MOLECULAR IDENTIFICATION AND CHARACTERISATION OF TICKS OF ONE-HUMPED CAMELS (*Camelus dromedarius)* FROM CAMEL REARING COUNTIES IN KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any institution.

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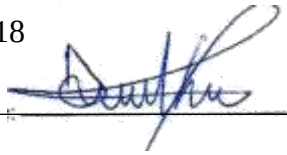
Signature:  Date: 8/12/2023

RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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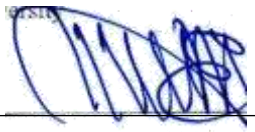
Signature:  Date: 9/12/2023

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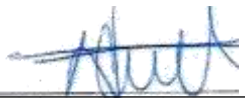
Signature:  Date: 9/12/2023

DEDICATION

To my parents, sister, wife and son Quinton Njoroge for their support and understanding throughout this M.Sc program. It has been a season of growth for all of us.

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ABSTRACT

Ticks are economically significant ectoparasites of livestock due to their ability to maintain and transmit various pathogens of medical and veterinary importance. Currently, knowledge of the diversity of ticks affecting camels in Kenya is scanty. This study aimed to assess the diversity of ticks infesting camels and the genetic diversity of *Hyalomma* tick species, the major tick species infesting camels. Adult ticks (*n* =1517) were collected from one-humped camels (*Camelus dromedarius*) from five counties in Kenya at the Athi-River abattoir and identified to the species level using taxonomical and molecular methods. Ticks comprised three genera (*Rhipicephalus, Hyalomma,* and *Amblyomma*). *Rhipicephalus pulchellus* was the most abundant tick species (*n* =739; 48.71%), while *Hyalomma albiparmatum* (*n* = 11; 0.73%) was the least abundant. Other tick species included *Hyalomma rufipes* (*n* = 306; 20.17%), *Amblyomma gemma* (*n* = 201; 13.28%), *Hyalomma dromedarii* (*n* = 97; 6.39%), *Hyalomma truncatum* (*n* = 70; 4.61%), *Amblyomma lepidum* (*n* = 45; 2.97%), *Hyalomma impeltatum* (*n* = 30; 2.01%), and *Rhipicephalus pravus* (*n* = 18; 1.18%). The nucleotide diversity (π) and haplotype diversity values of *Hyalomma* species were 0.095±0.005 and 0.953±0.020, respectively, and the average number of nucleotide differences (k) was 58.003. Tajima's D, Fu and Li's D, and Fu and Li's F test statistics were all non-significant with values of 1.008 ($p > 0.10$), 1.320 ($0.10 > p > 0.05$) and 1.440 ($p > 0.10$) respectively. Wright's F statistics for genetic differentiation between all population species was also non-significant ($p > 0.05$). This study found the existence of various tick species in camels. Awareness of the impact of these ticks on camels, plus limited veterinary infrastructure, poses a challenge to the tick control measures. In conclusion, this study recommends further detailed investigations on the population genetics of *Hyalomma* tick species to identify the presence of introgression and hybridisation and which species is driving the gene flow. The findings of this study might provide insights about hybridisation among other tick species on the possibility that it could introduce new alleles that alter the vector competence, capacity to utilize various microhabitats, or even host use.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACDS	Acute Camel Death Syndrome
AMOVA	Analysis of Molecular Variance
AFLP	Amplified Fragment Length Polymorphism
ASALs	Arid and Semi-Arid Lands
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
BTBPs	Bacterial Tick-borne Pathogens
CCHFV	Crimean Congo Haemorrhagic Fever Virus
CDC	Centre for Disease Control and Prevention
CO1	Cytochrome C Oxidase subunit 1
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
gDNA	Genomic DNA
GDP	Gross Domestic Product
ILRI	International Livestock Research Institute
ITS2	Internal Transcribed Spacer Region 2
KMC	Kenya Meat Commission
MEGA	Molecular Evolutionary Genetics Analysis
ML	Maximum Likelihood
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphism DNA
RFLP	Restriction Fragment Length Polymorphism
RPM	Revolutions Per Minute
rRNA	Ribosomal RNA
SSA	Sub-Saharan Africa
TBPs	Tick-borne Pathogens
TBDs	Tick-borne Diseases
TTBPs	Tick and Tick-borne Pathogens

CHAPTER ONE
INTRODUCTION

1.1 **Background information**

Livestock keeping is an important economic activity in Kenyan communities and is highly essential in providing food, income, and ox power labour. The animals kept include cattle, sheep, camels, donkeys, and poultry. The main species reared by the various ethnic communities are primarily dependent on customs and beliefs associated with them, the animal's ability to adapt to different climatic conditions and the prevailing environmental conditions. Economic benefits from the livestock sector vary from rural to urban households. This includes sales from meat and milk, skins used in the hides industry, transport and labour provision in ploughing, and paying of bride price. The Kenyan livestock sector contributes 10% and 42% of the country's and agricultural gross domestic product (GDP), respectively (SNV, 2018). This, therefore, is an essential pillar of the economy.

In livestock keeping, camels stand out as unique animals. They possess unique physiological, anatomical, and behavioural adaptive features. These features play a huge role in helping them cope with the torrid heat and extreme desiccation in the Arid and Semi-arid lands (ASALs). Some of the features include a long loop of Henle, which is six times longer than that of cattle helping in concentrating urine and reducing urine flow (Fesseha and Desta, 2020), thermoregulation, specialised digestive tract, large footpads, body size, and height (Hoter *et al.*, 2019). These features help them survive the harsh conditions ensuring they can go without water for long periods. About 80% of the world's camel population is in Africa, with Kenya being ranked fifth worldwide after Somalia, Sudan, Ethiopia, and Mauritania (FAO, 2018; Isako and Kimindu, 2019). In rural households, camels provide milk, meat, blood, transport, and performing traditional rites such as dowry payment and burial ceremonies (Gitao *et al.*, 2021). Nationally, they contribute approximately KES 3 billion annually (Yazan and Oliver, 2015).

Due to camels' inadequate care, the presence of a diversified environment and different agro-climatic zones in Kenya, a wide range of endo and ectoparasites can thrive in camels. Among these ectoparasites, ticks are of economic importance (Bezerra-Santos *et al.*, 2023). Ticks are obligate blood-feeding parasites of terrestrial vertebrates. There are 13 genera and more than 800 species of ticks distributed worldwide, with seven genera and 650 species found

in Africa. There are two prominent families of ticks: Argasidae or the soft tick and Ixodidae or hard tick, differentiated by a sclerotised cuticle (scutum) that the hard tick possesses. During feeding, a tick can draw up to 8ml of blood from its host. In heavy infestation cases, significant blood is lost from the host causing anaemia, tick worry, skin injury, and sometimes tick paralysis (Hurtado and Giraldo-Ríos, 2018). They also act as essential vectors for a wide range of pathogens, including rickettsia, protozoal, bacterial, and viral strains (Abdullah *et al.*, 2018, Al-Deeb *et al.*, 2015).

In tropical and sub-tropical countries, vector-borne diseases of economic importance in livestock include anaplasmosis, theileriosis, babesiosis, heartwater, and trypanosomiasis (Asmare *et al.*, 2017). *Theileria sp* and *Babesia sp* are apicomplexan haemoprotozoan parasites spread by ticks and cause significant morbidity and mortality in livestock. *Trypanosoma evansi*, on the other hand, is spread mechanically by biting insects, especially stomoxys, tabanids, and possibly by ticks (Al-Harrasi *et al.*, 2023). However, the role of ticks as potential vectors of *Trypanosoma evansi* through the ingestion of contaminated blood and engorged ticks has not been demonstrated (Vergne *et al.*, 2015).

Among the tick-borne pathogens of veterinary importance in Sub-Saharan Africa (SSA), *Theileria parva* is the most important. It causes East Coast Fever (ECF) which is characterised by enlarged lymph nodes, laboured breathing, weight loss, anaemia, nasal discharge, fever and recumbency (Amzati *et al.*, 2019). Bacteria such as *Ehrlichia ruminantium* which causes heartwater, characterized by sudden onset, lethargy, extreme respiratory distress, occasional nervous signs and corneal opacity, and mortality of close to 100% in adult animals in the absence of antibiotic treatment (Cangi *et al.*, 2017) is also a menace to the veterinary health of livestock in SSA. Also, intracellular alpha-proteobacteria, including the families Rickettsiaceae, Anaplasmataceae, and Bartonellaceae, may be spread by ticks (Driscoll *et al*., 2021). *Anaplasma marginale,* transmitted by at least 20 tick species, has the most comprehensive prevalence among the tick-borne bacteria of livestock worldwide (Akwongo and Byaruhanga, 2024). *Pasteurella multocida* has been shown to cause disease in both livestock and humans, including bovine haemorrhagic septicaemia in cattle and buffalo and fowl cholera in poultry (Mostaan *et al.*, 2021).

Ticks and tick-borne pathogens (TTBP) are of global importance to humans and livestock. Principally, camel ticks are responsible for economic losses due to high morbidity, animal deaths and the costs associated with disease control. This includes tick and parasite control, along with losses in body weight, fertility, and milk production. *Hyalomma dromedarii* is the most common tick affecting camels and other livestock, resulting in interspecies transmission of parasites. Some of the TBPs that have been found in camels include *Theileria camelensis, Theileria dromedarii, Babesia bovis, Babesia bigemina, Anaplasma camelii and Erhlichia sp.*

1.2 Statement of the problem

Over the years, ticks have been a significant challenge to livestock farming in Kenya (Kasaija *et al.*, 2021). This is mainly due to their capacity to spread a wide array of pathogens, including protozoa, bacteria, rickettsia, spirochetes, and viruses. In 2016, economic losses due to ticks and tick-borne diseases (TTBDs) globally were estimated to range from US$ 20-30 billion per annum (Lew-Tabor and Valle, 2016). Ticks also cause mild to severe anaemia, a reduction in growth rate due to lack of appetite, and decreased production. Additionally, direct damage includes damage to udders, myiasis due to infection of injured sites by maggots and microorganisms, and damage to teats and scrotum. This results in considerable losses in the camel sector, which in Kenya is estimated to be worth KES 3 billion (Yazan and Oliver, 2015; Isako and Kimindu, 2019). Currently, there is inadequate knowledge of the tick species infesting camels in Kenya, and the role they play in the transmission of tick-borne diseases in camels. Therefore, it is crucial to understand the prevalence of tick species, the tick-borne pathogens (TBPs) they transmit and their geographical distribution to generate knowledge that will help control ticks and the diseases caused by the pathogens.

1.3 Objectives

1.3.1 General objective

To explore the diversity of tick species infesting camels and assess their potential impact on the health and well-being of camelids in Kenya.

1.3.2 Specific objectives

i. To identify the tick species infesting camels in selected camel-rearing counties using taxonomical tools.
ii. To confirm the identified tick species infesting camels in selected camel-rearing counties using the CO1 gene marker.
iii. To assess the genetic diversity of *Hyalomma* ticks in order to determine the occurrence of interspecific hybridisation between the species

1.4 Hypotheses

This study was built on the following hypotheses:

i. There is no difference in taxonomical characteristics of tick species infesting camels in selected camel-rearing counties.
ii. There is no difference in the genetic identity of the tick species infesting camels in selected camel-rearing counties.
iii. There is no interspecific hybridisation between the species of *Hyalomma* ticks.

1.5 Justification

Dromedary camels are important food-producing animals in northern Kenya. An unknown acute and sudden death syndrome was last reported in Wajir County between December 2015 and May 2016. The syndrome manifested as mass acute deaths of adult camels in Wajir West, Wajir East, Wajir South and Eldas sub-counties (Gitonga, 2016). In January 2016, a similar outbreak occurred in Marsabit where 257 camels died in North Horr and Moyale sub-counties. The disease was also reported in Isiolo County. The accumulative mortality rate from the 2015-2016 outbreaks ranged from 2.2% to 2.6% per location and herd mortality of 7.5%. These mortality rates were high in camel species.

