Tick species composition and associated haemoparasites of cattle in a semi-arid area of Karamoja, Uganda

By

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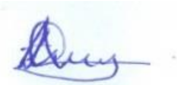
October 2019

DECLARATION

I declare that this dissertation, which I hereby submit for the degree **Master of Science** in Tropical Animal Health to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science University of Pretoria, is my original work and has not been submitted by me for a degree to any other University or institution.

I carried out sample collection with support from the veterinary officers and community animal health workers of Moroto and Kotido. Laboratory work was also done with technical guidance from Ms Milana Troskie and Ms Ilse Vorster from the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science University of Pretoria.



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Patience Christine Akure

October 2019

DEDICATION

To the highest God, my Redeemer, my Refuge, my Provider, my greatest help and forever faithful God, and to the memory of my late father Peter Hosea Akure (R.I.P)

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LIST OF ABBREVIATIONS

°C	degrees celsius
µl	microlitre
CAT	card agglutination test
CFT	complement fixation test
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxy-ribonucleic acid
EDAC	1-ethyl-3- (3-dimethyl-amino-propyl) carbodiimide
ECF	East Coast Fever
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization
IFAT	indirect fluorescent antibody test
IICD	Institute for International Cooperation and Development
ITM	Institute of Tropical Medicine
KCL	potassium chloride
MAAIF	Ministry of Agriculture Animal Industry and Fisheries
$MgCl_2$	magnesium chloride
ml	millilitre
mM	millimolar
NaOH	sodium hyroxide
PCR	polymerase chain reaction
qPCR	quantitative real-time polymerase chain reaction
RLB	reverse line blot
rRNA	ribosomal ribonucleic acid
Rpm	rotations per minute
SDS	sodium dodecyl sulphate
SNV	Netherlands Development organization

TBD	tick-borne disease
UBOS	Uganda Bureau of Statistics
USA	United States of America

DISSERTATION SUMMARY

Tick species composition and associated haemoparasites of cattle in a semi-arid area of Karamoja, Uganda

Candidate: Patience Christine Akure

Supervisor: Dr Hein Stoltsz

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Department: Veterinary Tropical Diseases

Degree: MSc (Tropical Animal Health)

Ticks and tick-borne diseases (TBDs) cause significant losses, through their effects on health, production of animals and humans worldwide. Notably, the countries located within the tropics and subtropics such as Uganda are the most affected due to abundance and distribution of the tick vector. Unfortunately, there is little data in Karamoja Region regarding tick species and the pathogens they transmit. Therefore, a cross-sectional study was undertaken to determine the various tick species, and to detect the tick-borne pathogens within the ticks collected from cattle in Karamoja Region, northeastern Uganda. Between June 2017 and early September 2017 (wet season), a total of 4,897 ixodid ticks were collected from 100 cattle in 20 purposively-selected herds. Three genera of ticks, namely *Amblyomma* (96.8%), *Hyalomma* (0.6%) and *Rhipicephalus* (2.6%) were identified. From the ticks collected, the most dominant species was *A. lepidum* (93.85%), followed by *A. variegatum* (2.0%), *R. evertsi evertsi* (1.0%) and *A. gemma* (0.98%). Tick species that have not been reported in recent studies in Uganda were found amongst cattle in Karamoja, and these were *R. pravus*, *R. praetextatus* and *R. turanicus.* A representative number of ticks, from each tick species identified in the present study were placed in pools of 1 to 10. Subsequently, a reverse line blot (RLB) hybridization assay was performed to screen for the presence of tick-borne pathogens. Out of the 40 tick pools, 30 (75%) were positive for tick-borne pathogens of the genera *Ehrlichia*, *Anaplasma*, *Babesia* and *Theileria*. The RLB assay results showed that 57% (n=17) of the tick pools were positive for single infections, while 43% (n=13) had mixed infections. The most frequently detected tick-borne pathogens were

T. parva (10 pools), *T*. *velifera* (10 pools), *T*. *mutans* (9 pools) and *Theileria* sp. (sable) (5 pools). Other pathogens detected were *E. ruminantium*, *B*. *microti, B*. *rossi*, *T*. *separata* and *B*. *bigemina*. The tick-borne species *B*. *microti*, *B. rossi*, *Theileria* sp. (sable) and *T*. *separata* are not common in cattle, or not known to infect cattle, but were detected from the ticks collected. The detection of *B*. *microti* in this study may point to incidental infections with implications for human health. There could have also been a possibility of cross reactions during the RLB analysis leading to the detection of *B. microti* in this study. These findings provide knowledge of the distribution of ticks and epidemiology of tick-borne pathogens in cattle and may provide support for control of TBDs and improve cattle productivity.

Key words: Tick; tick-borne pathogen; cattle; reverse line blot; Karamoja; Uganda

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CHAPTER ONE

INTRODUCTION

1.1 Background

Ticks are important ectoparasites of animals and humans (de la Fuente et al., 2008). They are responsible for economic losses and impact the productivity of cattle, including local breeds (Okello et al., 2003). Ticks are cosmopolitan but are more prevalent in tropics and subtropics where the warmth and humidity play an important role for their survival across the entire year, as opposed to the winter season in the template climates (Olwoch et al., 2009). The economic importance of ticks is derived from their ability to transmit several protozoal, bacterial, fungal and viral pathogens that affect animals worldwide. Furthermore, some of the pathogens are of zoonotic significance (de la Fuente et al., 2008). Ticks also cause severe detrimental conditions like irritation, allergy, paralysis, and destruction to hides, skins, teats and genitalia. Other effects are lameness, and the tick-bite wounds can become infected (Jongejan and Uilenberg, 2004). Additionally, ticks suck large volumes of blood from their hosts, leading to anaemia.

Widespread occurrence of tick vectors and tick-borne diseases (TBDs) has greatly affected the livestock industry in many developing countries (Brown, 1997; Perry and Randolph, 1999). Tick-borne diseases are associated with high morbidity and mortality in animals globally (Dantas et al., 2014; Dantas et al., 2015), and the risk of tick infestation and TBDs was estimated at 80% of the cattle populations (FAO, 2004). A study by De Castro (1997) showed that the annual global loss attributed to ticks and TBDs was US$ 13.9 to US$ 18.7 billion (De Castro, 1997). In Uganda, it was estimated that 75.4% of the losses to cattle farmers were due to ticks and TBDs (Ocaido et al., 2009b), and the cost of controlling ticks and TBDs amounted to 85% of disease control costs (Ocaido et al., 2009a).

The tick species reported in Uganda include *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus*, *Rhipicephalus evertsi evertsi*, *Ambylomma variegatum* and *Hyalomma* species (Kaiser et al., 1982; Rubaire-Akiiki et al., 2004; Magona et al., 2011; Byaruhanga et al., 2015a; Tayebwa et al., 2018). Kaiser et al. (1982) reported the occurrence of *Rhipicephalus*

simus and *Rhipicephalus compositus* in southern Uganda. More recently, tick species *Amblyomma lepidum*, *Amblyomma gemma* and *Rhipicephalus pulchellus* were found amongst cattle in Karamoja Region, and these had not been reported in previous studies from other parts of Uganda (Byaruhanga et al., 2015a). The most prevalent and most important protozoal diseases amongst cattle in Uganda are East Coast fever and babesiosis, while rickettsial diseases are anaplasmosis and heartwater (Uilenberg, 1992; Uilenberg, 1994).

East Coast fever caused by *Theileria parva* and transmitted by *R. appendiculatus* is considered the most important TBD of cattle in Uganda. The disease is distributed in eastern, central and southern Africa (Thumbi et al., 2013; Kabi et al., 2014; Muhanguzi et al., 2014). Anaplasmosis is mainly caused by *Anaplasma marginale*, and is the most predominant rickettsial tick-borne pathogen of cattle globally (Aubry and Geale, 2011). *Anaplasma centrale* causes a milder form of anaplasmosis and is used as a live vaccine to reduce clinical disease in cattle (Bell-Sakyi et al., 2015). In endemic areas, anaplasmosis is associated with high morbidity and mortality amongst cattle (Aubrey and Gaele, 2011); the disease is prevalent in various parts of Uganda (Magona et al., 2011; Byaruhanga et al., 2015c). Heartwater (cowdriosis) is caused by *Ehrlichia ruminantium* (Dumler et al., 2001), which is transmitted by *Amblyomma* ticks. The disease is responsible for high production and economic losses to the livestock industry and causes high death rates (reaching 80%) in susceptible hosts (Thumbi et al., 2013). *Amblyomma variegatum* is the species that transmits *E*. *ruminantium* in Uganda (Byaruhanga et al., 2015a; Tayebwa et al., 2018). *Babesia bovis* and *Babesia bigemina* cause babesiosis, which is one of the most devastating diseases of cattle, associated with high mortality due to the acute nature of the disease. *Babesia bovis* is more virulent than *B*. *bigemina*, and causes neurological signs due to the ability of the parasite to sequestrate in the brain capillaries (McCosker, 1981). The main tick vector of *B*. *bigemina* and *B*. *bovis* is *Rhipicephalus microplus*, which is widely distributed in the tropical and sub-tropical areas. In Uganda, *B. bigemina* is the known cause of bovine babesiosis, transmitted by *R*. *decoloratus* (Byaruhanga et al., 2016; Tayebwa et al., 2018). *Babesia bovis* on the other hand has never been reported in the country. Babesiosis is less severe in younger animals due to innate acquired resistance following infection of the dam. Once an animal is infected, it can become a carrier for life and the acquired immunity may

protect the animals against subsequent infections for babesiosis (Zintl et al., 2005).

Karamoja Region in northeastern Uganda is a pastoral area, and livestock keeping is the main source of livelihood for the communities (IICD, 2010). Cattle in Karamoja are important for cultural, social and economic reasons, and the animals contribute to people's self-worth, and the status in the communities is linked to livestock ownership. Noteworthy, the population of cattle in Karamoja Region outnumbers the population of people (MAAIF, 2003). In recent years, ticks and TBDs have become a major problem to livestock in Karamoja, which has adversely affected livestock productivity (Field veterinarians, personal communication). In Karamoja, seasonal movement of cattle in the dry season, in search of pastures and water, and communal grazing, increases exposure of the animals to ticks and TBDs (Ayana et al., 2013). Despite the high tick burden amongst cattle, tick control practices are often inappropriate, including hand picking and use of inadequate acaricides, and this tends to leave high tick numbers on cattle (Byaruhanga et al., 2015a).

1.2 Problem statement and justification

In a recent study, Byaruhanga et al. (2015a) investigated the tick species diversity and detected tick-borne parasites from cattle blood samples from the Karamoja Region during the dry season (November 2013 through January 2014). It is therefore imperative to follow up with a similar study in the wet season (in my case: June through early September 2017), but this time detecting pathogens in ticks, instead of blood, so as to obtain more comprehensive information about the epidemiology of TBDs. With more comprehensive information, the likelihood of TBDs occurrence or establishment of endemic stability can be estimated, and this could guide effective control measures against tick and tick-borne pathogens. In Uganda, there is generally scanty information about the occurrence and possible transmission of protozoal and ehrlichial pathogens by ticks. In one study, Nakayima et al. (2014) collected ticks from cattle in Tororo, Soroti and Amuria Districts in eastern Uganda, and used nucleic acid-based methods to identify the pathogens in the ticks; however, no study of that kind has been conducted in Karamoja, despite the relatively high burden of ticks reported in that region (Byaruhanga et al., 2015a). Therefore, the present study will provide valuable information regarding the tick species diversity and the tick-borne pathogens carried by the different tick species found in Karamoja Region. The findings

will be useful towards establishing ticks and TBD prevention and control measures in Karamoja Region.

1.3 Aim

To identify tick species infesting cattle and use molecular techniques to detect tick-borne parasites from ticks sampled during the rainy season in Karamoja Region, Uganda.

1.4 Objectives

(i) Collect representative samples of ticks from cattle (focusing on predilection sites) in four different sub-counties in two districts in the Karamoja Region of Uganda during the wet season and identify them to establish their diversity.

(ii) Select a representative sample of each tick species from each of the four sites, extract DNA and subject it to the reverse line blot hybridization assay to diagnose the tick-borne pathogens in the tick population.

1.5 Research Questions

Which tick species are present amongst cattle in Karamoja Region during the wet season?

Which tick-borne pathogens are present in ticks found on cattle in Karamoja Region?

CHAPTER TWO

LITERATURE REVIEW

2.1 Geographical and climatic features of Uganda

Uganda, “the Pearl of Africa”, is an East African country crossed by the equator, and lies between latitude 4°12' N and 1°29' S, and longitudes 29°34' E and 35°0' E (UBOS, 2014). The country covers a total land area of 241,551 sq. kms with most of the topography occurring as a plateau, small hills, valleys and extensive savannah plains. Most of the country’s land lies at an altitude above 900 m and generally has a slope from the south to the north, with the lowest recorded point (Lake Albert) being 620 m above sea level while the highest (Mt Rwenzori) lies at 5,110 m (UBOS, 2014). The temperatures range from 14˚C to 32°C and the precipitation ranges from 750 to 1,500 mm in the northeast part of the country, and rainfall areas of Lake Victoria, highlands around Mt. Elgon in the east and Mt. Rwenzori in the southwest, respectively. Uganda’s climate is mainly tropical with bi-annual rainy and dry seasons.

