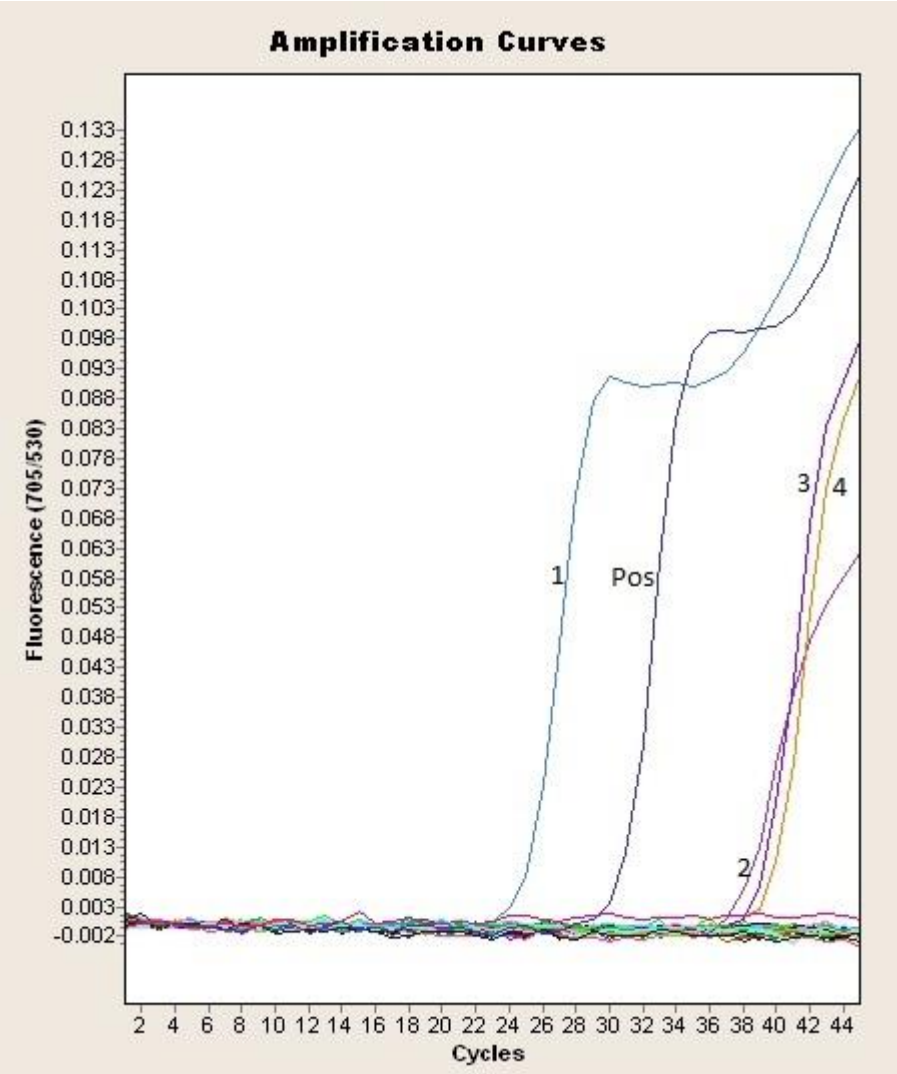
The C_t (threshold cycle) is the intersection between the amplification curves and the threshold line (running horizontally). Reactions are characterised by the point in time during cycling when amplification of a PCR product is first detected. The C_t is a relative measure of the concentration of target in the PCR reaction. Lower C_t values correspond to higher starting copy number of the nucleic acid target. There was no amplification for negative samples and the negative control (lines running horizontally). The amplification curves correspond to the samples: Pos, positive control (from cattle in Mnisi community area, Mpumalanga, South Africa; confirmed positive by qPCR and species-specific gene sequence analysis); 1, MT2-9; 2, MT3-18; 3, MT5-19; 4, MT4-12; 5, MT3-19; 6, KN6-27; 7, MT5-17; 8, MT3-20; 9, MT5-20; 10, MT1-27; 11, KN6-23; 12, MT4-10; and 13, MT5-18.

Appendix 6: An amplification plot of fluorescence signal versus cycle number during the detection of *Anaplasma centrale* in blood samples collected from cattle in Karamoja Region of Uganda, using TaqMan hydrolysis probes in a duplex quantitative real-time PCR for *A. marginale* (*msp1β* gene) and *A. centrale* (*groEL* gene) as previously described [Decaro et al., 2008].



There was no amplification for negative samples and the negative control (lines running horizontally). The amplification curves correspond to the samples: Pos, positive control (*A. centrale* vaccine strain from Onderstepoort Biological Products, Pretoria, South Africa; confirmed positive by qPCR and species-specific gene sequence analysis); 1, KR1-1; 2, KR4-3.

Appendix 7: Amplification of *Theileria parva* DNA from cattle from Karamoja Region, Uganda, using a hybridisation probe-based real-time PCR specific for *T. parva* (18S rRNA gene) as previously described (Sibeko et al., 2008).



There was no amplification for negative samples and the negative control (lines running horizontally). The amplification curves correspond to the samples: 1, MT2-1; pos, positive control (a known infected buffalo, KNP102, Onderstepoort Veterinary Institute); 2, MR4-9; 3, MT2-4; 4, MT2-2

Appendix 8: Letter approval for the PhD study 'Diversity and epidemiological dynamics of tick-borne haemoparasite infections amongst transhumant zebu cattle in Karamoja Region, Uganda' issued by the Animal Ethics Committee, University of Pretoria, South Africa

Appendix 9: Letter approval for the PhD study 'Diversity and epidemiological dynamics of tick-borne haemoparasite infections amongst transhumant zebu cattle in Karamoja Region, Uganda' issued by the Research Ethics Committee, Faculty of Humanities, University of Pretoria, South Africa

Appendix 10: Permission to do research in terms of Section 20 of the Animal Diseases ACT, 1984 (ACT No. 35 of 1984) for the research project 'Diversity and epidemiological dynamics of tick-borne haemoparasite infections amongst transhumant zebu cattle in Karamoja Region, Uganda', issued by the Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa

Appendix 11: Veterinary Import Permit for the transportation of 532 bovine serum samples and 400 FTA filter papers containing bovine blood spots, from Uganda to South Africa, for the research project 'Diversity and epidemiological dynamics of tick-borne haemoparasite infections amongst transhumant zebu cattle in Karamoja Region, Uganda' issued by the Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa

LIST OF PUBLICATIONS

C. Byaruhanga, N.E. Collins, D. Knobel, M.E. Chaisi, I. Vorster, H.C. Steyn, M.C. Oosthuizen. Molecular investigation of tick-borne haemoparasite infections among transhumant zebu cattle in Karamoja Region, Uganda. Veterinary Parasitology: Regional Studies and Reports 3-4, 27-35.

Byaruhanga, C., Collins, N.E., Knobel, D., Oosthuizen, M.C. 2015. Endemic status of tick-borne infections and tick species diversity among transhumant zebu cattle in Karamoja Region, Uganda: support for control approaches. Veterinary Parasitology: Regional Studies and Reports, 1-2, 21-30.

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, 287-297.