To date, the exact causative agent of this disease is not known. However, clinical and post-mortem findings from the 2016 outbreak indicated the presence of several disease conditions including heartwater, a tick-borne disease of sheep, goats, and cattle that is endemic throughout sub-Saharan Africa but has only been described in camels in other parts of Africa on three occasions in the past 60 years (Bornstein and Younan, 2013; Karrar, 1960). The disease threatens over three million camels which support the camel meat and milk industry worth

approximately US$11 million annually and the livelihoods of millions of herders in northern Kenya. The losses accrued during these outbreaks are enormous both to the owner and the economy. The loss of a camel results in approximately KES 100,000 loss in terms of assets to the owner, while the costs of treatment and the control of ticks are generally high. Therefore, it is vital to have profiles of TTBPs affecting camels. This could make it easy to develop tick control strategies and prevent and manage the existing diseases caused by ticks therefore safeguarding the livelihoods of pastoral communities and enhancing food production in northern Kenya as part of the Sustainable Development Goal (SDG) 2.

1.6 Limitations of the study

The number of samples analysed during the study reduced to 91 from 230 as some of the falcon tubes were poorly labelled, or the labels had come off. This also resulted in a reduced number of tick samples. Therefore, the data analysis from the reduced sample size may not have been the same if it would have been done from a larger sample size. However, the data obtained will form a basis for extensive studies in the future.

This study only relied on the COI gene alone to detect genetic hybridisation which has its limitations. This made it difficult to conclusively infer the existence of hybridisation in members of *Hyalomma* species.

The study relied on ticks collected opportunistically from another study, therefore it is unclear whether the ticks were sourced from only one-humped camels or two-humped camels were also sampled. This uncertainty may affect the interpretation of tick diversity and their associated health impacts specific to camelid species in Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Contribution of livestock to developing countries

Livestock plays a crucial role in the lives and livelihoods of close to 700 million people in the world. In Africa, livestock is a huge asset that contributes to economic growth and aids in bettering their owner's nutrition value (Zerfu *et al.,* 2023). Apart from the direct production of food, livestock contributes indirectly through skins, fibre, organic fertiliser, and fuel (FAO, 2018). In Kenya, the sector's contribution is 10% and 42% of the country's and agricultural gross domestic product (GDP), respectively (SNV, 2018). This, therefore, is an essential pillar of the economy. Among the livestock kept by nomadic pastoralists in Kenya, camels stand out as unique animals. Camels, referred to as 'ship of the desert,' are a capital reserve built up for the ASALs and come in handy when the crops and other livestock cannot thrive in harsh climatic conditions by providing meat and milk.

Some of the features which help camels cope with the extreme conditions in ASALs include a long loop of Henle, which is six times longer than that of cattle helping in concentrating urine and reducing urine flow (Fesseha and Desta, 2020), thick fur and reflective coat to reflect sunlight and keep them cool, specialized red blood cells which are oval to ensure smooth blood flow and prevent clotting during dehydration, specialised digestive tract, large footpads, body size, and height (Hoter *et al.,* 2019). These features help them survive the harsh conditions ensuring they can go without water for long periods.

Apart from the nutritional contribution, camels have a pivotal role in the local communities' social activities such as dowry payments and burial ceremonies (Gitao *et al.,* 2021). Nationally, the Kenyan camel meat and milk industry is worth approximately US$ 21 million annually (Yazan and Oliver 2015). However, ticks pose a major significant challenge to improved livestock productivity. They suck blood, causing general weakness of their hosts, and act as disease vectors between various livestock species that cause morbidity and mortality of livestock, leading to decreased productivity and income losses (Sparagano *et al.,* 2022).

2.1.1 Challenges faced by livestock farmers in Kenya

Livestock farming in Kenya is mainly confined to nomadic pastoralism. As a result, high competition for pastures, water and land arises between pastoral communities (Kagunyu and Wanjohi, 2014). Water and pasture scarcity results in pastoral migration to areas with adequate water and pastures resulting in interaction with other pastoralists. Interaction between livestock forms a hotspot for transmission of pathogens transversely with other livestock from different geographical zones (VanderWaal *et al*., 2017; Watson *et al*., 2016). Lack of water and pasture also results in wildlife-domestic animal interfaces as the pastoralists push their livestock into protected wildlife reserves (Oundo *et al*., 2020), which also facilitates the spread of pathogens across species. Ticks and tick-borne diseases are major hurdles to animal productivity in pastoralist communities (Kidambasi *et al*., 2019). Camel movement is not regulated in eastern Africa therefore camels in Kenya might have moved from Ethiopia, Sudan or Somalia which might result in the introduction of new tick species and tick-borne pathogens. It is therefore crucial for regular epidemiological studies in camels to ascertain the diversity of tick species and their role in the spread of TBPs in camels.

2.2 Biology of ticks

2.2.1 Systematics

Ticks belong to the Class Arachnida, Sub-class Acari, Order Parasitiformes, and Sub-order Ixodida (Dantas-Torres *et al.,* 2019). Globally, there exist about 878 species of ticks which are categorised into three families (Table 1), specifically the Argasidae (soft-bodied tick), Ixodidae (hard-bodied tick), Laelaptidae, and Nutalliellidae, with the latter two families having one species each which are of minor importance (Dantas-Torres, 2018; Du *et al.,* 2018; Sun *et al.,* 2019; Venzal *et al.,* 2019). The Ixodidae, characterised by a scutum, comprises about 670 species, making it the most extensive and essential tick family (Rubel *et al.,* 2021).

Tick identification to species level can be challenging (Sparagano *et al.,* 2022). However, many taxonomic keys exist, such as colour, size, scutum, anal groove, festoon, the shape of mouthparts, punctation, and colour of the legs that help identify ticks (Walker *et al.,* 2003; Dantas-Torres, 2018). First, the keys for the different feeding stages need to be used. Second, the adult Ixodids are dimorphic; therefore, separate keys are required to differentiate between males and females. Third, the larval, nymphal, and adult female stages vary in size depending on how

engorged they are. Fourth, the tick's external characters determine the morphology of ticks as they develop the keys needed for identification (Dantas-Torres *et al.,* 2019).

Table 1: Approximate number of tick species by genus (Sonenshine and Roe, 2014)

Family	**Genus**	**Approximate Number**
Ixodidae	*Ixodes*	245
	Amblyomma	102
	Aponomma	24
	Haemaphysalis	155
	Hyalomma	30
	Dermacentor	30
	Cosmiomma	1
	Nosomma	1
	Rhipicephalus	70
	Anomalohimalaya	3
	Rhipicentor	2
	Boophilus	5
	Margaropus	3
Nuttalliellidae	*Nuttalliella*	1
Argasidae	*Argas*	56
	Ornithodoros	100
	Otobius	2
	Antricola	8
	Nothoaspis	1
Total		**838**

2.2.2 Morphology of adult hard ticks

Hard ticks are ventro-dorsally compressed, with most segments fused into two parts (Balinandi *et al.,* 2020). The capitulum, which is the anterior end comprising the mouthparts (1 hypostome and two chelicerae), is used as an anchorage, feeding tube and cutting. Basis capituli is an integumental ring surrounding the mouthparts and the palps. The idiosoma is the posterior end that consists of the genital pore, regions where the legs attach and a region below the coxae

harbouring the spiracles and the anal aperture (Horak *et al.*, 2018). The male and female Ixodids can be differentiated by the fact that females are larger than males. The sclerotised dorsal shield (scutum) covers the whole back of males and only a third of the female's back (Sonenshine and Roe, 2014).

Identification of ticks to species level is based on various structural features such as palps and basis capitulum, size and shape of mouthparts, presence, and shape of the anal groove, presence or absence of eyes and festoons patterns, as well as nature of punctuations on the scutum. Figure 1 shows the general structure of a male and female hard tick.

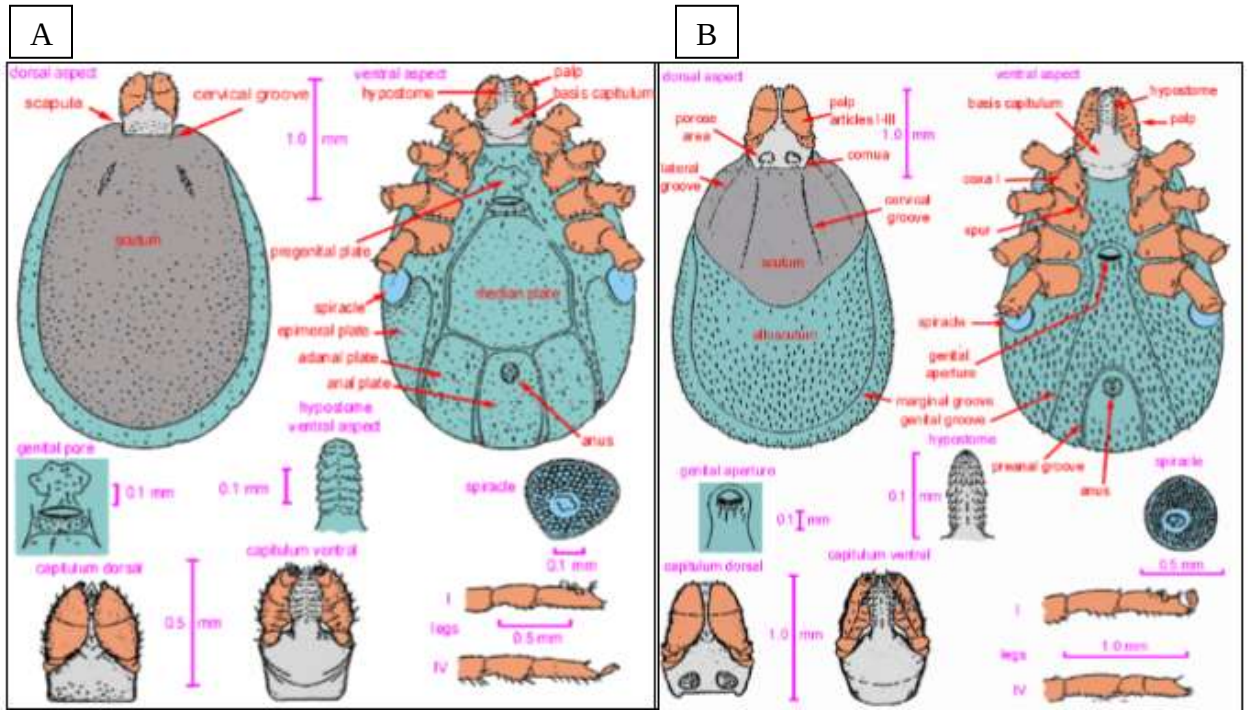

Figure 1: General structure of a male hard tick (a) and female hard tick (b). Retrieved August, 20 2019 from http://www.tickalert.org.au/index.htm based on Sonenshine and Roe, 2014.

2.2.3 Life cycle of hard ticks

Different tick species have life cycle patterns that involve feeding on one, two, or three hosts resulting in one-host, two-host, or three-host, respectively (Walker *et al.*, 2003; Dantas-Torres, 2018). In total, the life cycle may take up to 3 weeks. In one-host ticks, all three stages feed on the same animal, and two ecdyses also take place on the same animal. The larvae are the only stage involved in host location and do not fall off the host when engorged but moult into

nymphs that feed on the same host, and after three weeks, mature adults are engorged emerge (Boulanger *et al.,* 2019).