Figure 1. Map of Africa showing the location of Uganda (shaded in red)**.**
Source: Researchgate.net

2.2 Livestock production in Uganda

Livestock production is critical for the economic growth of Uganda. A report by MAAIF (2010) showed that livestock and its products provide income and high value protein to 70% of Uganda's population (MAAIF, 2011). More to that, statistics have shown the households involved in livestock production tend to be less poor than those that keep no livestock (UBOS, 2007). The cattle population in Uganda is estimated to be 14.2 million (UBOS, 2017). Several challenges face the livestock industry, and these include diseases, insufficient advisory and extension services, inadequate research and fluctuating market prices that discourage the farmers (MAAIF, 2010).

Livestock production systems in Uganda are stratified according to the agro-ecological zones and the socio-economic setting. According to Mbuza (1991), two main types of systems exist in Uganda namely; the traditional system (free range) that requires minimal inputs and

yields, marginal outputs, and the intensive farming systems which require investment capital in infrastructure, feeds, management and treatment to maximize the production. The most common species of livestock in Uganda are cattle, sheep, goats, pigs, rabbits and poultry (Benson and Mugarura, 2013).

Uganda's cattle production comprises of a mixture of livestock systems which include:

- Agro-pastoral: is a form of mixed farming, where both livestock and crop production are practiced. At times, animals migrate in the dry season in search of water and pasture as a few animals are left behind to provide milk for home. In the settled mixed system, the herds are usually smaller and the major household food and income is cropping. There are mutual benefits from the system, where the crops benefit from the manure and animals feed on the crop residues.
- Pastoral system: this is an extensive low input-low output system in which owners graze relatively large herds of livestock including cattle, goats and sheep. Pastoral production is usually practiced in the semi-arid parts of Uganda where the pastures and water becomes scarce during the dry season or the drought spell often occurs. Overtime, this practice became rooted as a culture in some of the livestock keeping areas such as in Karamoja region. Livestock keepers in Karamoja move long distances with their animals, and they often cross borders to neighbouring districts in Uganda but also cross borders to neighbouring countries, mainly Kenya. The livestock are comprised mainly of the indigenous relatively disease resistant or tolerant breeds, and due to the challenges such as inadequate feed resources and water, the animals are usually less productive (Mahadevan and Parsons, 1970).

2.3 Karamoja Region and livestock production systems

Karamoja Region is located in northeastern Uganda, and borders Kenya to the east and South Soudan to the north (Anderson and Robinson, 2009). The region occupies approximately 27,511 km^2 (about 10% of Uganda), and is estimated to have a human population of 1,455,200 (UBOS, 2014). Presently, seven districts that include Abim, Amudat, Kaabong, Kotido, Moroto, Nakapiripirit, and Napak make up the Karamoja Region (as of June 2018). Each district is further sub-divided into sub-counties with several parishes and villages/manyattas (Mubiru, 2010). Karamoja region is the poorest in Uganda with high

illiteracy rates, low household income, food insecurity, poor infrastructure and poor health care services (Anderson and Robinson, 2009; Mubiru, 2010).

The vegetation in Karamoja is predominantly savannah grassland, with thorny plants, scattered shrubs, small trees with thickets, and patches of forest cover along the river basin (Grade' et al., 2009). The region is semi-arid with one rainy season per annum and average rainfall ranging from 500 mm to 700 mm, but up to 1,000 mm in a few areas. The dry season lasts six to seven months (commonly from mid-September to February) (Anderson and Robinson, 2009; Mubiru, 2010). Temperatures range from 15°C to 32.5°C (Mubiru, 2010).

In Karamoja, livestock production is the main source of livelihood for the communities, followed by seasonal farming of cereals. The region is characterised by three livestock production systems, namely pastoral, agro-pastoral and agricultural systems (Aklilu and Catley 2009). The common livestock kept are cattle, sheep, goats and backyard poultry. The predominant cattle type is the short-horned East African Zebu breed, which has been described as relatively tolerant to ticks and TBDs, compared to the *Bos taurus* cattle (Mattioli et al., 2000). Other adaptations of the short-horned East African Zebu include a pendulous dewlap and a thoracic hump, which apparently allows them to survive well in the semi-arid environment. The cattle in Karamoja make up a 20% of Uganda's population of cattle (UBOS, 2017). The predominant management system of cattle practiced in Karamoja is transhumant, characterized by movement of herds in search for pasture and water during the long dry spell.

Figure 2. Map of Karamoja Region, with inset location of Karamoja in Uganda. Source: Kara-Tunga (www.kara-tunga.com/karamoja-travel-information/)

The herds are organized into groups with guidance and leadership of kraal leaders (Anderson and Robinson, 2009). Movement of cattle for long distances and the communal grazing system exposes the herds to high tick burdens and also exposes them to tick-borne pathogens (Ayana et al., 2013).

2.4 Ticks of cattle

2.4.1 Classification of ticks

Ticks are obligatory blood sucking external parasite of vertebrate animals, including man, birds and reptiles. Globally, about 800 species and subspecies which belong to the Ixodidae (hard ticks) and the Argasidae (soft ticks) families are documented (Kettle, 2000).

The tick taxonomy is classified as follows;

Phylum: Arthropoda

Subphylum: Chelicerata (anterior fangs/chelicerae)

Class: Arachnida Order: Acarina Sub-order: Ixodoidea

Family (1): Argasidae (soft ticks)

Genus: *Argas, Otobius, Ornithodoros*

Family (2): Ixodidae (hard ticks)

Genus: *Amblyomma, Dermacentor, Hyalomma, Haemaphysalis, Ixodes, Margaropus, Rhipicephalus.*

2.4.2 Morphology

Soulsby (1982) and Kettle (2000) described ticks as dorsoventrally compressed arthropods without a distinct division between the head, thorax and abdomen.

2.4.3 Family Argasidae (soft ticks)

There are several species and subspecies of argasid ticks documented all over the world (Hoogstraal, 1956). Three genera of argasid ticks, which comprise 183 species and subspecies are recognized; the genera are *Argas*, *Ornithodoros*, and *Otobius*, and which are of medical and veterinary importance. Soulsby (1982) description of argasid ticks emphasized that they have a leathery integument with mouth parts situated anteriorly on the ventral surface and not visible from the dorsal side. Eyes may be present or absent. There are two pairs of eyes situated laterally in supracoxal folds in some species of *Ornithodoros* while one pair of spiracles is cited between the third and the fourth coxa. Sexual dimorphism is not marked however the female can be differentiated by the presence of a large genital opening as compared to that in males. The female Argasidae tick lays several batches of eggs that hatch into larvae which later moult into two or more nymphal stages. Larvae, nymphs and adults, depending on a specific genus, may either not feed or feed repeatedly. The adult female usually lays eggs after each blood meal except in the case of *Otobius* which does not feed on blood but rather infests nests, burrows, buildings and attach to sleeping hosts.

2.4.4 Family Ixodidae (hard ticks)

For ixodid ticks, sexual dimorphism is marked with the scutum (conscutum) covering the entire dorsum of the male and only part of the dorsum of the female ticks. The mouth parts (capitulum) project forwards and are visible from the dorsal side. Larvae have three pairs of legs while nymphs have four and they lack porose areas, scutum and genital openings (Hoogstraal, 1956). As opposed to the argasid ticks, Ixodid ticks lay their eggs in a single batch of thousands. The hatched larvae develop into nymphs then to adults, engorge and fall off the host to lay eggs and continue the lifecycle (Soulsby, 1982; Kettle, 2000). Males usually remain on the host for longer and females die a natural death after laying eggs.

Each of the tick species is adapted to its environment in order to survive. For instance, the ticks in the semi-arid areas such as Karamoja become active, feed and lay eggs at the start of the rains so that their larvae hatch in time when the humidity is still high lest they will be desiccated by heat.

2.5 Life cycle

The life cycle of argasid ticks differs considerably from that of ixodid ticks. After a blood meal, male and female argasid ticks copulate off the host, after which the female lays small batches of a few hundred eggs repeatedly and often taking off time to take a blood meal. The larvae feed on blood, with the exception of *Ornithodoros* larvae, and then moult into nymphs. The nymphs of all argasid ticks feed on blood and moult into adults after 2-4 nymphal stages.

The life cycle of ixodid ticks is classified according to their feeding behaviour on blood into three types as follows:

2.5.1 One-host ticks

One-host tick lifecycle is the least common, and is characteristic of all *Rhipicephalus* (*Boophilus*) species, namely Rhipicephalus *decoloratus, Rhipicephalus microplus*, *Rhipicephalus geigyi* and *Rhipicephalus annulatus*. The ticks lay their eggs in the environment and after several weeks of development hatch into larvae which crawl onto vegetation to wait for a host (Walker et al., 2013). The larvae remain attached on the host

and moult into nymph and later into adult. This life cycle favors a rapid multiplication of ticks within three to four weeks compared to two months for the other ticks (Walker et al., 2013). One host ticks are likely to be exposed to acaricides regularly making their control easier; however, more regular exposure of the ticks to chemicals implies that are likely to be amongst the first to become resistant to acaricides.

2.5.2 Two hosts ticks

Two-host ticks differ from the one-host ticks at the point of development from the nymph to adult. The larvae and nymph feed on the same host, and subsequently the nymph detaches from the host to moult into adult, which seeks 'another' host to engorge, mate and lay eggs. The adult male and female attached to the host copulate during the feeding process after which the engorged female drops to the ground to lay eggs and thereafter dies a natural death. Examples of ticks with this type of life cycle are *R. evertsi evertsi* and *Rhipicephalus bursa* (Kettle, 2000; Walker et al., 2013).

2.5.3 Three hosts ticks

Of the three life cycles for ticks, the three-host life cycle is the most common. The larvae attach to the host, feed, engorge and detach. They then hide in soil or vegetation and moult into nymphs which then attach to a second host, feed, engorge and drop to the ground to moult into adults. The adult male and female attach to the third host and copulate during the feeding process. The engorged females drop to the ground to lay eggs. The females after laying eggs die a natural physiological death. This life cycle is slow, taking six months to several years. Examples of this type of ticks are all *Amblyomma* spp., *Haemaphysalis* spp. and most *Rhipicephalus* and *Hyalomma* species (Soulby, 1982; Kettle, 2000; Walker et al., 2013).

2.6 Tick distribution in Africa

In Africa, the distribution of ticks depends on their adaptability to various ecological and climatic factors present in their habitat (Halpin, 1975 cited by Sower, 2002). Ticks, within their habitats, experience several environmental factors such as temperature, relative humidity and rainfall. Host density, host susceptibility and specificity, vegetation type and host grazing behavior are also important factors that affect tick distribution. For instance,

Ambylomma pomposum occurs in Angola, *Ambylomma hebraeum* occurs in most parts of southern Africa, while *A*. *variegatum* has wide geographical distribution in Africa. The latter occurs across the continent from Senegal in West Africa into Central African Republic, Southern Sudan, Ethiopia, and extends to Somalia and East African countries including Uganda (Walker and Olwage 1987; Norval et al., 1992; Walker et al., 2013). Other factors that influence tick distribution are movements of animals, tick control strategies and rainfall variation (Barre and Uilenberg, 2010). In Uganda, the ticks previously reported to infest cattle include *R*. *appendiculatus*, *R. evertsi evertsi*, *A. variegatum*, *R*. *decoloratus* and *Hyalomma* spp. (Kaiser et al., 1982; Rubaire-Akiiki et al., 2004; Magona et al., 2011; Tayebwa et al., 2018). Kaiser et al. (1982) reported the occurrence of *R*. *simus* and *R*. *compositus* in southern Uganda. Recently, Byaruhanga et al. (2015a) reported the presence of *A*. *lepidum, A. gemma* and *R. pulchellus* from cattle in Karamoja, for the first time in Uganda.

2.7 Seasonal occurrence

The seasonal temperature and humidity can be major determining factors in the way ticks adapt to different environments (Walker et al., 2013). For example, *R*. *appendiculatus* in southern Africa employs a diapause mechanisms by reducing activity at times when the conditions are hot but increases activity at the beginning of the wet season to ensure that larvae hatch towards the end of the wet season when humidity is highest (Walker et al., 2013). Mooring et al. (1994) observed that *R. appendiculatus* adult infestation on host were 2-3 times more during the high rainfall. Adult ticks may usually be available throughout the year but depending on the species, they are present more or less in the wet or dry season. The nymphs are usually present during the dry and wet seasons and the larvae are more abundant towards the peak of the wet season when humidity is still very high.

In eastern Africa, which includes Karamoja Region, generations of ticks tend to overlap and therefore all stages of ticks occur simultaneously amongst cattle (Kaiser et al., 1982; Walker et al., 2013). In addition, the non-seasonality of *R*. *appendiculatus* may also largely be explained by the absence of marked variations in day light length between the seasons in East Africa (Norval et al., 1992). Karamoja experiences cycles of drought which are a serious danger to ticks especially the questing larvae which are susceptible to fatally drying out. Like

many tick species. A previous study in the dry season in the Karamoja Region (Byaruhanga et al., 2015a) and another study in southern Uganda (Kaiser et al., 1982) showed no differential correlation pattern in the occurrence of different stages of various tick species, including *R*. *appendiculatus*. Tick species with higher adult counts also had higher numbers of larvae and nymphs (Byaruhanga et al., 2015a; Kaiser et al., 1982). The long dry season in Karamoja may therefore have no implication on the occurrence of different stages of *R*. *appendiculatus* in the region.