Two-host ticks, on the other hand, usually locate the hosts as larvae and again as adults. Larvae feed and moult on the host, the nymph engorges and then falls to the ground where it moults into an adult, finding a new host. On the other hand, most hard ticks are three-host ticks in which the larvae, nymph, and adults find and then feed on a separate host. Each stage feeds on a different host and falls off the ground once engorged, and moulting occurs (CDC, 2017). The larvae, nymph, and adult stages of hard ticks have a single instar, and they can feed on the host for approximately ten days with blood ingestion taking two to three days in both larvae and nymph and more than a week in adults (Boulanger *et al.,* 2019).

2.2.4 Tick ecology and climate change

Ticks have to adjust to their physical surroundings to survive and feed on their host. Because proper hosts are necessary for adult reproduction, the availability of hosts and host behaviour is essential to the maintenance of tick populations. As such, the distribution of ticks on hosts will be influenced by their distribution (Sparagano *et al.,* 2022). Ticks that are impacted by unfavourable environmental conditions during their moulting and questing stages depend heavily on their physical surroundings (Walker *et al.,* 2003; Dantas-Torres, 2018).

The intricate relationship between hosts, landscape features, and climate influences the dynamics of the tick life cycle, among other things (Ogden *et al.,* 2021). Due to its direct and indirect effects on host availability and environmental circumstances, climate has a major influence on tick population dynamics. Thus, tick distribution is being significantly shaped by recent, mostly human-caused ecological disturbances that have led to changes in the global climate. Ticks and other arthropod vectors have been able to survive and spread throughout their geographic range as a result of these modifications (Nuttall, 2022).

2.2.5 Predilection sites of ticks in camels

Ticks can shelter anywhere on the camel's coat but mostly prefer the soft regions of the camel's body. Their presence is usually unnoticed until clinical signs such as irritation or debility develop (Walker *et al.,* 2003; Dantas-Torres, 2018). Once on the camel's coat, the tick will scan through the body to locate the warmer and moist regions where it can quickly sink its

mouthparts. Although various species prefer different attachment sites, most camel ticks appear in the inguinal, perineal, and axillary areas around the lips, eyes, and ears and between the toes (Sonenshine and Roe, 2014).

2.3 Molecular markers for tick identification

It is crucial for any research to correctly identify various species. Identification based on morphology has been widely and accurately used (Kumsa *et al.,* 2016). However, for closely related species or poorly studied, morphological markers can be challenging to use. Molecular markers, therefore, are essential to circumventing this problem (Abouelhassan *et al.,* 2019).

These markers include biochemical markers that detect the gene products such as proteins and amino acids and molecular markers that detect DNA variations such as deletion, insertion, or duplication (Marwal and Gaur, 2020). Molecular markers consist of two major groups, based on DNA-DNA hybridisation and based on Polymerase Chain Reaction (PCR). PCR-based markers include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), mitochondrial genes such as mitochondrial protein-coding and ribosomal genes, and nuclear ribosomal genes (Kaur and Singh, 2020).

2.3.1 Random amplified polymorphism DNA

Introduced in 1990, random amplified polymorphism DNA (RAPD) involves testing various short (8-10 bp) random primers to amplify the target DNA in a hit-and-trial PCR without prior knowledge of the sequence to be amplified. RAPD has been used widely in genome analysis and population genetics, especially in species whose morphology fails to resolve species identity (Babu *et al.,* 2021). The amplified PCR products are resolved in agarose gel and visualised in a UV machine where the unique bands observed are used to profile the DNA. Despite its application in differentiating genetically different species, it has many limitations. This technique needs large intact DNA and mostly fails in the event of fragmented DNA. Another limitation of RAPD is it is not reliable in mapping phylogeny since the random primers have low-resolution power compared to specific primers therefore, it is highly used in DNA profiling for intraspecific genetic variation (Kaur and Singh, 2020).

2.3.2 Restriction fragment length polymorphism

This technique is mainly used for diagnostic purposes since it is easy, fast and cheaper (Teama, 2018). In restriction fragment length polymorphism (RFLP), the PCR products are digested using various restriction enzymes which are then resolved using gel electrophoresis. It is mostly used in gene mapping and genetic disease diagnosis however; it cannot detect polymorphism (Wulff *et al.*, 2017).

2.3.3 Amplified fragment length polymorphism

This technique combines both classical hybridisation techniques and PCR-based techniques for genotyping. Amplified fragment length polymorphism (AFLP), does not require one to have prior knowledge of the template DNA sequence and involves digesting the genomic DNA using restriction enzymes. Restriction half-site-specific adaptors are then attached to the restriction fragment which is followed by selective PCR amplification using specific primers having sequences specific for the adaptors and the restriction site (Kaur and Singh, 2020). AFLP has been applied in constructing genetic maps and in studying phylogenetic relationships with a higher resolving power compared to RAPD and RFLP (Sheeja *et al.*, 2021).

2.3.4 Nuclear ribosomal genes

The second internal transcribed spacer region (ITS2), the non-coding region of the nuclear ribosomal gene, has been used widely to study genetic variation in organisms (Kelava *et al.*, 2023). ITS2 serves as one of the DNA candidates due to its valuable characteristics, including the ease of its amplification and the availability of conserved regions useful in designing universal primers and adequate variations important in distinguishing closely related species (Tan *et al.*, 2020). ITS2 comprises three genes (18S rDNA, 5.8rDNA, and 28 rDNA) interpreted to RNA but not converted to proteins. These genes are usually interpreted as a single transcript of RNA and are separated by ITS1 and ITS2. ITS1 and ITS2 are then spliced out, and they no longer serve other purposes. This makes them accumulate substitutions quickly due to little selection pressure, which forms the basis for distinguishing closely related species (Kelava *et al.*, 2023).

2.3.5 Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been widely used in molecular biology because it facilitates the choice of appropriate genes for phylogenetic studies. It is divided into two

categories: ribosomal genes and protein-coding genes. The protein-coding genes are made up of 13 protein subunits (three cytochrome oxidase subunits (COI, COII and COIII), two ATPase subunits (ATP6 and ATP8), seven NADH dehydrogenase subunits (ND1, ND2, ND3, ND4, ND5, ND6 and ND4L), and one cytochrome b. The 12S and 16S rDNA make up the two mitochondrial ribosomal genes (Kowalczyk *et al.,* 2021). Due to their high copy number, these genes are easier to work with, and their maternal inheritance is strict, which is helpful at the intraspecific level (Kelava *et al.,* 2023). The mitochondrial 12S rDNA sequence has been adopted to investigate relationships between diverged ticks of the genus *Rhipicephalus* (Kowalczyk *et al.,* 2021; Panicker *et al.,* 2019).

Mitochondrial protein-coding genes have been widely used to infer phylogenetic relationships in different metazoan taxa. Thirteen subunits are divided into three groups to ascertain which subunits have better resolving power. COI, ND2, ND4 and ND5 had the best resolving power and were therefore considered good markers, NDI, COII, and COIII were deemed as medium markers while ATPase 6, ND3, ATPase 8 and ND4L were concluded as poor makers in inferring phylogenetic distances (Donath *et al.,* 2019).

Cytochrome c oxidase subunit I (COI) has been used for DNA barcoding since it is one of the most conserved mitochondrial proteins (Aslam *et al*., 2019). COI has been used as a suitable marker due to two advantages. First, it has powerful primers that are universal, enabling recovery of its 5′ ends from most animal phyla (Hoque *et al.,* 2022; Lv *et al.,* 2014). Second, its phylogenetic signal has a more excellent range compared to other mitochondrial genes. Due to this gene's rapid change allows discrimination of related species and geographic distribution of groups within a single species (Aslam *et al.,* 2019; Pedraza-Marrón *et al.,* 2019). COI can provide deeper phylogenetic insights as its amino-acid sequence evolution occurs much slower than its alternative cytochrome b and COII (Dong *et al.,* 2021; Seddigh and Darabi, 2018).

Besides COI, COII has also been widely used to infer phylogenetic relationships in different metazoan taxa. This gene varies between 673-690 bp and encodes about 226-229 amino acids (Kaur and Singh, 2020). COII is useful at both intraspecific and interspecific levels since its 3’ end is highly variable in both sequence and length (Singh *et al,* 2022). Cytochrome b is also a powerful marker to identify species together with DNA analytical techniques (Seddigh and Darabi, 2018). This gene shows a high substitution rate at the third codon which is a synonymous

one resulting in no change in the amino acid and is therefore used for nucleotide sequence studies in lower taxa while the amino acid sequence is applied in higher taxa (Kaur and Singh, 2020). Cytochrome b is an accurate, rapid as well as economic technique and it possesses similar sequence variation and an A-T bias as COI (Subbanna *et al.,* 2016)

Some of the limitations of working with mitochondrial DNA include numts, heteroplasmy and introgression. Numts are copies of mitochondrial DNA translocated in the nuclear genome. These genes are inactive as they lack start and stop codons and may easily or unintentionally be amplified using universal primers (Xue *et al.,* 2023). Impaired DNA and application of universal primers increases the probability of numt amplification which could be minimised by using specific primers and purification of DNA before PCR amplification (Kaya *et al.,* 2018). The presence of more than one type of mitochondrial genome in a cell or an organism known as heteroplasmy is another limitation to the use of mitochondrial DNA. This occurs mostly in control regions making these regions suitable for polymorphic studies however for phylogenetic studies and identification of species, the protein-coding genes which are immune to heteroplasmy are usually reliable (Pereira *et al.,* 2021). Finally, introgression which is the transfer of blocks of genes from one population to another is another limitation of mtDNA. Introgression only occurs in a small group of a population and not the whole population which might result in misidentifications as the small population portion of mtDNA might be amplified

2.4 Molecular evolution and tick phylogeny

Evolution is a slow and gradual process in which complex creatures evolve from simple ancestors through natural processes over time (Bonneaud and Longdon, 2020). Since 1900, fossil records have been used to infer evolutionary changes and generate phylogenetic trees. However, this suffers a drawback in that the fossil record is incomplete and fragmentary (Lautenschlager, 2016). Advancement in molecular biology has helped solve this problem since the blueprint of all organisms is written in their DNA; hence evolutionary relationships can be inferred by comparing different DNA (Nater *et al.,* 2015). Studies on the phylogeny of tick families have been progressing over the years, primarily through the application of molecular markers in the identification of ticks and phylogenetic analyses. A consensus on the phylogeny of the three tick families despite the several changes in the nomenclature of some species is outlined in Figure 2.