2.8 Economic importance of ticks

The economic importance of ticks is due to their effects on animal productivity and cost of ticks and TBD control. Tick infestation causes irritation, damage of hides and skins, and wounds which serve as good environment for bacterial and fungal infections, as well as myiasis after dipteral flies have deposited their eggs and larvae in these wounds (FAO, 1983). Since ticks are some of the largest ectoparasites, they consume large quantities of blood which may lead to anemia. It has been estimated that animals lose 1 to 3 ml of blood for every tick to complete its life cycle.

Norval et al. (1992) documented that about 80% of the world's cattle population is at risk of ticks and TBDs, resulting to US$ 7,000 million in losses globally. In Tanzania, Kivaria (2006) estimated a US $364 million total annual national loss attributed to TBDs accompanied by 1.3 million cattle killed by TBDs. In Uganda, TBDs accounted for 75.4% loss in cattle production (Ocaido et al., 2009b) and 85% of the total disease control budget (Ocaido et al., 2009a).

2.9 Important tick-borne diseases of cattle

2.9.1 Theileriosis

Aetiology and transmission

Theileriosis is caused by a group of protozoan parasites of genus *Theileria.* There are several species of *Theileria* that infect cattle namely; *Theileria parva, Theileria annulata*, *Theileria mutans*, *Theileria velifera, Theileria taurotragi* and *Theileria buffeli*/*Theileria orientalis* with *T*. *parva* and *T*. *annulata* being the most pathogenic and economically important species. *Rhipicephalus appendiculatus* and *Rhipicephalus zambiziensis* transmit *T. parva* that causes

East coast fever (ECF), Corridor disease and January disease*,* while *Hyalomma anatolicum anatolicum* transmits *T*. *annulata* that causes bovine tropical theileriosis or Mediterranean Coast fever (Fujisaki et al., 1994). The other species of *Theileria* are of low pathogenicity and generally cause mild infection but their presence complicates the epidemiology of theileriosis in cattle. *Theileria velifera* causes benign theileriosis while *T*. *buffeli*/*orientalis* are mostly nonpathogenic (Uilenberg, 1981). The *Theileria* spp. of cattle documented in Uganda are *T*. *parva*, *T*. *mutans*, *T*. *taurotragi* and *T*. *velifera* (Muhanguzi et al., 2010b; Byaruhanga et al., 2016). In Uganda, *T. parva* only causes ECF (Kabi et al., 2014; Tayebwa et al., 2018), while Corridor disease and January disease have not been reported.

Pathogenesis and clinical signs

East Coast fever presents with clinical signs such as high temperatures (>40°C), peripheral lymphadenopathy, anorexia, congestion of the visible mucous membranes, conjunctivitis, lacrimation and corneal opacity. The disease is devastating because it can cause up to a 95% mortality of susceptible cattle. However, mild cases can be reported in ECF tolerant cattle breeds. These often recover but become carriers. The ECF infected cattle especially calves develop a lifelong immunity post-infection (Norval et al., 1992). Some of the infected cattle may develop neurologic signs, due to the capability of parasitized cells to cluster and block the cerebral vessel. At post-mortem, major pathological findings include enlarged lymph nodes, lung oedema, froth in trachea, abomasal ulcers and kidney infarcts.

2.9.2 Babesiosis

Aetiology and transmission

Babesiosis is a tick-borne protozoan disease in cattle caused by parasites of genus *Babesia*. The species of *Babesia* that mostly cause babesiosis in cattle are *B. bovis*, *B*. *bigemina* and *B*. *divergens* (Suarez and Noh, 2011)*. Babesia bigemina* and *B. bovis* are transmitted mainly by the ticks *R*. *microplus*, *R*. *annulatus* and may be *R*. *geigyi* (Bock et al., 2004). In addition, *B*. *bigemina* is transmitted by *R*. *decoloratus* and *R*. *evertsi evertsi*. Transmission of *B*. *bigemina* and *B*. *bovis* is transovarial in most tick vectors, and is trans-stadial in the two-host tick *R. evertsi evertsi* (Hall, 1985). *Babesia bigemina* is the reported species that causes cattle babesiosis in Uganda transmitted by *R. decoloratus* (Magona et al., 2011; Byaruhanga et al.,

2015b; Tayebwa et al., 2018). *Rhipicephalus evertsi evertsi* has also been found on cattle in Uganda (Magona et al., 2011; Byaruhanga et al., 2015a).

Pathogenesis and clinical signs

Common signs related to acute babesiosis are fever, depression and haemoglobinuria. As compared to the *B. bigemina*, *B. bovis* causes more severe disease associated with neurological signs such as hyperesthesia, convulsions, nystagmus and paralysis, due to its ability to sequestrate into the brain capillaries and cause neural damage. Death occurs within 24 hours in acutely infected cattle due to shock (Brown and Palmer, 1999). Both calves and adults are susceptible to the disease when exposed for the first time to *Babesia* parasites although calves (less than about nine months) have inverse age resistance. Cattle that have recovered from acute infection retain low parasitaemia levels and are reservoirs for transmission (Bock et al., 2004; Zintl et al., 2005). Pre-immunity occurs in most *Babesia* species after recovering from natural infection (Constable et al., 2016).

2.9.3 Anaplasmosis (Gall sickness)

Aetiology and transmission

Anaplasmosis in cattle is caused by *A. marginale*, *A. centrale*, *Anaplasma bovis* and *Anaplasma phagocytophilum. Anaplasma marginale* and *A. centrale* are found within erythrocytes near the margin or center of the cell respectively (Imam, 1999). Globally, bovine anaplasmosis is mainly caused by *A. marginale* (Kocan and de la Fuente, 2003). *Anaplasma marginale* is widely distributed throughout the world particularly because it can be transmitted by over 20 species of ticks (Battilani et al., 2017). Some of the common tick species implicated are *Dermacentor* and *Rhipicephalus* (Dikmans, 1950; Kocan et al., 2003); however, *Rhipicephalus* (*Boophilus*) spp. are the most significant vectors of anaplasmosis (FAO, 1983; Constable et al., 2016). In ticks, transmission occurs interstadially or intrastadially (Stich et al., 1989; Kocan et al., 1992). Apart from ticks, anaplasmosis can be transmitted by mechanical and transplacental means (Dikmans, 1950; Zaugg, 1985). Mechanical transmission occurs through biting flies such as *Tabanus* (Horse flies), *Stomoxys* (Stable flies), Culicidae (mosquitoes) or blood contaminated instruments (Ristic, 1968 and De Wall, 2000). When transplacental transmission of *A. marginale* occurs, there is delivery of infected calves (Grau et al., 2013). *Anaplasma centrale* is mainly found in tropics and

subtropics (Rar and Golovljova, 2011), and causes mild symptoms in cattle as compared to *A*. *marginale*. Since *A*. *centrale* can induce pre-infection it can induce immunity against *A*. *marginale*; therefore, a live vaccine for control of bovine anaplasmosis has been developed after exploiting the antibody cross-reactivity of the two species (Kocan, 2003). Using a quantitative real-time PCR (qPCR) assay on blood samples from cattle in Karamoja Region, the reported occurrence of *A*. *marginale* was 82.9% (Byaruhanga et al., 2016).

Pathogenesis and clinical signs

Clinical signs of anaplasmosis include rise in body temperature, jaundice with no haemoglobinuria, anorexia, lethargy and often death due to severe anemia (Potgieter and Stoltsz, 2004; Aubry and Geale, 2011). If infected cattle are untreated, they develop rumen stasis and constipation accompanied by dehydration and weight loss (Potgieter and Stoltsz, 2004). Post-mortem lesions include enlarged spleen, liver and distended gall bladder hence the name gall sickness (Hall, 1985). Calves are relatively resistant to infection, but cattle more than three years of age suffer per-acute or acute conditions, which may lead to death within 24 hours (Kettle, 2000). Severely affected animals usually die, although chronic or recurrent infections of anaplasmosis are common (Potgieter and Stoltsz, 1994; Kocan et al., 2003).

2.9.4 Heartwater (cowdriosis)

Aetiology and transmission

Heartwater is a tick-borne rickettsial disease of domestic and wild ruminants and is one of the most devastating diseases of cattle in sub-Saharan Africa (Deem, 1998; Collin et al., 2003). The causative agent, *E*. *ruminantium*, is transmitted by *Ambylomma* ticks (FAO 1983, Sumtion, 1996). The most important tick vectors are *A. variegatum*, *A*. *hebraeum, Amblyomma pomposum*, *A. lepidum, Amblyomma astrion, Amblyomma cohaerens, A*. *gemma* and *Amblyomma marmoreum (*Bezuidenhout, and Bigalke 1987; Walker and Olwage, 1987; Peter et al., 2000). *Amblyomma variegatum* is the reported tick vector of *E*. *ruminantium* in Uganda (Otim et al., 2004; Rubaire-Akiiki et al., 2004; Rubaire-Akiiki et al., 2006; Magona et al., 2011a), although *A*. *lepidum* and *A. gemma* were recently found on cattle in Karamoja Region (Byaruhanga et al., 2015a).

Pathogenesis and clinical signs

In domestic ruminants, heart water ranges from a subclinical infection to a peracute disease, with signs ranging from mild transient fever in the former to acute death (Van de Pypekamp et al., 1987). The acute form manifests by rapid onset of fever, tachypnea, inappettence; neurological signs are the most common presentation in susceptible naive hosts and often results in death (Van de Pypekamp et al., 1987). Additionally, it is reported that profuse, fetid, hemorrhagic diarrhea is common in domestic animals. Hydrothorax and hydropericardium are the main postmortem lesions, hence the name heartwater (Yunker, 1996; Constable et al., 2016).

2.9.5 Epidemiology of tick-borne diseases

Understanding the epidemiology of TBDs is vital for the development and implementation of control strategies (Gachohi et al., 2013). Factors including environment, host characteristics (size and coat), tick vector and the pathogen affect the transmission, occurrence and severity of TBDs. Global climatic changes as well as resistance to chemotherapeutics and acaricides are also factors that influence the occurrence and distribution of ticks and TBDs (Norval et al., 1992b; Kivaria, 2010; Kocan et al., 2010; Marufu et al., 2010). In addition, wild animals serve as reservoirs for ticks and tick-borne pathogens thereby sustaining the vector population and infections for livestock especially those that inhabit the wildlife-livestock interface (Smith and Parker, 2010; Fyumagwa et al., 2013; Walker et al., 2013).

In the free-living phases of their life cycles, ticks need certain levels of humidity and temperature (Léger et al., 2013). Any slight change may affect the suitability of their habitats as well as their distribution and abundance (Rubaire-Akiiki et al., 2006; Gachohi et al., 2012). Notably, host movements and transboundary movement of cattle can redistribute ticks to facilitate the colonization of new areas (Olwoch et al., 2008; Kocan et al., 2010).

The production system, grazing management and tick control practices facilitate or limit the exposure of cattle to ticks. For instance, animals raised under the open grazing system are more likely to harbor more ticks than zero-grazed animals (Rubaire-Akiiki et al., 2006; Swai

et al., 2009; Muhanguzi et al., 2010b; Gachohi et al., 2012). Furthermore, the tick control practices are more manageable in small herds of intensively managed cattle as opposed to hundreds of wild herds kept under an extensive system.

2.9.6 Diagnosis of tick-borne diseases

The diagnosis of TBDs is guided by presence of the vectors and presentation of clinical signs and/or necropsy findings (Lawrence et al., 2004b). However, clinical signs alone cannot diagnose TBDs due to related signs with other diseases (Allsopp et al., 2004; Lawrence et al., 2004b). The presence and distribution of tick vectors may also be useful, but this indicates circulation of a disease but not the level of risk. Moreover, some regions may have the vectors present but the disease absent and the ticks may have detached during animal examination (Minjauw and McLeod, 2003; Lawrence et al., 2004b).

Therefore, diagnosis of TBDs based on clinical signs, necropsy findings and epidemiological information may provide inadequate information that ought to be complemented by laboratory diagnostic tools and methods (Minjauw and McLeod, 2003).

Microscopic examination

Microscopic examination of Giemsa-stained blood smears or lymph node biopsy smears is commonly used to detect parasites. This method is the gold standard because it is cheap, quick and easy (Minjauw and McLeod, 2003). Because microscopy delivers results within as short as 1 to 2 h, it is a preferred technique in cases when the laboratory diagnosis is supposed to guide the prescription and treatment plan. (Minjauw and McLeod, 2003). However, it has low sensitivity especially when parasitaemia is low and the result depends on the experience of the technician (Minjauw and McLeod, 2003; Carelli et al., 2007; Sibeko et al., 2008). For instance, distinguishing piroplasms such as *T. parva*, *T. taurotragi, T. mutans, T. buffeli* and *T. velifera* using light microscopy might not be easy (Norval et al., 1992b). On the other hand, *Babesia* species are quite large and easy to distinguish at the merozoite stage; however, beyond that stage, they may be difficult to distinguish (Callow et al., 1993). Microscopic diagnosis of heartwater during post-mortem involves observing *E. ruminantium* in the cytoplasm of endothelial cells of the brain capillaries (Allsopp, 2010), where colonies of the organism are generally more numerous than in other tissues.

Although, distinctive colonies of *E. ruminantium* are easily detectable, sometimes it is hard to differentiate from other *Ehrlichia* species and might also be mistaken for *Chlamydia psittaci* (Allsopp, 2010). Taken together, microscopy has several shortcomings.

Serological methods

Serology has been vastly used in studies to detect tick-borne infections or their antibodies (Chenyambuga et al., 2010; Gachohi et al., 2010; Magona et al., 2011a; Malak et al., 2012). The most commonly used include; the enzyme-linked immunosorbent assay [ELISA] (Katende et al., 1998; OIE, 2015a), indirect fluorescent antibody test [IFAT] (Burridge and Kimber, 1972; Du Plessis and Malan, 1987b), complement fixation test [CFT], capillary agglutination assay and card agglutination test [CAT] (OIE, 2015a). However, serological tests have also proven to be unreliable because of low sensitivity, inability to accurately report the state of infection (acute and chronic infection), may not distinguish current from past infections, and cross-reactivity of antibodies. As such, it is inessential to use serological tests for diagnosis of clinical disease of suspected tick-borne infections as a guide for rational prescription of drugs (Oura et al., 2004a; Salih et al., 2010). So far, IFAT is mostly the gold standard assay for serological diagnosis of economically important parasites (Burridge and Kimber, 1972; Du Plessis and Malan, 1987b; OIE, 2015b). However, ELISA has proven better than IFAT in ways such as being less laborious, non-cross reactivity and can test several samples in a short time (Salih et al., 2010; Bilgiç et al., 2013).

Nucleic acid-based tests

Nucleic-acid based diagnostic techniques are the most reliable tests because they have a high specificity and sensitivity (Bekker et al., 2002; Sibeko et al., 2008; Salih et al., 2010; Liu et al., 2012). The frequently used methods in diagnosis of tick-borne infections include (i) isothermal amplification, (ii) reverse line blotting (RLB), (iii) conventional PCR, and (iv) qPCR. A combination of PCR with a specific hybridization by means of reverse line blot (RLB) was developed and is being used for the detection of mixed infections from one sample (Gubbels et al., 1999; Bekker et al., 2002; Nijhof et al., 2003; Schnittger et al., 2004). Unfortunately, these tests are very expensive and cannot easily be used for routine laboratory diagnosis.

2.9.7 Control of tick-borne diseases

The control of TBDs is concomitant with the control of ticks. Unfortunately, ticks are very complicated to control especially in the tropics and subtropics where conditions support reproduction across the year. Chemical control has been the most reliable by far, however the emergence of acaricide resistance across the world and the cost of acaricide has limited its success. Therefore, it is important that an integrated approach using immunization against ticks and TBDs, the use of chemotherapeutic and chemoprophylactic drugs, putting up appropriate farm infrastructure and rationally using chemicals (acaricides) to build or maintain endemic stability (Norval et al., 1992b; Bock et al., 2004; Mugisha et al., 2005) is used to manage the ticks.

Tick control

Tick control aims at reducing the burden of ticks on the animals, but complete eradication is impossible due to tick persistence or resistance especially the multi-host ticks and the ability of the adults to live longer away from the hosts. Tick control can be either intensive aimed at controlling all tick stages throughout the year or strategically regulating the tick numbers (Bezuidenhout and Bigalke, 1987; Norval et al., 1992b; Perry and Young, 1995; Allsopp et al., 2004). Tick control methods can be through direct application of acaricides to cattle (Gachohi et al., 2012). The use of acaricides is the most widely used method in controlling ticks (GALVmed, 2015a); however, acaricides are costly (Okello-Onen et al., 1998; Mugisha et al., 2005), and can contaminate food and the environment (Norval et al., 1992a; George et al., 2004). Furthermore, although livestock keepers use mainly acaricides for tick control, these are effective only for a short time and it may be difficult to effectively apply this method for cattle extensively managed or communally grazed. Nevertheless, the farmers living in tick endemic areas often have no choice but to use the acaricides to control tick and nuisance fly populations (Chenyambuga et al., 2010; Mugabi et al., 2010; George et al., 2004; SNV, 2013b; Kasozi et al., 2014). The farmers and their advisors should therefore be trained so that they use the chemicals in way that does not encourage resistance developing or disrupt endemic stability.

In Africa, traditional methods of ticks control include burning grass, hand removal of the ticks, use of extracts from leaves and dusting cattle with ash. The practice of hand-picking of

ticks is common amongst rural livestock keeping areas, but it is applied irregularly and is less effective (Dipeolu et al., 1992). Habitat modification such as vegetation management as well as burning and heavy grazing (Imam, 1999) can also contribute to tick control by reducing tick population. In other communities, livestock keepers have used crude extracts of plant parts to control ticks; for example, in western Ethiopia, juices of crushed leaves of *Phytolacca dodecandra, Vernonina amygdalina* and crushed seed of *Lepidium sativum* mixed with fresh cattle faeces have been used to control ticks (Regassa, 2000). In Karamoja, the livestock keepers use crude extracts of the plants including *Azadirachta indica*, *Acacia gerrardii*, *Adenium obesum* and *Acacia drepanolobium* to control ticks, and the removal of ticks by hand from the livestock is also common (Byaruhanga et al., 2015b). The use of traditional methods to control ticks is dictated by limited access to conventional drugs and due to unaffordable costs (Byaruhanga et al., 2015c).

Biologically tick control mainly relies on natural enemies, both predacious and parasitoid or pathogens. Many bacteria, fungi, beetles, rodents, ants, birds, and other living things naturally feed largely on insects including ticks and this indirectly reduces tick population (Hoogstraal, 1956).

Immunization is another way of control ticks based on identifying polypeptide antigens extracted from the mid gut of ticks and used as vaccines. A number of tick vaccines have been developed, of which the most common one is the recombinant Bm86 antigen derived from the mid gut of *Rhipicephalus microplus* (Rodriquez et al., 1994). Ingestion of blood containing antibody to Bm86 causes lysis of the gut cells and damage to the gut wall of the ticks. This results in high mortality of feeding ticks, reduction of engorged weight and egg laying capacity causing reduction of tick population (Hassan, 2003). However, these vaccines have not been available to livestock keepers in Africa. Given the high tick infestation amongst cattle in Karamoja Region, the ideal anti-tick vaccines and vaccination approaches would be those that reduce tick infestation and reproductive capacity (de la Fuente et al., 1998; Merino et al., 2011) of the most important tick species. Extension workers need to advise the farmers so that vaccination targets particular seasons and grazing areas with high infestations. One application of the vaccine in a season of 5 to 7 months is sufficient to provide protection. This can reduce the tick numbers and reduce the exposure of

susceptible animals to infected ticks. In this way, infections levels will be kept low; to only levels necessary for the attainment of acquired immunity amongst animals, but reduce the incidence of TBDs. However, the application of anti-tick vaccinations will need to be integrated with other methods, including treatment of clinical cases and grazing management to reduce exposure of animals to areas that are highly infested. On the other hand, the feasibility of using anti-tick vaccines in Karamoja is likely to be affected by the difficulty in maintaining a cold chain and inadequate extension veterinary services for advice to the herders and the administration of the vaccines. In addition, a single vaccine will not protect against all tick species present.

Chemotherapy and chemoprophylaxis

Once the cattle are infected with TBDs, the farmers have no choice but to incur costs of treatment for their cattle. Theileriosis in cattle is successfully treated with parvaquone and buparvaquone (hydroxynapthoquinones). These drugs are efficient when given in time because they target the causative parasite but they are costly thus limiting their usage in the field (Lawrence et al., 2004b).

Treatment of bovine anaplasmosis mainly involves the use of tetracyclines and imidocarb drugs (Potgieter and Stoltsz, 2004), aimed both at control of active infection and treatment of clinical cases. Bovine babesiosis is treated with diminazene aceturate and imidocarb dipropionate (imidocarb); however, they have shown side effects to the host such as toxicity and residues in meat, and should therefore be used carefully. Several other candidates such as nitidine chloride, epoxomicin, nerolidol and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) have been investigated to treat babesiosis (Bock et al., 2004), but have not been commercially available, and therefore diminazene aceturate and imidocarb dipropionate are still the most commonly used drugs for treatment of babesiosis.

Vaccination

Vaccination is a reliable alternative for the control of TBDs, although vaccines are not readily available for all TBDs (Marcelino et al., 2012). The infection-and-treatment method (ITM) developed by Radley et al. (1975a) offers a viable option for control of ECF (Lynen et al., 2006; Oura et al., 2007; Lynen et al., 2012). In this technique, the animal is inoculated with

T. *parva* sporozoite and concurrently administered with long-acting formulations of oxytetracycline to subdue any clinical manifestations of the disease, but induce immunity (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 challenge, but it is life-long if infected ticks continuously challenge the immunized cattle (Burridge and Kimber, 1972).

The original trivalent Muguga cocktail that comprises of three stocks of *Theileria*, namely *T*. *parva* Muguga, *T*. *parva* Kiambu 5 and *T*. *parva* Serengeti, has been widely used in the ECF endemic areas of East Africa (Radley et al., 1975b; McKeever, 2007). In Uganda, the ITM has been used in over 43 districts to vaccinate cattle particularly the exotic breeds that are more susceptible to ECF, fortunately, up to 85% protection among vaccinated cattle has been achieved (SNV, 2013b).

Although anaplasmosis has severe economic effects, there is no readily available vaccine yet, although studies and tests are currently underway (Aubry and Geale, 2011; Ducken et al., 2015). The live vaccines have been used to stimulate defensive immunity, although their use remains limited (Kocan et al., 2003). Vaccination against babesiosis is by use of attenuated or live *Babesia* parasites (Suarez and Noh, 2011). Although the vaccine against babesiosis is used in some countries in Africa and South America, it is not used in Uganda. Currently, cryopreserved vaccines prepared from the blood of sheep infected with *E. ruminantium* parasites of the Ball 3 isolate are the only commercially available vaccines used against heartwater (Bezuidenhout and Wright, 1989; Nakao et al., 2012).

Animal genetics

The *Bos indicus* are more resistant to ticks than *Bos taurus* and tolerate higher levels of challenge by TBDs (Bock et al., 1999; Minjauw and McLeod, 2003). In areas where ticks and TBDs constrain livestock production, the *Bos indicus* could be the best cattle to keep. Unfortunately, their productivity is quite low compared to the *Bos taurus*; perhaps guided cross breeding might generate a very productive but also TBD-resistant cross breed (Mattioli et al., 2000; De Vos et al., 2004).

Integrated control

Considering the limitations of different control approaches against TBDs, the best approach then would be one that integrates several methods to achieve the best result. One could combine the use of acaricides, vaccines and keeping of tick- resistant breeds of cattle to achieve a successful control programme for ticks and TBDs (Norval et al., 1992a; Perry et al., 1998; Minjauw and McLeod, 2003; George et al., 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Karamoja Region in northeastern Uganda covers an area of 27,200 square kilometers lying between longitude 33° E to 35 °E and latitude 1°N to 4°N. The region comprises seven administrative units/districts, namely Moroto, Nakapiripirit, Napak, Amudat, Abim, Kotido and Kaabong (as of June 2018). The region shares a border with Kenya to the east and South Sudan to the north (FAO, 2014). Karamoja is semi-arid with the majority of the population engaged in pastoral or an agro-pastoral lifestyle. Geographically, Karamoja Region has widely savannah plains (1500 m above sea level), thorny plants and scattered shrubs/small trees, with long episodes of up to 7 months of no rain (IICD, 2010). In Karamoja, generally the dry season is from mid-September to the end of February and the wet season runs from the beginning of March to mid-September. Unlike other parts of the country with a bimodal rainfall pattern, the rainfall in Karamoja is unimodal and low, with peaks from April through May and from July through August (Anderson and Robinson, 2009). The human population in Karamoja is approximately 1,455,200 (UBOS, 2014). The communities in Karamoja are mainly agro-pastoralists and have a huge dependence on cattle for their livelihood, as well as for cultural and spiritual purposes (FAO, 2014). This region harbors a high population of livestock, with highest proportion of cattle (2.3 million cattle; 19.8% of the national herd), 2.0 million goats (16.3% of national population) and 1.7 million sheep (49.4% of national flock) (UBOS, 2014). The rainfall in Karamoja is on average 500 to 700 mm in the central lowland areas, and 700 to 1,000 mm in the wetter western areas (Mugerwa et al., 2014).