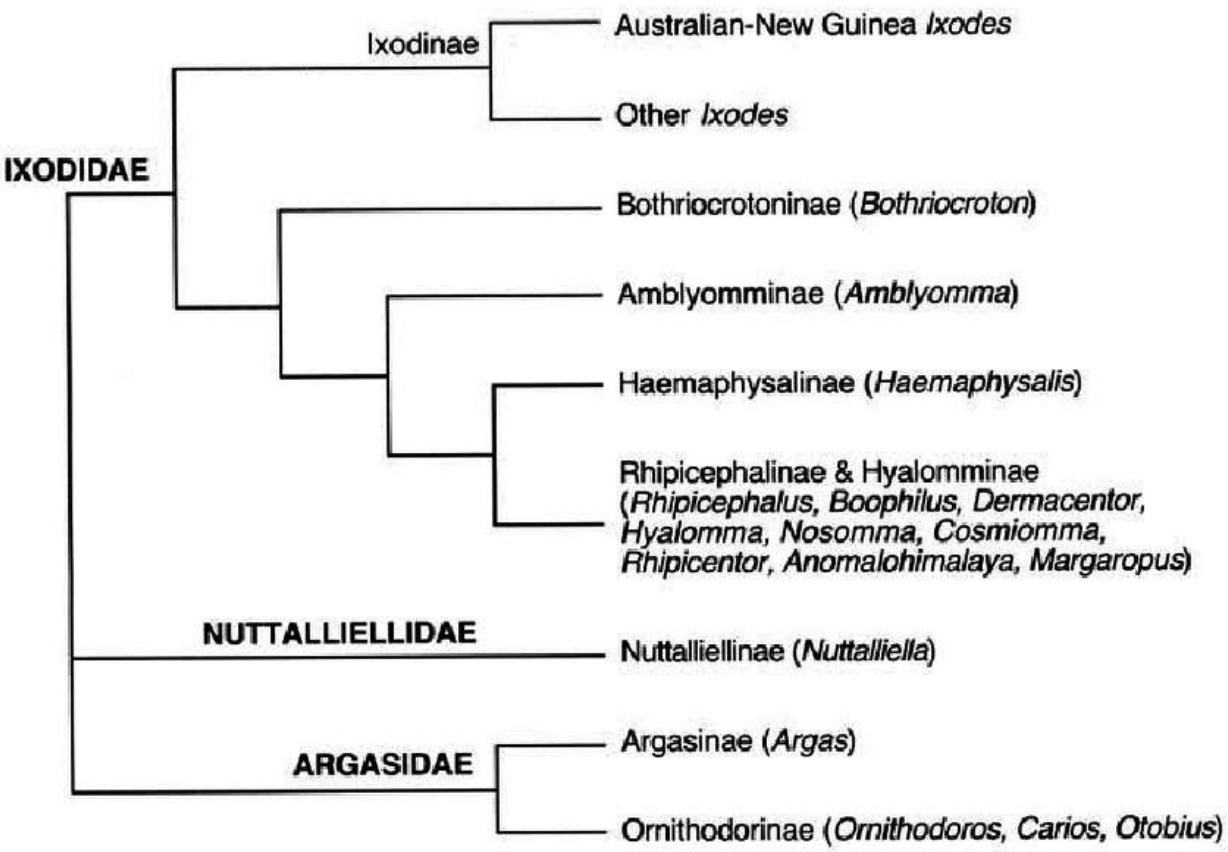

Figure 2: Current hypothesis of the phylogeny of tick families indicating the relationships between different tick taxa proposed according to analyses on nucleotide sequences as well as phenotypes (Wang *et al.,* 2019)

2.4.1 Interspecific hybridisation of ticks

It has been demonstrated that gene introgression and genetic hybridization are important sources of genetic variation within and between populations. This results in the transfer of gene blocks across various systems, causing evolutionary change that is then selected against by natural selection (Feuerstein *et al.,* 2024; Henderson *et al.,* 2017). Through gene flow, populations or species may mingle and become accustomed to new ecological niches, or the hybrid might backcross with either of its parent species, extending the genetic diversity of the parent species.

Most of the consequences of hybridization in disease vectors are unclear and have not been thoroughly studied. Inter-specific gene exchange, however, is thought to have an impact on the biology of vectors, their interactions with hosts, and even the infections they spread (Bitume

et al., 2017). The diseases carried by hybrids may spread more quickly as a result of their wider host preference and expanded range. To fully comprehend the potential role that hybridization may play in the interactions between the pathogen, vector, and host, more investigation is necessary.

Changes in the distribution of ticks have an impact on other tick species that they come into contact with in addition to their hosts through the viruses they carry. It is known that some tick species exhibit parapatric distributions, which result in species boundaries with a tiny zone of overlap between adjacent regions (Barnden *et al.,* 2023). Although resource competition and reproductive incompatibility are frequently considered to be the main causes of parapatry, other ecological characteristics like ecotones, microhabitats, and interspecific competition can also have an impact (Taylor *et al.,* 2015). These kinds of boundaries can be extremely vulnerable to environmental changes brought on by significant climatic events, including heavy downpours. A species may benefit from changes in the environment more than another, which would enable it to thrive and spread farther. Understanding the effects of extended climate change requires monitoring parapatric species where climate events cause changes to species distribution, population growth, and/or boundary movements (Harr and Price, 2014; Hunter *et al.,* 2017; Taylor *et al.,* 2015).

When interspecies reproductive incompatibility preserves parapatry, hybrid zones can arise under two circumstances (Barnden *et al.,* 2023). First, changes in the natural range of allopatric species can result in secondary interaction due to changes in the environment (Gao *et al.,* 2020; Pfaffle *et al.,* 2014). According to Westram *et al*. (2021), species can diverge along a habitat gradient or cline, resulting in a short contiguous overlap of ranges and majority segregation. It is believed that parapatric borders occur in natural systems more frequently than is presently known (Taylor *et al.,* 2015). Adjacent boundaries between morphologically similar species can be challenging to notice, as is frequently the case with many bird species (Barnden *et al*., 2023). Ticks have been known to hybridize within adjacent borders on multiple occasions (Bournez *et al.,* 2015; Sungirai *et al.,* 2017). Given that hybrids frequently carry novel and highly transmissible diseases, the possibility of tick hybridization along a parapatric boundary may influence host health (King *et al.,* 2015).

Three things usually happen to species boundaries as a result of hybrid zones: first, distinctive features of one species are lost; second, the line separating the two species becomes hazy, which might finally result in full introgression. Second, through phenomena like hybrid swarms, hybrids can endure in a certain area and maybe establish a population of their own (Hasselman *et al.,* 2014). According to Brauer *et al*. (2023), these events would be especially favourable if there was little migration occurring between the parental species and the hybrid population, or if hybrids were less susceptible to environmental changes like climate change. Finally, the hybrid zone might be significantly impacted by an unrelated biological feature, such as an ecotone, meaning that the hybrids won't have much of an impact on the boundary (Theodosopoulos *et al.,* 2019). It can be challenging to discern between hybrid morphology and parental traits in all situations where hybridization may have an impact on the morphology of the parent species (Abbott *et al.,* 2016).

Particularly with their cryptic form, ticks are challenging to taxonomically describe (Dantas-Torres, 2018). As two species of *Rhipicephalus* ticks, *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*, demonstrate, the sex and species of the hybrid parents can influence which morphotype is presented by the hybrid offspring (Barnden *et al.,* 2023). Across a broad spectrum of tick families, cryptic species and morphological variation linked to different degrees of hybridization are also frequently described (Dantas-Torres, 2018; Kovalev *et al.,* 2016). In the future, individual tick genomic analysis may be necessary to identify hybrid ticks.

2.5 Ticks as vectors and reservoirs of disease pathogens

Ticks act as vectors for a broad range of pathogens, including protozoal, viral, rickettsia, and bacterial strains (Abdullah *et al.,* 2018; Al-Deeb *et al.,* 2015). *Babesia sp* and *Theileria sp* are the major TBPs of livestock in tropical and sub-tropical parts of the world that cause babesiosis and theileriosis, respectively (Asmare *et al.,* 2017). Both *Theileria sp* and *Babesia sp* are haemoprotozoan parasites that induce various clinical manifestations that range from subclinical presentation to catastrophic disease depending on the animal species, the host's age, and the species of the pathogen (El-Naga and Barghash, 2016). *Babesia sp* is spread mainly by ticks belonging to the sub-genus *Boophilus* and is suspected to affect the camel, but the data published about its role in disease transmission is limited (Mirahmadi *et al*., 2022; Swelum *et al.,* 2014). A study done in Egypt by Barghash *et al*. (2016), indicated the presence of two *Babesia*

sp in camels at low prevalence, which were *Babesia bovis* (18.18%) and *Babesia bigemina* (27.27%). On the other hand, *Theileria sp* are prevalent in livestock and are widely distributed from North Africa to China (Gharbi *et al.,* 2020). Unlike the other pathogens, it can also be spread mechanically by biting flies of the family Tabanidae or blood-contaminated fomites (Hornok *et al.,* 2020). The most prevalent *Theileria sp* in camels is *Theileria camelensis.* Their disease transmission role is also unknown, with reports published claiming that it is non-pathogenic in camels (Moezi *et al.,* 2016). Other reports also show that *Theileria annulata,* mainly spread by ticks of the genus *Hyalomma,* is transmitted to camels in areas where camels co-habit with cattle and sheep. Still, no case of the disease has been reported (Barghash *et al.,* 2016).

Among the tick-borne bacteria of veterinary importance, *Ehrlichia ruminantium* is the most important. It causes heartwater, characterised by sudden onset, lethargy, extreme respiratory distress, occasional nervous signs and corneal opacity, and mortality of close to 100% in adult animals in the absence of antibiotic treatment (Cangi *et al.,* 2017). Besides, intracellular alpha-proteobacteria, including *Rickettsiaceae, Anaplasmataceae,* and *Bartonellaceae,* could be transmitted by ticks (Driscoll *et al.,* 2021). *Anaplasma marginale,* transmitted by at least 20 tick species, is the most prevalent tick-borne rickettsial haemoparasite of animals, including camels world-wide (Akwongo and Byaruhanga, 2024).

Anaplasma marginale causes subclinical anaplasmosis in camels characterised by loss of appetite, depression, reluctance to carry a load, pale conjunctiva, and slightly enlarged superficial lymph nodes (Ghafar *et al.,* 2014; Sudan *et al.,* 2014). A study by El-Naga and Barghash (2016) in Egypt showed that 47.4% of camels examined suffered from subclinical anaplasmosis caused by two pathogens, *Anaplasma marginale* and *Anaplasma centrale*. In Nigeria, the prevalence of blood parasites in camels was 21.5%, with *Anaplasma sp* being the most common (Azmat *et al.,* 2018).

2.6 Tick-host-pathogen interaction

The presence of suitable mammalian hosts, which can also function as carriers or reservoir hosts of infections and infect naïve ticks, determines the occurrence of ticks in a given geographic area (Sparagano *et al.,* 2022). Ticks pick up pathogens by feeding on an infected reservoir host; nevertheless, a tick's ability to spread a pathogen is contingent upon the

pathogen's ability to survive and proliferate inside the tick. The majority of infections have a biphasic life cycle. According to De la Fuente *et al*. (2017), they have multiple modifications that allow them to survive inside the tick vector and the mammalian host, hence guaranteeing their effective transmission. For instance, the majority of bacteria carried by ticks express surface proteins that adhere to tick cells. Furthermore, some, like *Anaplasma marginale*, proliferate inside the membrane-bound vacuoles of tick vector cells (Akwongo and Byaruhanga, 2024). Furthermore, successful transovarial transmission is made possible by certain viruses' capacity to grow and infect organs like the ovaries (Maqbool *et al.,* 2022).

To thwart immune system attacks, the majority of viruses have developed a variety of successful evasion techniques (Rosbjerg *et al.,* 2017). Pathogens carried by ticks in the family Anaplasmataceae order Rickttesiales, such as *Ehrlichia sp.* and *Anaplasma sp,* have effective defence mechanisms against their hosts. These bacteria enter the host cell quickly and spend less time in the extracellular space because they secrete adhesins and invasins that change the shape of the cell membrane (Moumene and Meyer, 2016). These infections release proteins into the cell that aid in taking over the functions of the host cell. Transient receptor potential proteins (TRPs), which are secreted by *Ehrlichia sp.* members, are carried to the nucleus of the host cell and alter host cell signalling pathways (Tsagareli and Nozadze, 2020). *Theileria sp.* and *Babesia sp.* are two examples of piroplasms that show antigenic diversity. Using this technique, *Babesia sp* modifies the Tp1 and Tp2 genes, while *Theilera parva* modifies the VESA1a gene on its surface (Amzati *et al.,* 2019; Hakimi *et al.,* 2020).