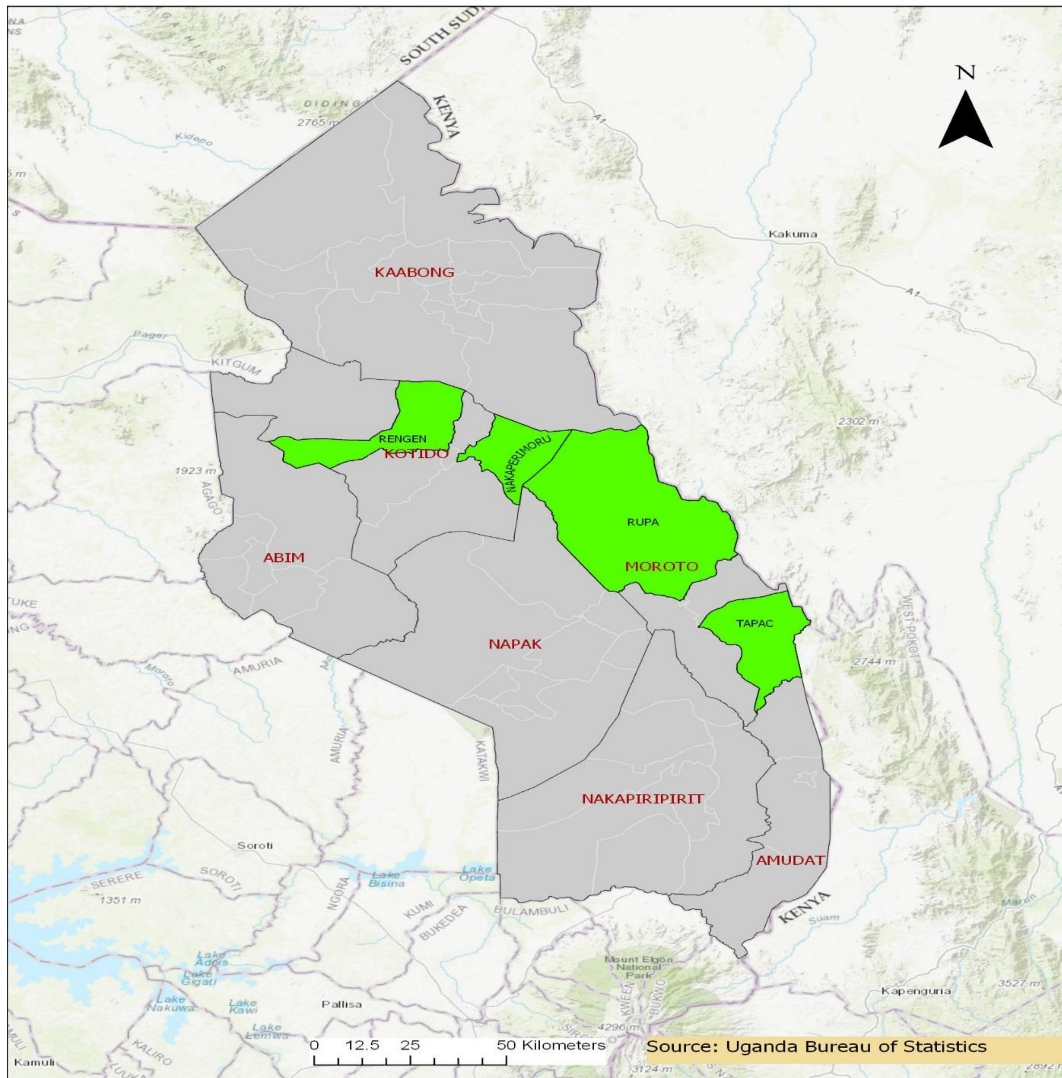

Figure 3. Map of Karamoja showing the study areas in Kotido and Moroto Districts. The sub-counties where the research was carried out are shaded in green.

3.2 Study design

A cross-sectional study was carried out from June through the beginning of September 2017 in in four sub-counties (Rupa, Tapac, Rengen and Nakapelimoru) of two districts of Karamoja Region (Moroto and Kotido), which were purposely selected to represent the pastoral and agro-pastoral zones, respectively. The pastoral zone is semi-arid, and the livelihoods are mainly dependent on livestock production (cattle, goats and sheep) with some crop growing in times when rainfall is adequate. In the agro-pastoral zone, annual rainfall is 500 to 800 mm, and this is erratically distributed; the livelihoods are supported by some cropping and livestock production (Aklilu, 2016; Mubiru, 2010). Twenty super herds were selected in the two districts based on ease of accessibility and guidance from the kraal leaders and

extension workers. A super herd is a group of herds composed of 500 to 600 animals (Mamo et al., 2013), and these share grazing and watering points during the wet season and belong to the same cattle camp in dry season. One herd was randomly selected from each super herd from the sites visited in each district for this study. Once consent was sought from the herdsmen, the cattle were restrained with ropes and examined for presence of ticks prior to sampling.

3.3 Collection and identification of ticks

Five animals with the highest tick infestation were purposively selected for tick collection, and in total 100 cattle were examined. Representative tick samples from different predilection sites of the whole body were collected. The ticks collected from each location were well-preserved in separately labeled vials containing 70% ethanol. Subsequently, identification was conducted using a stereoscopic microscope at the ectoparasitology laboratory at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa, using morphological keys described by Walker et al. (2013).

3.4 DNA extraction from ticks

Up to 10 ticks of each species collected from the four sub-counties were pooled for subsequent DNA extraction. Ticks were washed with distilled water, dried with absorbent paper towels, dissected into small pieces then placed into sterile MagNA lyser green bead tubes. DNA was extracted from ticks using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The 300 µl of ATL buffer was added to the tubes and placed on ice. The tick samples were homogenized twice for 1 min at 6,800 revolutions per minute (rpm) and the tubes placed back on ice to cool. Twenty microliters of Proteinase K were added to the tubes and incubation was done overnight at 56˚C to completely lyse the ticks. The next day, samples were briefly vortexed and 200 ul of supernatant was drawn from each tube and put in Eppendorf tubes of 2.5 mls. Into the Eppendorf tubes was added 200 µl of AL buffer, followed by vortexing and incubation at 70˚C for 10 minutes.

The 200 µl of ethanol was added to each tube, followed by vortexing, after which the mixture was transferred to mini spin columns and centrifuged for 1 minute at 8,000 rpm and

the flow-through was poured. The 500 µl of AW1 buffer was added to each column and centrifuged at 8,000 rpm for 1 min and the flow-through was discarded again. Then 500 µl of AW2 buffer was also added to each column and centrifuged at 14,000 rpm for 3 min and the flow-through poured again.

Columns were then placed in fresh collection tubes and centrifuged at 14,000 rpm for one minute to get rid of any AW buffer. The columns were placed in marked 2.5 ml tubes of 2.5 ml and 100 µl of AE, an elution buffer, was added to the QIAamp membrane. The columns were incubated for 2 min at room temperature, followed by centrifugation at 8,000 rpm for one minute to elute the DNA. DNA was stored at -20˚C for further analysis.

3.5 Polymerase chain reaction (PCR)

The PCR was performed using a set of primers that amplify the hypervariable V4 region of the 18S rRNA gene of *Theileria* and *Babesia* species (Nijhof et al., 2003) and the hypervariable V1 region of the 16S rRNA gene of *Anaplasma* and *Ehrlichia* (Bekker et al., 2002). The sequences of the primers that were used for the *Theileria/Babesia* and *Erlichia/Anaplasma* PCRs are shown in Table 1. The PCR mixture was made using 1x of Platinum® Quantitative PCR SuperMix-UDG (ThermoFisher Scientific Massachusetts, U.S.A), 8 pmol of each primer, 9.5 µl of nuclease-free water and 2.5 µl of DNA to make 25 µl final volume. DNA from live blood vaccine strains of *A*. *centrale* and *B*. *bovis* (Onderstepoort Biological Products, Pretoria, South Africa) were used as positive controls for the 16S rRNA and 18S rRNA genes PCR, respectively. PCR grade water was used as a negative control.

The PCR was conducted on the Gene Amp ® PCR system 9700 (Applied Biosystems Foster City, California, U.S.A) programmed to include enzyme activation at 37°C for 3 min, initial denaturing at 94°C for 10 min, then followed by 2 cycles of 94°C for 20 s, 67°C for 30 s and72°C for 30 s for complete denaturation. To anneal, the temperature was lowered by 2°C, every second cycle for the step-down PCR followed by 40 cycles of 94°C for 20 s, 57°C for 30 s and 72°C for 30 s, then final extension of 1 cycle at 72°C for 7 min.

Table 1. Primer sequences for the 18S rRNA and 16S rRNA polymerase chain reactions during a reverse line blot hybridisation assay

Genus	Primers	Primer Sequence (5'-3')
Theileria/Babesia	RLB-F2	GACACAGGGAGGTAGTGACAAG
	RLB-R2	biotin - CTAAGAATTTCACCTCTGACAGT
Ehrlichia/Anaplasma	EHR-F	GGAAATCAGAGTTGGATCMTGGYTCAGCGGGATCCGAG
	EHR-R	biotin -TTTGCCGGGACTTYTTCTC

3.6 Reverse line blot (RLB) hybridization assay

Reverse line blot hybridization was performed as described by Bekker et al. (2002) and Nijhof et al. (2003). Genus (catch-all) and species-specific oligonucleotide probes (Table 2), designed based on the 18S rRNA and 16S rRNA genes and linked to an RLB membrane, were used for the specific detection of *Babesia/Theileria* and *Anaplasma/Ehrlichia* species, respectively. A blotting membrane was activated by a 10-minute incubation in 10 ml of 16% (wt/vol) 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC) at room temperature. The membrane was washed for 2 minutes with distilled water and placed in an MN45 mini blotter (Immunetics Cambridge, Mass.). Specific oligonucleotides (Table 2) were diluted to a 200- to 1,600 pmol/150 ml concentration in 500 mM NaHCO3 (pH 8.4) and were subsequently covalently linked to the membrane with the amino linker by filling the mini blotter slots with the oligonucleotide dilutions. They were then incubated for 1 min at room temperature. The oligonucleotide solutions were aspirated, and the membrane inactivated by incubation in 100 ml of a 100 mM NaOH solution for 10 min at room temperature. The membrane was washed by gentle shaking in 125 ml of 2 X SSPE–0.1% sodium dodecyl sulfate (SDS) for 5 min at 60°C.

Before use, the membrane was washed for 5 min at 42°C with 125 ml of 2 X SSPE–0.1% SDS and placed in the mini blotter with the slots perpendicular on the previously applied specific oligonucleotides. A volume of 40 µl of PCR product was diluted to an end volume of 150 ml of 2 X SSPE–0.1% SDS, heated for 10 minutes at 100°C, and cooled on ice immediately. Denatured PCR samples were applied into the slots and incubated for 60 minutes at 42°C. PCR products were aspirated, and the blot washed twice in 125 ml of 2 X SSPE–0.5% SDS for

10 min at 42°C with gentle shaking. The membrane was subsequently incubated in 10 ml of peroxidase-labeled streptavidin (Boehringer, Mannheim, Germany) in 2 X SSPE–0.5% SDS for 30 min at 42°C. The membrane was washed twice in 125 ml of 2 X SSPE–0.5% SDS for 10 minutes at 42°C with gentle shaking. This was followed by two rinses in 125 ml of 2 X SSPE for 5 minutes each at room temperature, an incubation for 1 min in 10 ml of ECL detection fluid (Perkin Elma, U.S.A) before exposure to an x-ray film for development and visualisation by chemiluminescence. After use, all PCR products were stripped from the membrane by two washes in 1% SDS for 30 minutes each at 80°C. The membrane was rinsed in 20 mM EDTA (pH 8.0) and stored in fresh EDTA solution at 4°C for reuse (Nijhof et al., 2003).

Table 2. List of genus and species-specific oligonucleotide probes used on the reverse line blot membrane

Oligonucleotide probe	**Sequence (**5′-3′)	**References**
Ehrlichia/Anaplasma genus-specific (“E/A catch-all”)	GGG GGA AAG ATT TAT CGC TA	Bekker at al. (2002)
A. bovis	GTA GCT TGC TAT GAG AAC A	Bekker at al. (2002)
A. centrale	GAC CAT ACG CGC AGC TT	RLB manual, Isogen
A. marginale	GAC CGT ATA CGC AGC TTG	Bekker at al. (2002)
A. phagocytophilum	TTG CTA TAA AGA ATA ATT AGT GG	Bekker et al. (2002)
A. platys	AAG TCG AAC GGA TTT TTG TC	Beall et al. (2008)
Anaplasma sp. (Omatjenne)	CGG ATT TTT ATC ATA GCT TGC	Bekker at al. (2002)
E. canis	TCT GGC TAT AGG AAA TTG TTA	Bekker at al. (2002)
E. chaffeensis	ACC TTT TGG TTA TAA ATA ATT GTT	RLB manual, Isogen
E. ruminantium	AGT ATC TGT TAG TGG CAG	RLB manual, Isogen
Theilera/Babesia genus-specific (“T/B catch-all”)	TAA TGG TTA ATA GGA RCR GTT G	Gubbels et al. (1999)
Babesia genus–specific 1 (“B catch-all 1”)	ATT AGA GTG TTT CAA GCA GAC	Nijhof (unpublished)
Babesia genus–specific 2 (“B catch-all 2”)	ACT AGA GTG TTT CAA ACA GGC	Nijhof (unpublished)
B. bicornis	TTG GTA AAT CGC CTT GGT C	Nijhof et al. (2003)
B. bigemina	CGT TTT TTC CCT TTT GTT GG	Gubbels et al. (1999)
B. bovis	CAG GTT TCG CCT GTA TAA TTG AG	Gubbels et al. (1999)
B. caballi	GTG TTT ATC GCA GAC TTT TGT	Butler et al. (2008)
B. canis	TGC GTT GAC GGT TTG AC	Matjila et al. (2004)