2.7 Tick control methods

2.7.1 Biological control

This method involves the use of natural organisms against ticks (Boulanger *et al.,* 2019). Predators, parasitoids, and pathogens are the three primary types of species that naturally oppose ticks that prey on livestock (Jamil *et al.,* 2022; Ramzan *et al.,* 2021). Ticks that are affixed to their hosts or engorged females that have fallen off the host into the ground are consumed by birds, ants, and some mite species. Wasps and other parasitoids lay their eggs in ticks, and the developed larvae consume the tissues of the ticks until they die. Ticks are infected and killed by pathogens such as bacteria, fungi, and nematodes (Jamil *et al.,* 2022). According to studies conducted in the US, wasps can achieve 25–50% natural parasitisation rates, which is an efficient

way of eradicating *Ixodes scapularis* ticks (Ramos *et al.,* 2023). A parasitisation rate of almost 50% was found in another study on wasps against *Amblyomma varium* (Dominguez *et al.,* 2023). The safest, most economical, and most efficient method of controlling ticks is biological control. It is advisable to support the integration of chickens as natural predators for livestock tick control (Pegram *et al.,* 2014).

2.7.2 Chemical control

This is the common method of tick control. Acaricides are sprayed or dipped on animals as part of the tick management technique (Boulanger *et al.,* 2019). Synthetic pesticides called acaricides are applied to cattle or the environment to eradicate ticks. Arsenicals, carbamates, amidines, organophosphorous chemicals, pyrethroids, and chlorinated hydrocarbons are a few general classes of substances that are used to kill ticks (Jamil *et al.,* 2022; Ramzan *et al.,* 2021; Pegram *et al.,* 2014). Particularly when used in conjunction with other tick management strategies, acaricides can effectively lower tick populations.

Chemical tick management techniques fall into two categories: tick host-targeted and habitat-targeted. Applications of acaricides focused on specific habitats mostly target tick habitats, such as the wooded regions surrounding homes and grazing grounds, as well as the borders along woodland edges, stonewalls, and decorative plantings (Jamil *et al.,* 2022). Although it can also be successful against adult ticks, this approach works best when controlling ticks in the nymphal stage. Using an acaricide to treat tick hosts to eliminate any ticks that might be feeding on them is known as a tick-host targeted application. Since some ticks, like *Rhipicephalus microplus,* are extremely adaptive and will likely respond to any challenge posed by a novel acaricide, the usage of chemical acaricides continues to cause resistance in ticks (Gerem *et al.,* 2016). Therefore, to extend their efficacy as tick control agents, the effective acaricides currently in use must be used correctly and efficiently. Lack of water in arid and semi-arid regions makes it difficult to apply acaricides correctly. If handlers do not use protective gear, they are subject to the hazardous effects of acaricides.

2.7.3 Mechanical control

This is the conventional method of controlling ticks, which entails manually eliminating ticks from confined animals by hand (Pegram *et al.,* 2014). Infested pastures must also be burned, farms and pasture areas must be fenced off, and the tick's natural habitat must be

disrupted or changed. The majority of mechanical control techniques are used in integrated tick management strategies, which combine several techniques to successfully lower tick populations. The method's drawback, though, is that it takes a lot of time and is inefficient for large herds.

2.7.4 Use of anti-tick vaccines

Vaccinating cattle against specific protein antigens can help prevent tick infestation. Immunity against tick infestation is induced by vaccination (De la Fuente *et al.,* 2016). This approach is a more ecologically friendly way to control tick infestations than the mechanical and chemical methods. Targeting the common tick vector can effectively manage some TBPs (De la Fuente *et al.,* 2016; Rego *et al.,* 2019). Since vector-borne pathogens use tick proteins to spread, targeting a pathogen in the vector and preventing its spread is a novel and promising way to manage vector-borne infections. Nonetheless, a significant barrier to the production of vaccines is the identification of appropriate antigens (De la Fuente *et al.,* 2016).

2.7.5 Use of tick-resistant livestock breeds

According to Bhowmick and Han (2020), tick resistance is an acquired trait that allows an animal to control the number of ticks that they encounter and their growth and persistence. According to studies, alien European cattle breeds (*Bos taurus*) are less susceptible to tick infection than native breeds (*Bos indicus*) and Sanga (*Bos taurus* and *Bos indicus* crossbreed) (Itenge *et al,* 2020; Shyma *et al.,* 2015). Tick resistance can appear in domestic animals in a variety of ways, such as increased familiarity with native cattle from the grazing region with the infected zone or host morphological variations that reduce the likelihood of tick attachment (Itenge *et al,* 2020). According to Shyma *et al.* (2015), skin thickness also seems to have a significant impact on a host's ability to resist ticks. Research has indicated that skin secretions, coat type, and hair density could all contribute to livestock resistance to ticks. Lighter-coloured animals are more resistant to ticks than dark-coloured animals, according to studies (Bhowmick and Han, 2020). Compared to their peers, females, pregnant animals, and younger animals have greater resistance (Bhowmick and Han, 2020; Shyma *et al.,* 2015).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Sampling site and study animal

This retrospective study was conducted using samples collected opportunistically during a field survey at the Athi River abattoir in Athi River district, Machakos County. Located between 1° 27′ 0″ S and 36° 59′ 0″ E, Athi-River abattoir is the largest abattoir in Kenya. The slaughterhouse can handle 1000 large animals and 1500 small stocks per day. The samples were collected from dromedary camels (*Camelus dromedarius*) from Isiolo, West Pokot, Marsabit, Baringo, and Wajir counties meant for slaughter at the abattoir.

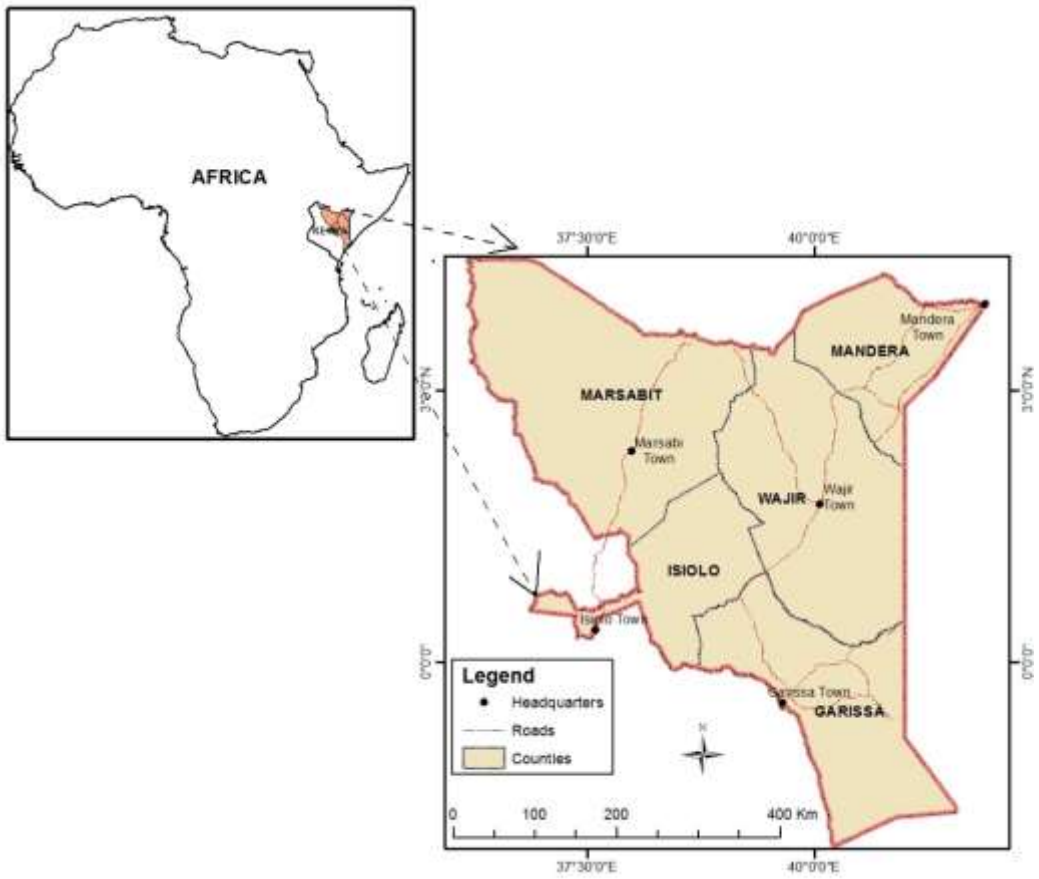

Figure 3: Map showing the counties in which the camels originated and are considered camel rearing

3.2 Tick sample collection

Ticks were collected during the dry and wet seasons of 2015 from a total of 230 camels (Table 2) between February and August. A comprehensive physical examination was conducted on each camel. Information concerning sex, age, collection date, and holding duration in the abattoir was recorded carefully, according to Mwamuye *et al*. (2017). After proper restraining of the camel, the ticks were collected from the different predilection sites using steel forceps. Ticks from each camel were pooled together and preserved in falcon tubes containing 70% for identification under a binocular stereomicroscope. Falcon tubes were labelled regarding the site and animal number, and the information was recorded in a data sheet.

Table 2: Number of camels sampled in the wet and dry season of 2015 (Deem *et al.*, 2015)

Season	**Month (2015)**	**No of camels sampled**	**No of males**	**No of females**
Wet	February	37	21	16
	March	74	41	33
Dry	June	39	20	19
	August	80	35	45
Total		**230**	**117**	**113**

3.3 Tick sample preparation and morphological identification

The samples were washed twice with distilled water and then dried on a bleached pulp. The ticks were then placed in Petri dishes and examined under a binocular stereomicroscope (ZEISS Stemi 508, Zeiss, Oberkochen, Germany), where identification of ticks was done to species level according to Hoogstraal (1956), Walker *et al.* (2003) and Horak *et al.* (2018), taking into consideration recent names of the genus and species. The identification features of the ticks were colour, size, scutum, anal groove, festoon, the shape of mouthparts, punctation, and colour of the legs. Tick images were then captured using the ZEISS Axiocam ERc 5s camera and ZEN 3.2 (blue edition) software, Carl Zeiss Microscopy GmbH, Germany.

3.4 DNA extraction for molecular identification

After morphological identification, the ticks were pooled into pools of 3-8 adult ticks according to the genus and animal number (Mwamuye *et al.,* 2017). Representative species from each pool were removed and genomic DNA was extracted using QIAGEN® DNeasy® Blood & Tissue Kit (QIAGEN GmbH-Germany) according to the manufacturer's instructions. Briefly, individual ticks were placed into 1.5mL microcentrifuge tubes pre-cooled in liquid nitrogen, and a few drops of liquid nitrogen were added. Using a sterile pestle, the ticks were ground, and 180µL Buffer ATL was added, followed by 20µL Proteinase K. The contents were mixed thoroughly by vortexing and incubated at 56 °C overnight in a shaking water bath. After the overnight incubation, the tubes were vortexed thoroughly, and 200µL Buffer AL was added. The tubes were incubated in a shaking water bath at 70 °C for 10 minutes, after which 200µL of absolute ethanol (96–100%) was added and the contents vortexed again.

The mixture was then transferred into a DNeasy Mini spin column in a 2mL collection tube and centrifuged at 8000 rpm for 1 minute. The flow-through was discarded, the spin column was placed in a new 2mL collection tube, 500µL Buffer AW1 was added, and the contents were centrifuged again for 1 minute at 8000 rpm. The flow-through was discarded, and the spin column was placed in a new 2mL collection tube, and 500µL Buffer AW2 was added. The contents were centrifuged for 3 minutes at 14,000 rpm. The flow-through was discarded, the spin column was put in new collection tubes, and the contents were centrifuged for 1 minute at 14,000 rpm to remove excess residual buffer AW2. The spin-column was placed in a new 1.5mL microcentrifuge tube, and 60µL of warmed Buffer AE was added directly onto the DNeasy membrane for elution. The mixture was incubated at room temperature for 10 minutes. The microcentrifuge was then centrifuged for 2 minutes at 8000 rpm to obtain the eluate, and the DNA samples were aliquoted and stored at -20 °C for further processing.

3.5 PCR amplification and purification of CO1 gene

Amplification of a 700 bp CO1 gene fragment was done for each of the tick DNA extracts using a specific primer pair listed in Table 3. A volume of 50µL was used for the PCR reaction, which consisted of 25µL of 2x BioMix™ Red (Bioline, London, UK), 1µL 10pmol of both the primers, 5µL of the gDNA as the template, and the remaining volume made up by nuclease-free water. PCR amplification was done on a DNA Engine®Dyad Peltier Thermal

Cycler (BIO-RAD, Hercules, CA) conditions were an initial denaturation at 95 °C for 5 minutes. This was followed by 35 cycles of 94 °C for 1 minute, 40 °C annealing temperature for 1 minute, and extension at 72 °C for 1½ minutes. Final elongations were carried out at 72 °C for 10 minutes. The PCR products were then separated by agarose gel electrophoresis.

Table 3: Sequences of oligonucleotide primers used for PCR amplification of tick and pathogen DNA

Gene	Primer ID	Sequence	Reference
CO1 (Tick DNA)	F- LCO1490	5′-GGTCAACAAATCATAAAGATATTGG-3′	Hoque *et al.*, 2022
	R- HC02198	5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	
CO1 (Hybridisation)	F-C1-N-2312	5′- CATACAATAAAGCCTAATA-3′	Bilbija *et al.*, 2023
	R-TY-J-1449	5′- AATTTACAGTTTATCGCCT-3′	

Amplified CO1 PCR products were purified using GENECLEAN® III Kit (MP Biomedicals, USA) as per the manufacturer's protocol: Three volumes of NaI solution were added to 1 volume of the PCR sample and mixed. Five µl of EZ-GLASSMILK® was added to the DNA/NaI solution mixed and incubated at room temperature for 10 minutes. The mixture was then centrifuged at 14,000 rpm for 30 seconds to pellet the EZ-GLASSMILK® with the bound DNA. The supernatant was discarded, and 500µL of prepared NEW Wash was added, followed by centrifugation at 14,000 rpm for 30 seconds. The supernatant was discarded, and the wash step was repeated. The pellet was then dried at room temperature for 10 minutes, after which 5µL of buffer TE was added and mixed with the pipette tip. The samples were then centrifuged, and the supernatant containing DNA was removed and placed in labelled PCR tubes, which were sent for sequencing.

3.6 Hybridisation studies of *Hyalomma* species using CO1 gene

Forty-six *Hyalomma* ticks were separated and amplification of an 823 bp CO1 gene fragment was performed using primers listed in Table 3. A volume of 50µL was used for the

PCR reaction, which consisted of 25µL of 2x BioMix™ Red (Bioline, London, UK), 1µL 10pmol of both the primers, 5µL of the gDNA as the template, and the remaining volume made up by nuclease-free water. PCR amplification was done on a DNA Engine®Dyad Peltier Thermal Cycler (BIO-RAD, Hercules, CA). Conditions for amplification were an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 45 °C annealing temperature for 1 minute, and extension at 72 °C for 1 minute and final elongations were carried out at 72 °C for 10 minutes (Bilbija *et al.,* 2023). Successful amplification was determined by resolving 5µL of the PCR products by electrophoresis in 2% (w/v) agarose gels containing RedSafe™ nucleic acid staining solution (iNtRON Biotechnology Inc, Korea) and DNA fragments visualized under ultraviolent light using an Analytik Jena™ UVP ChemStudio imaging system (Fisher Scientific). The remaining volumes were sent for purification and Sanger sequencing to Macrogen Inc (Amsterdam, The Netherlands).

3.7 Data analysis

3.7.1 Sequence data analysis

The COI nucleotide sequence chromatograms that were produced were examined visually and manually modified using the CLC Main Workbench program (CLC bio). Consensus sequences were created from the sequenced fragments and sequences were edited to remove low-quality reads at the 5´ and 3´ ends. Through BLASTN searches of the COI sequences against the non-redundant nucleotide sequence database at GenBank, the molecular species identity of the tick specimens was verified. The COI sequences were subjected to several sequence alignments using ClustalW2 in the CLC Main Workbench. Using DnaSP v5.10.01, the COI sequences were compressed into haplotypes.

3.7.2 Phylogenetic analysis

Using the MEGA v.11.0 program and the Kimura 2-parameter model with Gamma distribution based on the Akaike information criteria, a maximum likelihood tree was created for phylogenetic analysis (Tamura *et al.,* 2021). Using the nearest neighbour interchange improvements, tree topologies were calculated across a thousand bootstrap replicates (McRoberts *et al.,* 2023). The phylogeny of the *Hyalomma sp* mtDNA was inferred using the maximum parsimony approach. FigTree v 1.4.4 was used to illustrate the resulting trees (Rambaut, 2020).

3.7.3 Genetic diversity and population genetics of *Hyalomma* spp

Relationships between *Hyalomma* spp haplotypes were estimated using a median-joining haplotype network constructed using parsimony criteria in PopArt (Leigh and Bryant, 2015). Population diversity indices (number of segregating sites (S), average number of pairwise nucleotide differences (K), number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π)) were determined overall and for each species using DnaSP v.6 (Rozas *et al.*, 2017). Using a median connecting haplotype network built using PopArt's parsimony criterion, relationships between *Hyalomma* spp haplotypes were inferred (Leigh and Bryant, 2015). DnaSP v.6 was used to calculate the population diversity indices for each species, including the number of segregating sites (S), average number of pairwise nucleotide differences (K), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π) (Rozas *et al.,* 2017). The neutrality indices (Tajima's D, Fu and Li's F, and Fu's F) were computed in DnaSPv6 to evaluate the selective neutrality hypothesis. Wright's F-statistics for estimating pairwise genetic differences (FST), mismatch-distribution, nucleotide substitution per site (Dxy), net nucleotide substitution per site (Da), and average number of pairwise nucleotide differences (Kxy) were also computed by DnaSP. Using the Tajima-Nei model of replacement with 1000 permutations in PopArt, analysis of molecular variance (AMOVA) was utilized to examine the population-genetic structure both within and between populations (Leigh and Bryant, 2015).

CHAPTER FOUR
RESULTS

4.1 Tick species obtained

Morphological identification of a total of 1517 adult ticks was performed. Of these, three genera and nine species were identified of which (884/1517, 58.27%) belonged to *Rhipicephalus*, followed by (486/1517, 32.03%) *Hyalomma* and finally (147/1517, 9.70%) *Amblyomma* as shown in Table 4. Specifically, the identified species were *Rhipicephalus pulchellus* (48.71%), *Hyalomma rufipes* (20.17%), *Amblyomma gemma* (13.28%), *Hyalomma dromedarii* (6.39%), *Hyalomma truncatum* (4.61%), *Amblyomma lepidum* (2.97%), *Hyalomma impeltatum* (2.01%), *Rhipicephalus pravus* (1.18%) and *Hyalomma albiparmatum* (0.73%) as shown in Table 5. All the tick species were present during the dry and wet seasons with (801/1517) collected during the wet season and (716/1517) collected during the dry season. *Rhipicephalus pulchellus* was the most abundant tick during both seasons while *Hyalomma albiparmatum* was the least abundant tick during both seasons as shown in Table 6.

Table 4: Number of ticks identified according to their genus and their percentage abundance

Genus name	**Total number of ticks**	**Relative abundance**
Amblyomma	147	9.70%
Hyalomma	486	32.03%
Rhipicephalus	884	58.27%
Total	**1517**	**100%**

Table 5: Number of tick species identified, their sex ratio, and percentage abundance

Tick species	No male ticks	No female ticks	Total number of ticks	Male to Female ratio	Relative abundance
Amblyomma gemma	117	84	201	1.40	13.28%
Amblyomma lepidum	45	0	45	0	2.97%
Hyalomma dromedarii	67	30	97	2.23	6.39%
Hyalomma rufipes	201	105	306	1.91	20.17%
Hyalomma albiparmatum	11	0	11	0	0.73%
Hyalomma truncatum	29	41	70	0.71	4.61%
Hyalomma impeltatum	12	18	30	0.67	2.01%
Rhipicephalus pravus	5	13	18	0.38	1.18%
Rhipicephalus pulchellus	407	332	739	1.22	48.71%
Total	**894**	**623**	**1517**		**100%**

Table 6: Number of ticks collected during the wet and dry season of 2015

Tick species	Season			
	Wet	Relative abundance	Dry	Relative abundance
Amblyomma gemma	133	16.60%	68	9.50%
Amblyomma lepidum	35	4.37%	10	1.40%
Hyalomma dromedarii	47	5.86%	50	6.98%
Hyalomma rufipes	169	21.12%	137	19.13%
Hyalomma truncatum	16	1.99%	54	7.54%
Hyalomma impeltatum	10	1.32%	20	2.79%
Hyalomma albiparmatum	8	1.00%	3	0.42%
Rhipicephalus pulchellus	375	46.81%	364	50.84%
Rhipicephalus pravus	8	1.00%	10	1.40%
Total	**801**		**716**	

4.2 Morphological identification of ticks

Morphologically, nine species were identified using morphological markers according to Hoogstraal (1956) and Walker *et al.* (2003). The identification features of the ticks were colour, size, scutum, anal groove, festoon, the shape of mouthparts, punctations, and the colour of the legs. Images of the ticks obtained are shown in Figures 4, 5, 6, 7, 8, 9, 10 and 11

Figure 4: *Amblyomma gemma*: Male (left), Female (right).

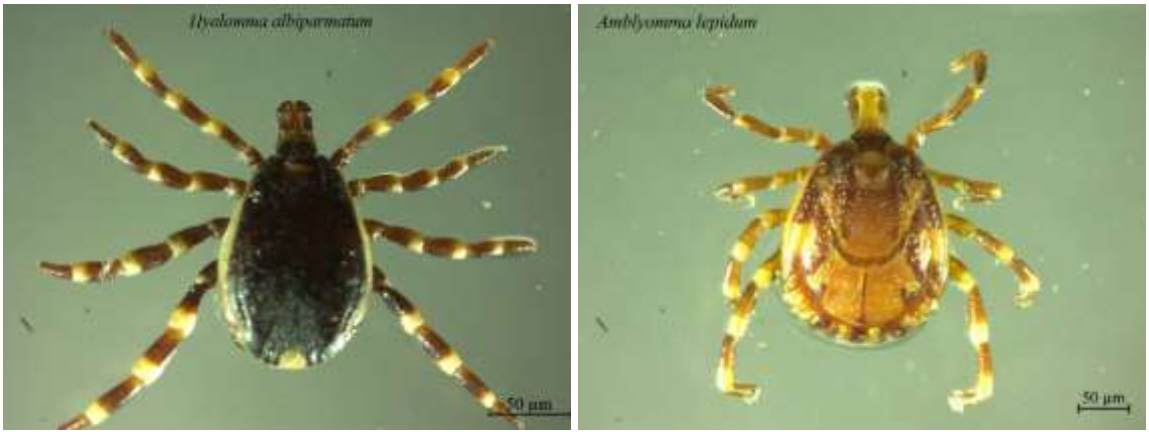

Figure 5: *Hyalomma albiparmatum* Male (left); Amblyomma lepidum Male (right)

Figure 6: *Hyalomma truncatum:* Male (left), Female (right).

Figure 7: *Hyalomma rufipes*: Male (left), Female (right).

Figure 8: *Hyalomma dromedarii:* Male (left), Female (right).

Figure 9: *Hyalomma impeltatum* Male (left), Female (right).

Figure 10: *Rhipicephalus pravus*: Male (left), Female (right).

Figure 11: *Rhipicephalus pulchellus*: Male(right), Female (left).

4.3 Molecular identification of ticks using CO1

Since most of the species were identified using morphological keys and due to the cost constraints of sequencing, only two representative ticks from each species were further characterized using CO1.