Oligonucleotide probe	Sequence (5′-3′)	References
B. divergens	ACT RAT GTC GAG ATT GCA C	Gubbels et al. (1999)
B. felis	TTA TGC TTT TCC GAC TGG C	Bosman et al. (2007)
B. gibsoni	CAT CCC TCT GGT TAA TTT G	Nijhof et al. (2003)
B. leo	ATC TTG TTG CTT GCA GCT T	Bosman et al. (2007)
B. microti	GRC TTG GCA TCW TCT GGA	Nijhof et al. (2003)
B. occultans	CCT CTT TTG GCC CAT CTC GTC	Oosthuizen et al. (2008)
B. rossi	CGG TTT GTT GCC TTT GTG	Matjila et al. (2004)
Babesia sp. (sable)	CCT CTT TTG GCC CAT CTC GTC	Oosthuizen et al. (2008)
B. vogeli	AGC GTG TTC GAG TTT GCC	Matjila et al. (2004)
Theileria genus-specific (“T catch-all”)	ATT AGA GTG TTT CAA GCA GAC	Nijhof[a] (unpublished)
T. bicornis	GCG TTG TGG CTT TTT TCT G	Nijhof et al. (2003)
T. buffeli/orientalis	GGC TTA TTT CGG WTT GAT TTT	Gubbels et al. (1999)
T. equi	TTC GTT GAC TGC GYT TGG	Butler et al. (2008)
T. lestoquardi	CTT GTG TCC CTC CGG G	Schnittger et al. (2004)
T. mutans	CTT GCG TCT CCG AAT GTT	Gubbels et al. (1999)
T. ovis	TTG CTT TTG CTC CTT TAC GAG	Matjila et al. (2004)
T. parva	TTC GGG GTC TCT GCA TGT	Gubbels et al. (1999)
T. separata	GGT CGT GGT TTC CTC GT	Schnittger et al. (2004)
Theileria sp. (buffalo)	CAG ACG GAG TTT ACT TTG T	Oura et al. (2004)
Theileria sp. (kudu)	CTG CAT TGT TTC TTT CCT TTG	Nijhof et al. (2005)
Theileria sp. (sable)	GCT GCA TTG CCT TTT CTC C	Nijhof et al. (2005)
T. taurotragi	TCT TGG CAC GTG GCT TTT	Gubbels et al. (1999)
T. velifera	CCT ATT CTC CTT TAC GAG T	Gubbels et al. (1999)

CHAPTER FOUR

RESULTS

4.1 Tick identification

In this study, a total of 4,897 adult ixodid ticks and 67 immatures (17 larvae and 50 nymphs) were collected and identified from 100 cattle from two districts in Karamoja Region, Uganda. From the adult ticks collected, 2,460 were females and 2,437 were males. All the 50 nymphs were from the genus *Amblyomma*, while the larvae were not identified. Over 80% of the female ticks collected and identified were engorged. Three genera and 12 tick species were identified. The genera identified were *Amblyomma* (96.8%), *Hyalomma* (0.6%) and *Rhipicephalus* (2.6%). The collected tick species were identified as *R*. *appendiculatus*, *R. evertsi evertsi*, *Rhipicephalus pravus*, *Rhipicephalus praetextatus*, *R. pulchellus*, *Rhipicephalus turanicus*, *Amblyomma lepidum* and *A. gemma*. Others were *A*. *variegatum*, *R*. *decoloratus*, *Hyalomma truncatum* and *Hyalomma rufipes*. The proportions of the various tick species collected from cattle are shown in Table 3. The dominant tick species collected was *A*. *lepidum* (93.9%), followed by *A*. *variegatum* (2%). A few *R*. *appendiculatus* were collected (0.1%), while the proportion of *R*. *decoloratus* ticks was only 0.7%. Out of the 100 cattle sampled, most (73%) had moderate (11-50) to abundant (>50) number of ticks (Table 4).

Table 3. Distribution of the various tick species collected from cattle in four sub-counties in Karamoja Region, Uganda from June through September, 2017

Tick species	Number of ticks collected (%)				
	Tapac	Rupa	Rengen	Nakapelimoru	Total (%)
Rhipicephalus appendiculatus	0 (0)	0 (0)	1 (20.0)	4 (80)	5 (0.1)
Rhipicephalus evertsi evertsi	30 (58.8)	6 (11.8)	2 (3.9)	13 (25.5)	51 (1)
Rhipicephalus pravus	8 (61.5)	5 (38.5)	0 (0)	0 (0)	13 (0.3)
Rhipicephalus praetextatus	2 (50.0)	0 (0)	0 (0)	2 (50)	4 (0.08)
Rhipicephalus pulchellus	9 (50.0)	2 (11.1)	2 (11.1)	5 (27.8)	18 (0.4)
Rhipicephalus turanicus	0 (0)	0 (0)	0 (0)	1 (100)	1 (0.02)
Amblyomma lepidum	509 (11.1)	379 (8.2)	277 (6)	3431 (74.7)	4596 (93.9)
Amblyomma gemma	25 (52.1)	12 (25.0)	2 (4.2)	9 (18.8)	48 (0.9)
Amblyomma variegatum	58 (59.2)	30 (30.6)	5 (5.1)	5 (5.1)	98 (2)
Rhipicephalus decoloratus	5 (15.2)	19 (57.6)	2 (6.1)	7 (21.2)	33 (0.7)
Hyalomma truncatum	5 (20.8)	1 (4.2)	8 (33.3)	10 (41.7)	24 (0.5)
Hyalomma rufipes	2 (33.3)	3 (50)	0 (0)	1 (16.7)	6 (0.1)

Table 4. Distribution (median and range) of ticks per head of cattle as collected from Karamoja Region, Uganda from June 2017 to September 2017

District	Sub county	Median ticks per animal	Minimum ticks per animal	Maximum ticks per animal	No. of cattle with >11 ticks (%)
Kotido	Nakapelimoru	134	59	224	25/25 (100)
	Rengen	10	2	32	12/25 (48)
Moroto	Rupa	15	3	54	15/25 (60)
	Tapac	24	1	60	21/25 (84)
Total					73/100 (73)

Total number of cattle sampled=100 (Cattle herds sampled per sub-counties=5; cattle sampled per herd=5)

4.2 Detection of haemoparasites in ticks using reverse line blot (RLB) hybridization

The occurrence of tick-borne pathogens in ticks in a RLB hybridization analysis of DNA extracted from 40 tick pools (with a panel of 43 oligonucleotide probes), representing 4897 ticks belonging to 12 species is shown in Table 5. Of the 40 pools, 30 (75%) tested positive for tick-borne pathogens of the genera *Ehrlichia*, *Anaplasma*, *Babesia* and *Theileria.* Pathogens identified in ticks were *E. ruminantium*, *A. centrale*, *B. bigemina*, *T. mutans* and *T. velifera*. Others were *Theileria separata*, *Babesia microti*, *Babesia rossi, Theileria* sp. (sable)

and *T. parva*. Out of the 40 tick pools analyzed, 17 pools were infected with a single pathogen, 11 with two pathogens and 2 were infected with three pathogens.

Theileria parva was detected in 10 pools from *R. pravus, R. praetextatus, R. pulchellus, A. lepidum, A. variegatum, A. gemma, R. decoloratus* and *H. truncatum*. *Anaplasma centrale* was detected in one pool from *R. pulchellus*, while *E. ruminantium* was detected in three pools, one from each of the tick species *A. gemma*, *A. lepidum* and *A. variegatum. Babesia bigemina* was detected in one pool from *A. lepidum.* No tick-borne pathogens were detected from *R. turanicus* and *R. appendiculatus*. From the RLB results, the most abundant pathogens were *T. velifera*, *T. parva* and *T. mutans*, found in 25% (*T. velifera/T. parva*) and 22.5% (*T. mutans*) of the tick pools, followed by *Theileria* sp. (sable) (12.5%), *B. microti* (9%) and *E. ruminantium* (7.5%) (Figure 4). Some pools showed positive signals with genus-specific probes only, i.e., *Ehrlichia/Anaplasma* (24) and *Theileria/Babesia* (2), *Theilera* (5) and *Babesia* 1 (12) on RLB hybridization.

Table 5. Infection rates of tick-borne pathogens in a reverse line blot analysis of DNA extracted from ixodid ticks collected from cattle in Karamoja Region, Uganda (June to September 2017)

Tick species	No. of tick pools examined	Number of tick pools positive for tick-borne infections (% of tick pools)									
		E. ruminantium	*A. centrale*	*B. bigemina*	*B. microti*	*B. rossi*	*Theileria* sp. (sable)	*T. mutans*	*T. separata*	*T. velifera*	*T. parva*
R. appendiculatus	2	0* (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R. evertsi evertsi	4	0 (0)	0 (0)	0 (0)	1 (25)	1 (25)	1 (25)	2 (50)	1 (25)	1 (25)	0 (0)
R. pravus	2	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
R. praetextatus	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
R. pulchellus	4	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	0 (0)	2 (50)	1 (25)
R. turanicus	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
A. lepidum	4	1 (25)	0 (0)	1 (25)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	1 (25)
A. gemma	5	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1(20)	0 (0)	1 (20)	1 (20)	2 (40)
A. variegatum	4	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	1 (25)	1 (25)	0 (0)	1 (25)	1 (25)
R. decoloratus	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (80)	0 (0)	2 (40)	2 (40)
H. truncatum	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	2 (50)	1 (25)
H. rufipes	3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)	0 (0)	0 (0)	1 (33.3)	0 (0)

* Zero (0) denotes samples that tested negative or below the detection limit of the assay.

Total number of tick pools = 40. *R*, *Rhipicephalus*; *A*, *Amblyomma*; *H*, *Hyalomma*; *E*, *Ehrlichia*; *A*, *Anaplasma*; *B*, *Babesia*; *T*, *Theileria.*

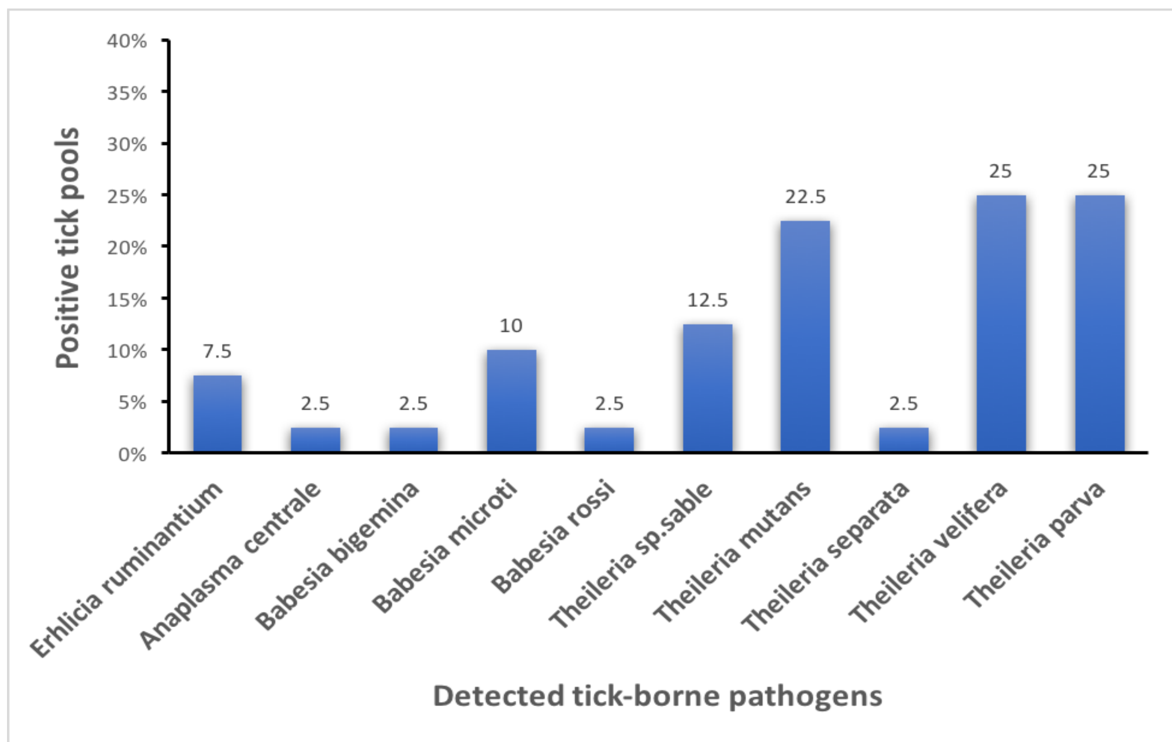

Figure 4. Proportions of tick pools that tested positive for tick-borne pathogens in a reverse line blot analysis of DNA extracted from ticks collected from cattle in Karamoja Region, Uganda, June to September 2017. Number of tick pools = 40

CHAPTER FIVE

DISCUSSION

In this study, we identified various tick species present on cattle during the wet season in Karamoja Region, Uganda. In addition, the tick-borne pathogens present in the ticks were also identified. Our findings complement the findings previously documented by Byaruhanga et al. (2015a), who reported tick-borne infections from cattle, but only from blood samples and in the dry season.

We found 12 tick species, namely *R. appendiculatus*, *R. evertsi evertsi*, *R. pravus*, *R. praetextatus*, *R. pulchellus*, *R. turanicus*, *A. lepidum*, *A. gemma*, *A. variegatum*, *R. decoloratus*, *H. truncatum* and *H. rufipes*. Previous studies in other parts of Uganda documented tick species *R. appendiculatus*, *R. evertsi evertsi, R. decoloratus* and *A. variegatum* (Rubaire-Akiiki et al., 2004; Magona et al., 2011). In another study in Uganda, Kaiser et al. (1982) reported *H. rufipes* in the southern part of the country. Other tick species found in this study are similar to those previously documented by Byaruhanga et al. (2015a) from cattle from Karamoja Region, i.e., *A. lepidum*, *H. truncatum*, *A. gemma*, *R. pulchellus*, and these had been reported for the first time in Uganda. In the current study, *R. pravus*, *R. praetextatus* and *R. turanicus* are reported for the first time in Karamoja, and have not been reported in recent studies in Uganda. The detection of unique ticks during different studies in Karamoja may be due to differences in the sampling periods for various studies. It is known that some tick species, through adaptation, are present towards the end of the dry season and may therefore be abundant at the beginning or during the wet season (Walker et al., 2013), and this could be the case for this study. The finding of tick species in Karamoja that have not been found in other parts of Uganda can be explained by the fact that Uganda's climate is not uniform for all regions; therefore, some tick species may not reproduce the whole year in some parts of the country. On the other hand, it could be that the newly reported ticks were picked from other areas in the neighbouring countries (especially Turkana in Kenya and South Sudan) as a result of the transhumant nature of the herding system in Karamoja, coupled with communal grazing and sharing water points.