4.3.1 DNA yield and concentration

Tick DNA concentration and yield determined by spectrophotometric measurement at 260nm and 280nm wavelengths (Thermo Scientific NanoDrop™ 2000 UV Spectrophotometer) with an average of between 93.4 ng/µl and 216.2 ng/µl as shown in Table 7.

Table 7: DNA yield and concentration (ng/µl) of individual tick species

Sample	***DNA conc (ng/µl)***	***A260***	***A280***	***260/280***	***260/230***
Amblyomma gemma	*216.2*	*3.283*	*0.817*	*4.02*	*7.62*
Amblyomma lepidum	*178.3*	*2.11*	*0.742*	*2.84*	*2.54*
Hyalomma dromedarii	*134.6*	*2.388*	*0.967*	*2.47*	*1.86*
Hyalomma albiparmatum	*197.2*	*2.072*	*0.711*	*2.92*	*2.52*
Hyalomma truncatum	*132.9*	*3.01*	*1.457*	*2.07*	*0.25*
Hyalomma rufipes	*112.3*	*3.135*	*1.556*	*2.01*	*0.26*
Hyalomma impeltatum	*93.4*	*1.762*	*0.899*	*1.96*	*0.1*
Rhipicephalus pravus	*173.4*	*3.581*	*1.29*	*2.78*	*4.3*
Rhipicephalus pulchellus	*180.1*	*3.075*	*1.54*	*2.41*	*3.6*

4.3.2 Sequenced products of CO1

PCR amplification of CO1 was successful and generated a product of about 700 bp, as shown in Figure 12.

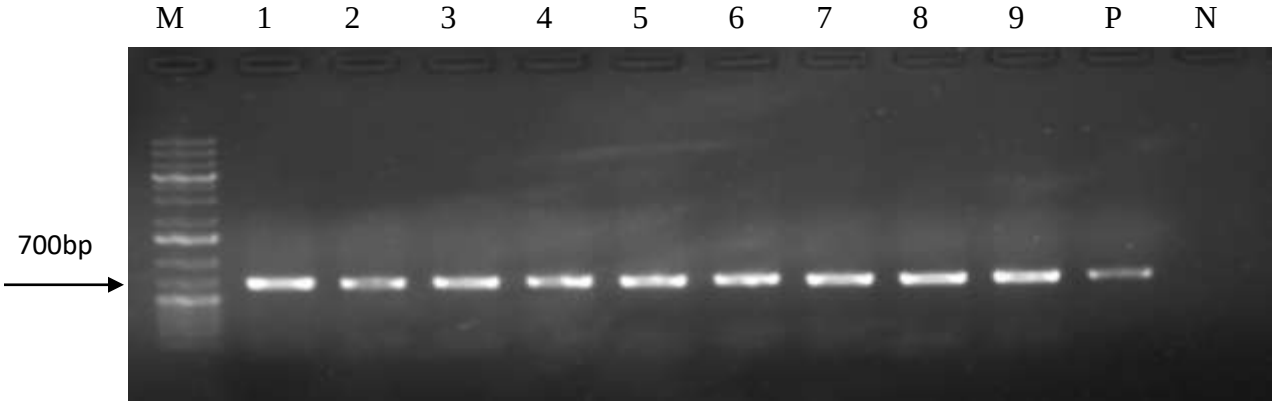

Figure 12: Agarose gel electrophoresis of CO1 PCR products of the tick species ran in 2% agarose gel.

M: 1kb molecular weight marker (HyperLadder™), 1-9: wells containing loaded DNA samples, P: positive control (*Rhipicephalus appendiculatus*), N: negative control (PCR water).

4.3.3 Molecular tick identification and phylogenetic analysis of CO1 sequences

Twenty sequences that had been modified were blasted into the GenBank and identified through a comparison with the CO1 gene's associated sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). As the closest matches to the sequences in this study, the sequences with the highest percentage similarity value were obtained from the database and used for phylogenetic analysis. Table 8 displays the GenBank sequences that had the highest percentage of similarity with the study sequences.

Table 8: Identity of tick species, their base pair sizes and percentage similarity value with the reference sequences

Genus	Tick Species	Reference Sequence	CO1 Fragment Size	% Identity	Country
Rhipicephalus	*Rhipicephalus pulchellus*	KY678133	786	99	Kenya
	Rhipicephalus pravus	KT307494	658	99	Kenya
	Rhipicephalus pravus	MF361720	658	99	Kenya
Hyalomma	*Hyalomma albiparmatum*	KU130577	644	99	Kenya
	Hyalomma albiparmatum	KU130436	644	99	Kenya
	Hyalomma impeltatum	MT896152	653	99	Kenya
	Hyalomma dromedarii	MT896151	709	99	Kenya
	Hyalomma dromedarii	KT920181	709	99	Iran
	Hyalomma truncatum	AJ437084	793	97	Ethiopia
	Hyalomma truncatum	KU568497	793	97	Guinea Bissau
	Hyalomma rufipes	KX000641	672	96	France
	Hyalomma rufipes	MT896154	672	96	Kenya
Amblyomma	*Amblyomma lepidum*	KP987775	698	99	Israel
	Amblyomma lepidum	KT307492	658	99	Kenya
	Amblyomma gemma	MT549815	674	86	China

BLASTn analysis of *Rhipicephalus pulchellus, Rhipicephalus pravus, Hyalomma albiparmatum, Hyalomma impeltatum, Hyalomma dromedarii, Hyalomma truncatum, Hyalomma rufipes and Amblyomma lepidum* sequences obtained in this study showed identities ranging from 96 to 99% with reference sequences from the GenBank while *Amblyomma gemma* sequence showed 86% similarity with *Amblyomma hebraeum* (Figure 13). Intraspecific pair-wise similarities of *Amblyomma gemma* ticks from this study compared with five *Amblyomma gemma* sequences retrieved from the Barcode of Life Database (BOLD) BIN (Cluster ID: BOLD:

ACG8914) ranged between 98.1% to 99.6% (Figure 14). Sequences from this study and the reference sequences obtained from the GenBank were aligned using the MUSCLE algorithm in MEGA v.11. Maximum composite likelihood (ML) method was used in computing evolutionary distance. Figures 13 and 14 show the phylogenetic trees generated from the CO1 sequence data with reference sequences from the GenBank and BOLD respectively. The branch length represents evolutionary changes that have taken place over time and the amount of genetic change is represented by a scale of 0.09 and 0.05 respectively. The number of substitutions related to the clustering together of the taxa as a bootstrap test that is, the number of substitutions per 100 nucleotide sites is shown above the branches. All sequences obtained in this study have been deposited in the GenBank under accession numbers OQ457668-OQ457742.

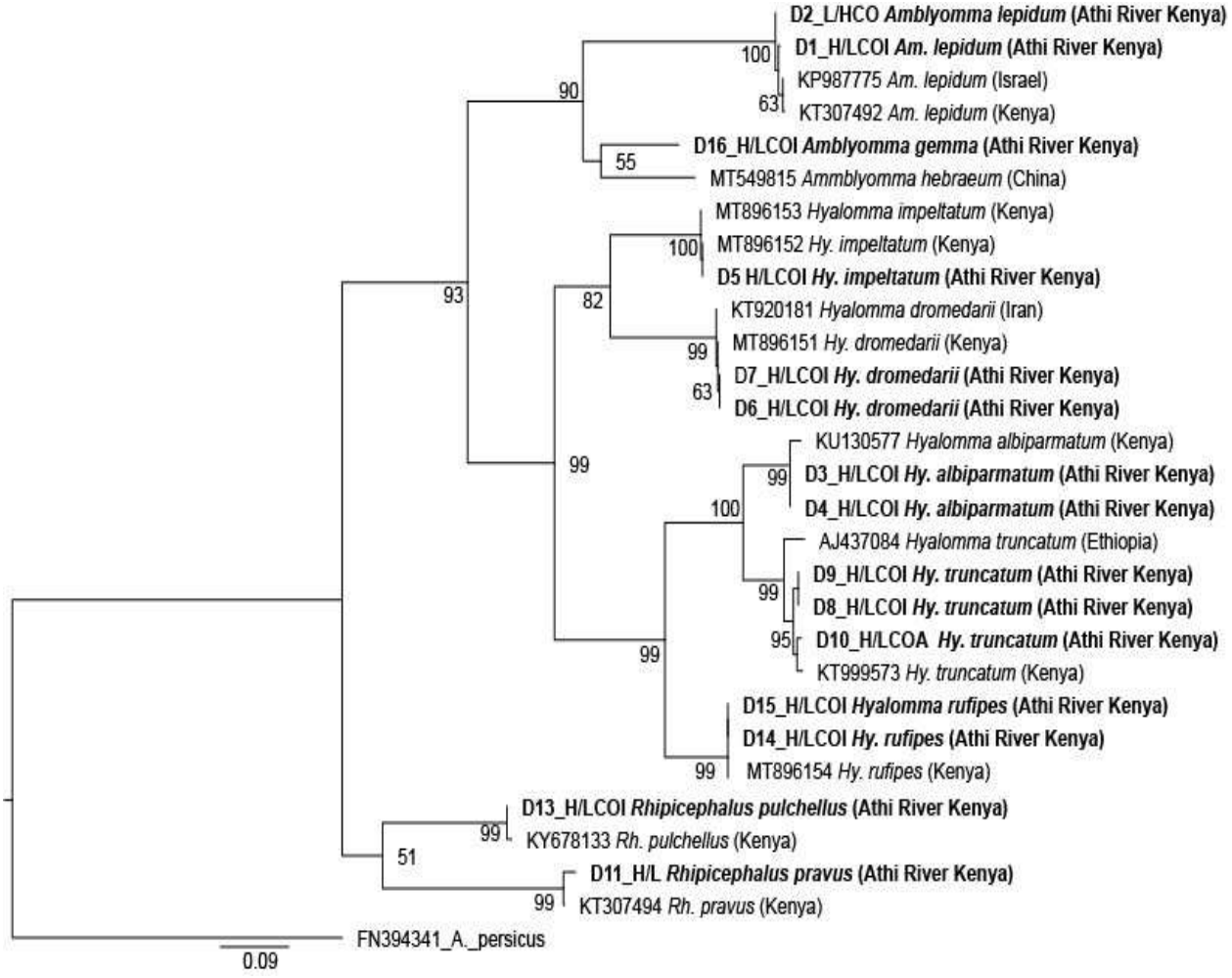

Figure 13: Maximum likelihood phylogenetic tree of tick CO1 mitochondrial gene sequences.

The information provided includes GenBank accession numbers, species identifications, and country of origin in brackets. Sequences obtained from this study are labelled D1-D1 and bolded. Bootstrap values at the major nodes are of percentage agreement among 1000 replicates. The branch length scale represents substitutions per site. *Argas persicus* was used to root the tree.