Nonetheless, *R*. *pravus* is usually linked with *A. gemma* in dryland areas and also common in wildlife like the antelopes and hares (Matthysse and Colbo, 1987; Walker et al., 2013), and these wild animals often share grazing areas with cattle in Karamoja Region (Byaruhanga et al., 2005c). Therefore, the semi-arid environment of Karamoja Region might be no strange place for this tick species. The co-grazing of cattle with wildlife as the pastoralists herd their cattle (Byaruhanga et al., 2015c) could have exposed the cattle to *R*. *pravus*.

In this study, the most dominant tick species was *A*. *lepidum* followed by *A*. *variegatum* and *R. evertsi evertsi.* In contrast to the present study in which the reported proportion of *A*. *lepidum* was 93.9%, the reported proportion for this tick species was 11.6% in the dry season [November 2013 through to January 2014] (Byaruhanga et al., 2015a). An increase in tick numbers in the wet season has been documented, but a 10-fold increase might indicate that other factors that may not have been determined in the current study could be involved. For example, in the present study, focus was placed mainly on common predilection sites with an obvious bias towards adult ticks by collectors. Therefore many immature ticks in some body parts are likely to have been missed. In addition, *Amblyomma* species are characterised by a three-host life cycle, and spend about 90% of the life cycle in the environment, making them more vulnerable to high temperatures in the dry season, than it would be in the wet season (Pegram and Banda, 1990). Therefore, a higher number of adult *Amblyomma* ticks are expected on the host in the wet season than the dry season. Also, adult *Amblyomma* ticks feed mainly in the rainy season, while the immature ticks feed mainly in the dry season, and although the immature stages feed mainly in the dry season, the shortened survival periods for the larvae and nymphs and the prolonged molting periods at higher temperatures limits the number of immature ticks that can make it from the environment to the host (Pegram and Banda, 1990). Collection of ticks from the environment (from grass) will be necessary in future studies for further analysis of the trend in tick species distribution in Karamoja. The abundancy of *A*. *lepidum*, coupled with co-occurrence with *A. variegatum* in Karamoja is likely to increase the risk of heartwater (*E*. *ruminantium*) amongst cattle (Walker and Olwage, 1987).

Unlike the previous study in the dry season (Byaruhanga et al., 2015a), in which *R*. *appendiculatus* was the most abundant tick species collected (77%), in this study, only 0.1%

of the ticks were *R.* a*ppendiculatus*. Also, a small proportion of *R. decoloratus* ticks (0.7%) were collected from cattle compared to a higher proportion in the previous study in Karamoja (17.7%) (Byaruhanga et al., 2015a) being infested with *R. decoloratus*. The differences in the period of sampling may have influenced the relative abundance of the different tick species.

About three quarters of the cattle sampled had moderate to abundant number of ticks. This relatively high number of ticks can be explained by poor tick control practices which include picking ticks with hands, irregular spraying with acaricides in little quantities and less than the recommended dilutions, as recently found during a participatory study with the pastoralists in Karamoja (Byaruhanga et al., 2015c). Furthermore, communal grazing and cross-border movement of cattle may also increase the likelihood of tick infestation amongst cattle (Byaruhanga et al., 2015c). The high tick infestation is likely to increase exposure of calves to tick borne pathogens when they are still protected by innate or colostral immunity and this may contribute to endemic stability to the various TBDs amongst the short-horned East African Zebu cattle in Karamoja. So, mortalities due to TBDs are expected to be low. However, endemic stability may not always be assumed in Karamoja; in the severe dry seasons, the infected tick numbers may not be sufficient to achieve adequate exposure in the new-born calves, and these can become susceptible to tick-borne infections when innate immunity wanes. Therefore, in a recent study in the dry season in Karamoja Region (Byaruhanga et al., 2015c), and in the same herds and locations as for this study, the pastoralists reported relatively high mortalities due to TBDs (ECF, 14% of all disease mortalities; anaplasmosis, 17.5%).

Moreover, during recent clinical examinations of cattle amongst these herds, seven cases of ECF (three calves< 6 months and four cattle> 12 months) and cases of anaplasmosis were found amongst various groups of cattle in nearly all the 20 herds sampled (Byaruhanga et al., 2015a). Cycles of drought occur in Karamoja and this may limit survival of ticks in some seasons, so some cattle may not be exposed adequately to tick-borne infections when they are still young. This reduced exposure may increase the proportion of susceptible animals therefore increase in mortalities among cattle can occur. Also, movement of animals from areas within and outside Karamoja that have low occurrence of tick-borne pathogens to

higher prevalence areas in Karamoja may contribute to mortalities as a result of a proportion of relatively naïve animals. Also, some of deaths in cattle can also be attributed to stress from nutritional deficiencies in the dry season and animal movements in search of pastures and water, which can increase susceptibility to TBDs.

Theileria velifera, *T*. *parva* and *T*. *mutans* were the most frequently detected tick-borne pathogens from the tick species (25%, 25% and 22.5% respectively). The relatively high occurrence of *T*. *mutans* and *T*. *velifera* amongst the ticks is consistent with the previous study in Karamoja, in which these *Theileria* species were the most prevalent in cattle (71.3% and 88.3%, respectively) (Byaruhanga et al., 2016). Apart from *A*. *lepidum* and *A*. *variegatum*, which are known vectors of *T*. *mutans* and *T*. *velifera*, DNA of the two organisms was also detected in various *Rhipicephalus* and *Hyalomma* spp. Although *Rhipicephalus* and *Hyalomma* ticks may not mechanically or biologically transmit *T*. *mutans* and *T*. *velifera*, the detection of these pathogens in these tick species could be as a result of their presence in the blood meal. *Theileria mutans* infection causes mild symptoms of theileriosis in cattle (Lawrence and Williamson 2004a). According to Lawrence and Williamson (2004b), *T. velifera* is considered not pathogenic for cattle hence its field control is not usually compulsory. Furthermore, Du Plessis (1990) and Wanduragala and Ristic (1993) also reported that *T. mutans* and *T. velifera* are generally non-pathogenic in cattle. The high occurrence of benign *Theileria* species may complicate the clinical and laboratory diagnosis for theileriosis; it is difficult to different immature stages of various *Theileria* species under in blood or other tissue smears, and some clinical symptoms may be similar.

The detection of *T. parva* in a quarter of the tick pools examined is relatively high and inconsistent with the low occurrence of the pathogen in blood samples of cattle reported in a recent study in the same area (2.9% by RLB and 3.3% by qPCR) (Byaruhanga et al., 2016), although there was relatively higher seroprevalence of 15% (Byaruhanga et al., 2015a) A higher seroprevalence can be observed even in low *R*. *appendiculatus* situations because anti-*T*. *parva* antibodies persist in animals that have previously been exposed to infection, and no longer have active infection or are in a carrier state with very low parasitaemia to be detected by nucleic acid-based methods. The carrier state in *T*. *parva* can persist for about 16 months without infection challenge from ticks in indigenous cattle (Kariuki et al., 1995).

Similarly, low prevalence of *T. parva* was reported in other parts of Uganda, for example on farms in central Uganda the prevalence of 7% using RLB (Oura et al., 2004) and 5.3% using p104-based PCR (Muhanguzi et al., 2014) in eastern Uganda. In this study (wet season), *T. parva* was detected in various tick species, namely *H. truncatum*, *R. pravus*, *R. praetextatus*, *R. pulchellus*, *A. lepidum*, *A. variegatum*, *A. gemma* and *R. decoloratus*, which are not known vectors of the pathogen. Accidental infections in ticks could have occurred while taking their blood meals as all the ticks were collected whilst attached to the host.

The other pathogenic tick-borne species of cattle, *B. bigemina*, was detected in only one tick pool of *A. lepidum*, signifying low occurrence of *B. bigemina* circulating in the tick populations. This also demonstrated less likelihood of infection in cattle, consistent with the low occurrence of the pathogen (5%, using RLB) in Karamoja (Byaruhanga et al., 2016) and other parts of Uganda (4%, Asiimwe et al., 2013) (2% Oura et al., 2004). Whereas *B. bigemina* infections are characterised by high parasitaemia, animals recovering from babesiosis result in tick infectivity for only 4 to 7 weeks and carriers just for a few months (Suarez and Noh, 2011), and this may explain low infection rates of *B. bigemina* detected from various studies.

Tick-borne pathogens, *B. microti* (10.0%), *B. rossi* (2.5%), *Theileria* sp. (sable) (12.5%) and *T. separata* (2.5%), which are not common to cattle, or not known to infect cattle, were detected from the ticks in this study. The detection in the present study may be due to presence of pathogens in the blood meal of engorged ticks which comprised majority of the female ticks collected in this study. *Babesia microti* is transmitted by hard-bodied ticks of the genus *Ixodes* as the definite and mice as the intermediate hosts. Humans acquire infection through tick bites (Westblade et al., 2017). The detection of *B. microti* from ticks in the study area could be indication of possible presence of the pathogen in the resident human population and may have implications for human health. Transmission to cattle in the study area is most likely due to high abundance of mice in the grazing areas, which are probably infected with the pathogen, with accidental infection occurring as the ticks from the mice feed on cattle. However, detection of *B. microti* may as well be due to cross-reactions amongst RLB probes used during analysis. The design of most of the current RLB probes has not taken into account all pathogen variants from various geographical regions.

Therefore, the possible presence of pathogen variants may manifest as false detection of other pathogens. However, there is need for further investigation about the possible occurrence of *B. microti* in Karamoja, including examination of *Ixodes* ticks in the environment.

Theileria sp. (sable) is a common pathogen of wild animals and its relatively high proportion here does not match its tick vector *R. appendiculatus*. Stoltsz and Dunsterville (1992) reported *Theileria* sp. (sable) being originally isolated from a sable antelope and then identified also in clinically healthy cattle in Tanzania (Nijhof et al., 2005) and South Africa (Yusufmia et al., 2010). The detection of *Theileria* sp. (sable) in ticks in this study may be due to incidental infections. However, there is a possibility of other species of haemoparasites circulating in Karamoja Region which may be significantly similar to the probes used in the RLB assay. For example, the oligonucleotide probes for *Theileria* sp. (sable) and *T. velifera* currently used in the RLB assay are highly similar (Mans et al., 2015), and therefore the reported occurrence of *Theileria* sp. (sable) from various studies (Nijhof et al., 2005; Yusufmia et al., 2010; Tembo et al., 2018) can be attributed to the occurrence of *T. velifera*. There is therefore urgent need to validate the RLB assay for some of the tick-borne pathogen species, including *Theileria* sp. (sable).

Erhlichia ruminantium was present in 7.5% of the ticks, and this can be attributed to the abundance of the tick vectors *A. lepidum* and *A. variegatum* in the study areas. As reported by Allsopp (2010), ticks of the genus *Amblyomma* transmit *E. ruminantium* which causes heartwater disease in ruminants, and in Uganda *A. variegatum* is the tick vector of heartwater disease (Magona et al., 2011a). DNA of *E. ruminantium* was previously also detected in *Amblyomma* ticks from Ethiopia (Tomassone et al., 2012; Teshale et al., 2015).

The observed proportion of *A. centrale* (2.5%) was low compared to the 20.4% detected using RLB and 12.1% using qPCR from a previous study in Karamoja (Byaruhanga et al., 2017); but similar to those found from other studies: 4.3% in central Uganda (Asiimwe et al., 2013) and 4.5% in western Uganda (Muhanguzi et al., 2010a) using RLB hybridization. The most likely explanation here could have been low infections in tick vectors.

Babesia rossi (*2.5%*) was detected from *R. evertsi. evertsi*. Although *R. evertsi evertsi* is a vector of *Babesia* and *Theileria* species, it is not a confirmed vector of *B. rossi*. The pathogen mainly causes babesiosis in dogs, and is restricted to sub-Saharan Africa, transmitted by *Haemaphysalis elliptica* (Penzhorn, 2011). Detection of pathogens in ticks may not necessary indicate that they are vectors to the pathogens. Although unlikely, it is possible that ticks that fed on dogs in the previous life cycle stage may be infected when they feed on cattle in the adult stage. A more likely explanation, however, is that the detection of *B. rossi* may also be due to cross-reaction with other pathogens during RLB analysis since there are sequence variants of different pathogens and the RLB probes have not been validated for all variants.

In some samples, reverse line blot analysis showed positive signals with *Ehrlichia*/*Anaplasma* and/or *Theileria/Babesia* genus-specific probes but no signals with species-specific probes. This may indicate the presence of variants of known species or novel species of *Ehrlichia*, *Anaplasma*, *Theileria* and *Babesia* which could not be detected by the RLB assay. Not all currently used RLB probes have been validated for worldwide detection of corresponding tick-borne pathogens. Variations in DNA sequences in RLB probe region have been found, for example for *B. bigemina* (Martin et al., 2010; Byaruhanga et al., 2016). Likewise, in South Africa, Oosthuizen et al. (2009) and Oosthuizen et al. (2008) demonstrated failure of PCR products from some samples to hybridize with any of the included *Babesia* or *Theileria* species-specific probes, but only with the *Babesia/Theileria* genus-specific probes, signifying the occurrence of a novel species or variant of a species.

The presence of pathogen DNA in ticks in this study does not necessarily mean they are vectors of these pathogens. During the collection of ticks, most were engorged and the pathogens present at the time of analysis could have been from the hosts' blood or the ticks might have been infected while feeding during their immature stages on naturally infected domestic or wild animals since some ticks have more than one host. Nevertheless, this is the first time that ticks were tested for tick-borne pathogens in Karamoja, and these results contribute to a better understanding of the epidemiology of tick-borne pathogens.

Proper diagnosis is a prerequisite for rational treatment and quick recovery. Therefore, it is important that proper and accurate diagnosis is performed to control TBDs. The veterinary authorities in Karamoja can assist the livestock farmers by routine microscopic examination of blood smears and organ impression smears from sick, diseased or dead animals to confirm or exclude haemoparasites as the cause of illness, in particular babesiosis, anaplasmosis and theileriosis. Heartwater can only be confirmed during post-mortem examination. The Karamoja regional veterinary laboratory in Moroto has the capacity to do routine microscopic examination of TBDs. Therefore, microscopy remains the most reliable and cost-effective diagnostic test for the livestock farmers in Karamoja Region. However, the microscopic technique has limited use for the detection of carrier animals with low parasitaemias (Carelli et al., 2007; Sibeko et al., 2008). The community-based animal health workers should be trained in basic diagnostic skills (for example detection of common clinical signs and preparation of smears for haemoparasite detection), and the livestock keepers should be informed about practical methods for early diagnosis and treatment of TBDs as well as strategic tick control to reduce tick burdens to only low levels that can provide sufficient exposure in young animals for them to acquire immunity. There is also need for government to support private sector in increasing access to and availability of veterinary drugs, since this is a major constraint to the control of ticks and TBDs in Karamoja Region (Byaruhanga et al., 2015c).

Some of the challenges experienced during field sampling included;

- Kraals were quite distant and were not easily accessible due to poor road infrastructure.
- Most kraals did not have crushes which made restraint challenging.
- Some livestock keepers whose herds were selected for sampling during mobilization would migrate without notice.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

In summary, 10 tick-bone pathogen species were identified from 12 tick species in this study. This has shed some light on infection of ticks by tick-borne pathogens in Karamoja Region. This study also demonstrated a high tick burden in the region, which indicates that ticks are important ectoparasites of cattle in Karamoja. Additionally, the abundance of some tick species may increase the risk of infections in naïve animals or animals from non-endemic areas, for example *A. lepidum* a tick vector of *E. ruminantium*, but may be beneficial in maintaining endemic stability in resident animals. The presence of *Hyalomma* species does raise concern with regard to the zoonotic risk of Crimean-Congo Hemorrhagic fever (CCHF), although the risk is considered to be extremely low since the disease has never been reported in Karamoja region. The CCHF is a zoonosis and affects various wild and domestic livestock, and a case of the disease was recently confirmed in Lyantonde District (central Uganda) in August 2019. There is therefore high need for continuous surveillance of the Crimean-Congo Hemorrhagic fever virus amongst humans, various animal species and amongst *Hyalomma* ticks in the Karamoja Region.

Recommendations

The recommendations derived from the findings in this study include;

- Integrated control of TBDs by farmers should be carried out.
- Herders should be encouraged to strategically apply acaricides to reduce tick infestation, but allow sufficient exposure for immunity at a young age in order to reduce the incidence of clinical disease.
- Breeding programmes need to utilize only breeds that are relatively tolerant to ticks and TBDs.
- Improved access to drugs so that clinical cases are treated on time to reduce mortalities.

- Teams should carry along some cotton and wound sprays for the sampled animals due to bites wounds that bleed after tick collection.
- The current study detected previously unreported tick species and pathogens not usually found in cattle. Further studies using more sensitive and more robust techniques, including DNA sequence analysis, are required to confirm the presence of tick-borne pathogens that were detected in this study, but are not common or not known to infect cattle, because these have public health implications or are of pathogenic significance in other animal species.

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APPENDICES

Appendix A: Details of tick pools from various tick species collected from cattle in Karamoja Region, Uganda, submitted for laboratory analysis (DNA extraction and RLB Analysis)

Tick pool	No. of ticks	Tick species present	Haemoparasite detected with RLB assay
1	10	*A. lepidum*	*B. bigemina*
2	10	*A. variegatum*	*T. mutans*
3	10	*A. gemma*	*E. ruminantium, Theileria* sp. (sable)*, T. velifera*
4	4	*R. decoloratus*	*T. mutans, T. velifera*
5	5	*H. truncatum*	*T. parva*
6	1	*H. rufipes*	Negative for species- specific probes
7	10	*R. e. evertsi*	*Theileria* sp. (sable)*, T. mutans, T. velifera*
8	8	*R. pravus*	*T. parva*
9	2	*R. praetextatus*	*T. parva*
10	9	*R. pulchellus*	*T. parva*
11	10	*A. lepidum*	*E. ruminantium, T. parva*
12	10	*A. variegatum*	*T. parva*
13	10	*A. gemma*	*T. parva*
14	5	*R. decoloratus*	*T. parva*
15	5	*R. decoloratus*	*T. mutans, T. parva*
16	1	*H. truncatum*	*Theileria* sp. (sable)*, T. velifera*
17	3	*H. rufipes*	*Theileria* sp. (sable)*, T. velifera*
18	6	*R. e. evertsi*	*B. microti, B. rossi*
19	5	*R. pravus*	*B. microti*
20	2	*R. pulchellus*	Negative for species- specific probes
21	10	*A. lepidum*	Negative for species-specific probes
22	5	*A. variegatum*	*E. ruminantium, B. microti*
23	2	*A. gemma*	*T. parva*
24	2	*R. decoloratus*	*T. mutans*

25	8	*H. truncatum*	*T. velifera*
26	1	*R. appendiculatus*	Negative for species-specific probes
27	2	*R. e. evertsi*	*T. separata*
28	2	*R. pulchellus*	*T. mutans, T. velifera*
29	10	*A. lepidum*	*B. microti*
30	5	*A. variegatum*	*Theileria* sp. (sable)*, T. velifera*
31	5	*A. gemma*	Negative for species- specific probes
32	4	*A. gemma*	*T. mutans*
33	5	*R. decoloratus*	*T. mutans, T. velifera*
34	10	*H. truncatum*	Negative for species-specific probes
35	1	*H. rufipes*	Negative for species-specific probes
36	4	*R. appendiculatus*	*Negative*
37	8	*R. e. evertsi*	*T. mutans*
38	*2*	*R. praetextatus*	Negative for species-specific probes
39	*1*	*R. turanicus*	Negative for species-specific probes
40	*5*	*R. pulchellus*	*A. centrale, T. velifera*

RLB, reverse line blot hybridisation

Appendix B: Distribution of ticks collected from cattle from four sub-counties in two districts of Karamoja Region, Uganda

District	Sub county	No. of ticks
Kotido	Nakapelimoru	3488
	Rengen	299
Moroto	Rupa	457
	Tapac	653
Grand total		**4897**

Appendix C: Animal Ethics Certificate, Import and Export Permit

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Determining the tick species composing and infection with associated tick born disease pathogens of the tick population present on cattle in a semi-arid area of Karamoja, Uganda**
PROJECT NUMBER	**V094-17 (Revised)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**PC Akure**

STUDENT NUMBER (where applicable)	**U17388687**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Cattle**	
NUMBER OF SAMPLES	**100**	
Approval period to use animals for research/testing purposes		**October 2017 – October 2018**
SUPERVISOR	**Dr. WH Stoltsz**	

Conditions: The AEC has noted that this project will be completed in a facility outside of South Africa. Since the AEC has not inspected the facility, please note that we cannot comment on the quality of the facility other than what provided in the study questionnaire

<u>KINDLY NOTE:</u>

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 30 October 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

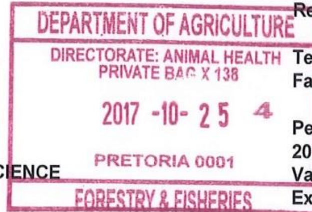
S4285-15

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate of Animal Health
Import-Export Policy Unit
Private Bag X138
Pretoria, 0001
Republic of South Africa

Tel: (27)-012-3197514
Fax: (27)-012-3298292



Permit No: 13/1/1/30/0-201710003705
Valid from: 2017-10-25
Expiry date: 2018-01-25
Valid for 3 months

IMPORTER
UNIVERSITY OF PRETORIA
FACULTY OF VETERINARY SCIENCE
PRIVATE BAG X 04
ONDERSTEPOORT
0110

VETERINARY IMPORT PERMIT FOR PRESERVED INSECT MATERIAL

[Issued in terms of the Animal Diseases Act, 1984 (Act 35 of 1984)]

Authority is hereby granted for you to import 100 X 50 ML VIALS CONTAINING PRESERVED ADULT TICKS by air/road from UGANDA

to South Africa through the port of entry OR TAMBO INTERNATIONAL AIRPORT subject to the following conditions:

1. The consignment must be accompanied by this original permit.
2. The insect material must be securely packed in sealed, leakproof containers, filled with formalin, alcohol or TRIzol lysis buffer, and be sufficiently preserved to ensure that it is completely immersed.
3. The consignment must be accompanied by this permit and it's arrival reported immediately to the inspecting veterinary official: KEMPTON PARK Tel: 011 973 2827, and may not be released without his/her written permission.
4. Upon arrival the inspecting veterinary official will inspect the consignment and release it to the importer only after he/she is satisfied that all the import conditions have been complied with in full.
5. The specimens must be kept and used for purposes of testing/research at the laboratories of DVTD, FACULTY OF VETERINARY SCIENCE under the personal supervision of DR W.H STOLTSZ.
6. On completion of tests/research the specimens, including all contaminated/infectious things or animal products (as defined by the Animal Diseases Act, 1984 [Act No. 35 of 1984]) derived/produced from or that came into contact with the above-mentioned specimens, must be destroyed by incineration. Records of the incinerations must be maintained for a period of 5 years, and made available for auditing to the Veterinary Authority upon request.
7. This permit is subject to amendment or cancellation by the Director Animal Health at any time and without prior notice being given.
8. This permit does not absolve the importer from compliance with the provisions of any other legislation relating to this import.

This permit is valid for three (3) months from date of issue and for one consignment only.

DIRECTOR: ANIMAL HEALTH

NOTE:

THE REPUBLIC OF UGANDA

MINISTRY OF AGRICULTURE,
ANIMAL INDUSTRY AND FISHERIES
WEBSITE: www.agriculture.go.ug

DEPARTMENT OF LIVESTOCK HEALTH AND ENTOMOLOGY
P. O. Box 513,
ENTEBBE, UGANDA
E-MAIL: dlhe.maaif@imul.com
TELEPHONE: 256 041 320 627, 320166, 320376
FAX: 256-041-321047, 256-041-321010, 256-041-321255, 320428

ORIGINAL

No.: IVHC-PRODUCTS 00046191

INTERNATIONAL VETERINARY HEALTH CERTIFICATE PERMITTING INTER-STATE MOVEMENT OF ANIMAL PRODUCTS (ALL SPECIES)

(Issued under the Animal Diseases Act Chapter 38)

i. **Identification of animal products** TICK PRESERVED SAMPLES

Type of animal species	Breed of origin	Type of animal product (semen, meat, milk, bee products, horn products, hides / skins, gall stone, silk worm products, biological / pathological samples etc)	Cartons / Bales / Pallets / Lts / trays / product area in m² for hides-skins / Grade	Gross / Net weight	Total Number / Batch Number
TICKS		TICK PRESERVED SAMPLES	100X50ML	=3.0=KG	

ii. **Origin of animal products**

Name and address of consigner / owner / exporter: DR. PATIENCE CHRISTINE AKURE, KARAMOJA, UGANDA

Place of origin of animal products (sub-county / division and district) KARAMOJA, UGANDA

iii. **Destination of animal products**

Country of final destination SOUTH AFRICA Name and address of consignee / importer: MS DEPARTMENT OF VETERINARY TROPICAL DISEASES, FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA.

Means of transport and routes of movement TRANSPORTATION BY AIR

Import Permit Number: REF: 13/1/1/30/0-2017/0003705

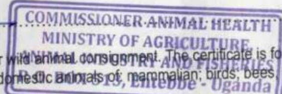
iv. **Zoo-sanitary information and attestation (delete inapplicable):**

I, the undersigned state authorized veterinary officer certifies that the animal products described above and examined on this day to the best of my knowledge:

a) ✓ Are from areas and animals free from disease. The products show no sign of disease/vectors / pests;
b) ✓ They have been produced under veterinary supervision and processed/preserved as per the required standards;
c) The breeding apiary/silkworm units are approved by veterinary authorities and the packing materials and accompanying products are to be moved directly for export from such units;
d) ✓ Are certified as fit for ~~human consumption~~ / industrial use / others – specify. RESEARCH PURPOSE

Issued at: (Location) ENTEBBE **Date** 01/11/2017 **Name, rank, address and telephone of authorized state veterinary officer** DR. SAMUEL OREE SENIOR VETERINARY INSPECTOR

Signature and stamp / seal [signature]



Veterinary inspection fee: 40,000/= per domestic animal product consignment, 60,000/= for wild animal consignment. The certificate is for a single consignment and issued in quadruplicate. Animal products here mean those derived from domestic animals of, mammalian, birds, bees, silk-worm and wild animals of terrestrial and aquatic origin.