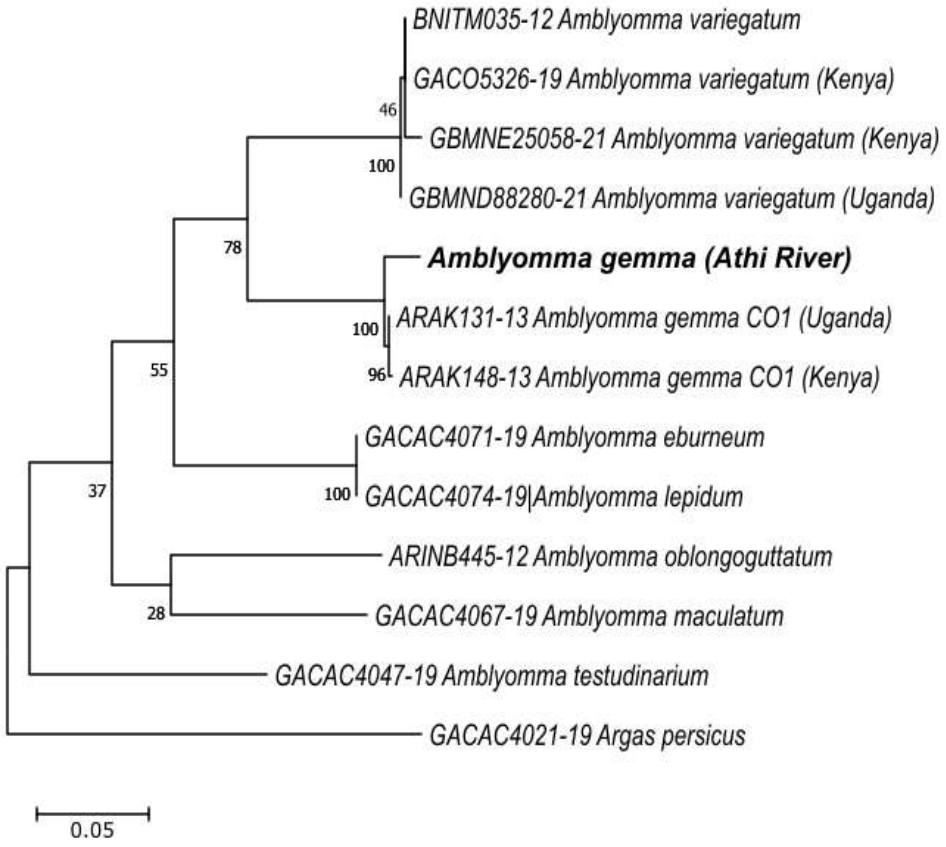

Figure 14: Maximum likelihood phylogenetic tree of *Amblyomma* species CO1 mitochondrial gene sequences obtained from BOLD database.

The data supplied include country of origin in brackets, species identifications, and BOLD accession numbers. The sequence found in this study is bolded. The major nodes' bootstrap values represent the percentage agreement across 1000 replicates. Substitutions for each location are represented by the branch length scale.

4.4 Hybridisation study of *Hyalomma* species using CO1 gene

4.4.1 Sequenced products of CO1

Ten *Hyalomma* ticks from each species were further characterized using CO1. Ten more pure colonies of *Hyalomma dromedarii* from ILRI's tick unit were also included to ascertain the purity of *Hyalomma dromedarii* from the field.

PCR amplification of the CO1 gene of representative ticks was successful and generated a product of about 823 bp fragment as shown in Figure 15.

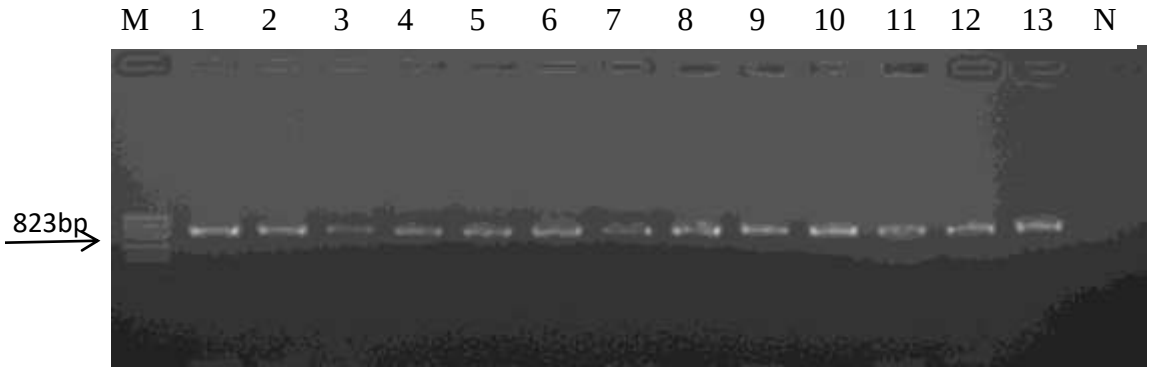

Figure 15: Agarose gel electrophoresis of CO1 PCR products of the tick species ran in 2% agarose gel.

M: 100bp molecular weight marker (HyperLadder™), 1-13: wells containing loaded DNA samples, N: negative control.

4.4.2 Phylogenetic analysis of *Hyalomma* CO1 gene

BLASTn analysis of *Hyalomma dromedarii* and *Hyalomma truncatum* sequences obtained in this study showed identities of 100% with reference sequences from the GenBank. Fourteen *Hyalomma rufipes* sequences had 100% identity with reference sequences from the GenBank while one sequence showed 98% similarity with *Hyalomma truncatum* sequences from both the GenBank and sequences from this study. Sequences from this study and the reference sequences obtained from the GenBank were aligned using the MUSCLE algorithm in MEGA v.11 and evolutionary distance was computed using the maximum parsimony method (Figure 16).

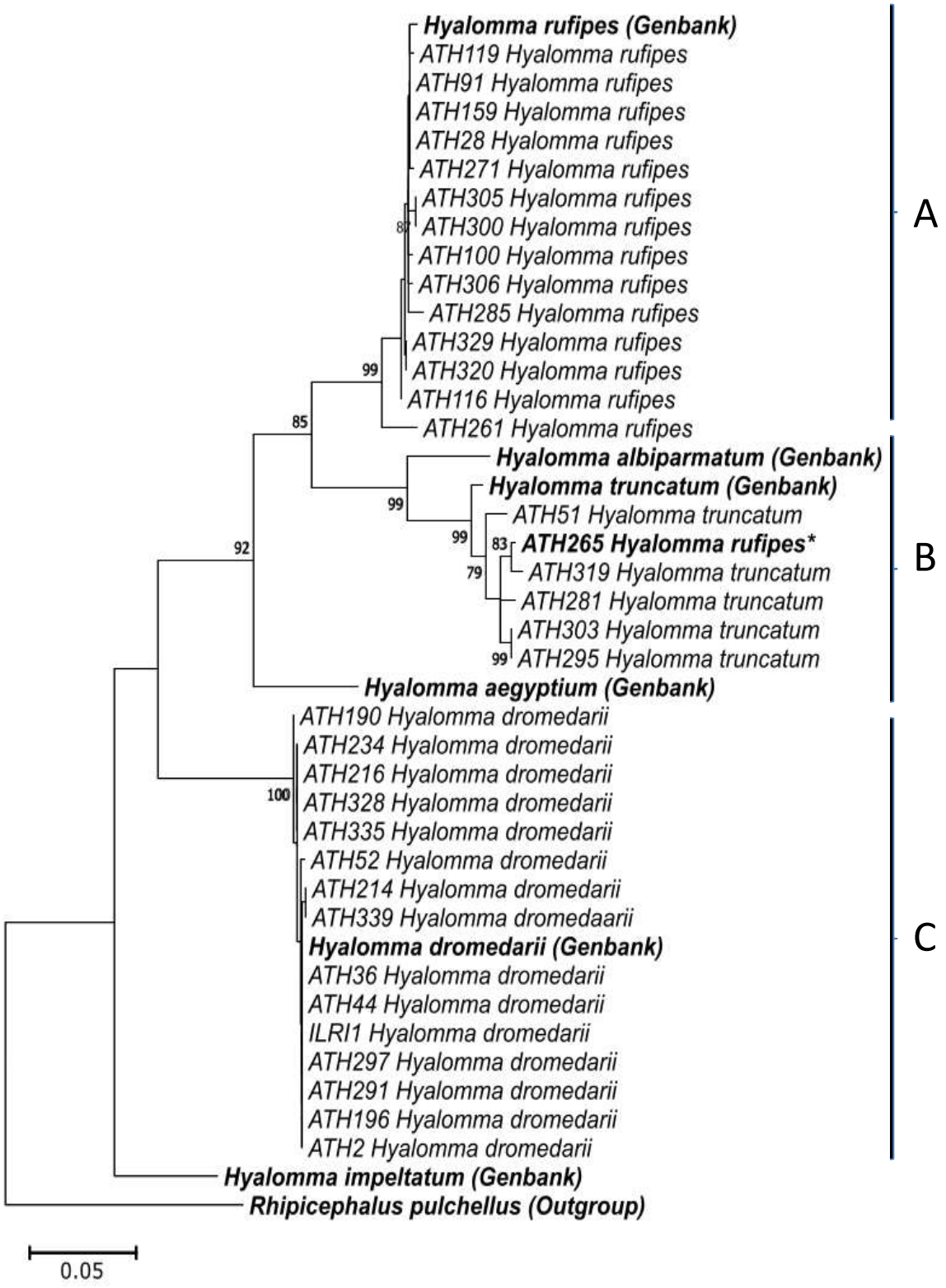

Figure 16: One of the ten equally parsimonious trees for species of *Hyalomma* using mitochondrial COI sequence data.

Branch lengths correspond to changes in substitution. Nodes getting more than 70% support (based on 1000 replicates) are indicated by bootstrap values. The letters A, B, and C stand for the major clades. Samples suspected of being hybrids and reference sequences from Genebank are bolded.

4.4.3 Genetic diversity of *Hyalomma* species

Forty sequences of the CO1 gene used to assess the relationship of *Hyalomma* species fell into 23 haplotypes (Figure 17) with 163 variable sites. *Hyalomma rufipes* had the largest number of haplotypes (h=11) followed by *Hyalomma dromedarii* (h=7), *Hyalomma truncatum* (h=4) and *Hyalomma albiparmatum* (h=1) (Table 9).

Table 9: Various haplotypes of *Hyalomma* species identified in the current study based on the CO1 gene

Haplotype	No of Sequences	Species Sample
Hap_1	2	ATH329_*Hyalomma rufipes*, ATH320 *Hyalomma rufipes*
Hap_2	1	ATH306_*Hyalomma rufipes*
Hap_3	3	ATH91_*Hyalomma rufipes*, ATH159_*Hyalomma rufipes*, ATH28 *Hyalomma rufipes*
Hap_4	1	ATH119_*Hyalomma rufipes*
Hap_5	7	ATH36_*Hyalomma dromedarii*, ATH44_*Hyalomma dromedarii*, ILRI1_*Hyalomma dromedarii*, ATH297_*Hyalomma dromedarii*, ATH291_*Hyalomma dromedarii*, ATH196_*Hyalomma dromedarii*, ATH2_*Hyalomma dromedarii*
Hap_6	1	ATH281_*Hyaloma truncatum*
Hap_7	1	ATH386_*Hyalomma dromedarii*
Hap_8	4	ATH335_*Hyalomma dromedarii*, ATH328_*Hyalomma dromedarii*, ATH234_*Hyalomma dromedarii*, ATH216_*Hyalomma dromedarii*
Hap_9	1	ATH285_*Hyalomma rufipes*
Hap_10	1	ATH265_*Hyalomma rufipes*

Hap_11	1	ATH100_*Hyalomma rufipes*
Hap_12	1	ATH116_*Hyalomma rufipes*
Hap_13	1	ATH261_*Hyalomma rufipes*
Hap_14	1	ATH281_*Hyalomma truncatum*
Hap_15	1	ATH190_*Hyalomma dromedarii*
Hap_16	1	ATH52_*Hyalomma dromedarii*
Hap_17	1	ATH271_*Hyalomma rufipes*
Hap_18	1	ATH212_*Hyalomma dromedarii*
Hap_19	2	ATH150_*Hyalomma albiparmatum*, ATH232_*Hyalomma albiparmatum*
Hap_20	2	ATH214_*Hyalomma dromedarii*, ATH339_*Hyalomma dromedarii*
Hap_21	2	ATH303_*Hyalomma truncatum*, ATH295_Hyalomma truncatum
Hap_22	1	ATH319_*Hyalomma truncatum*
Hap_23	2	ATH305_*Hyalomma rufipes*, ATH300_*Hyalomma rufipes*

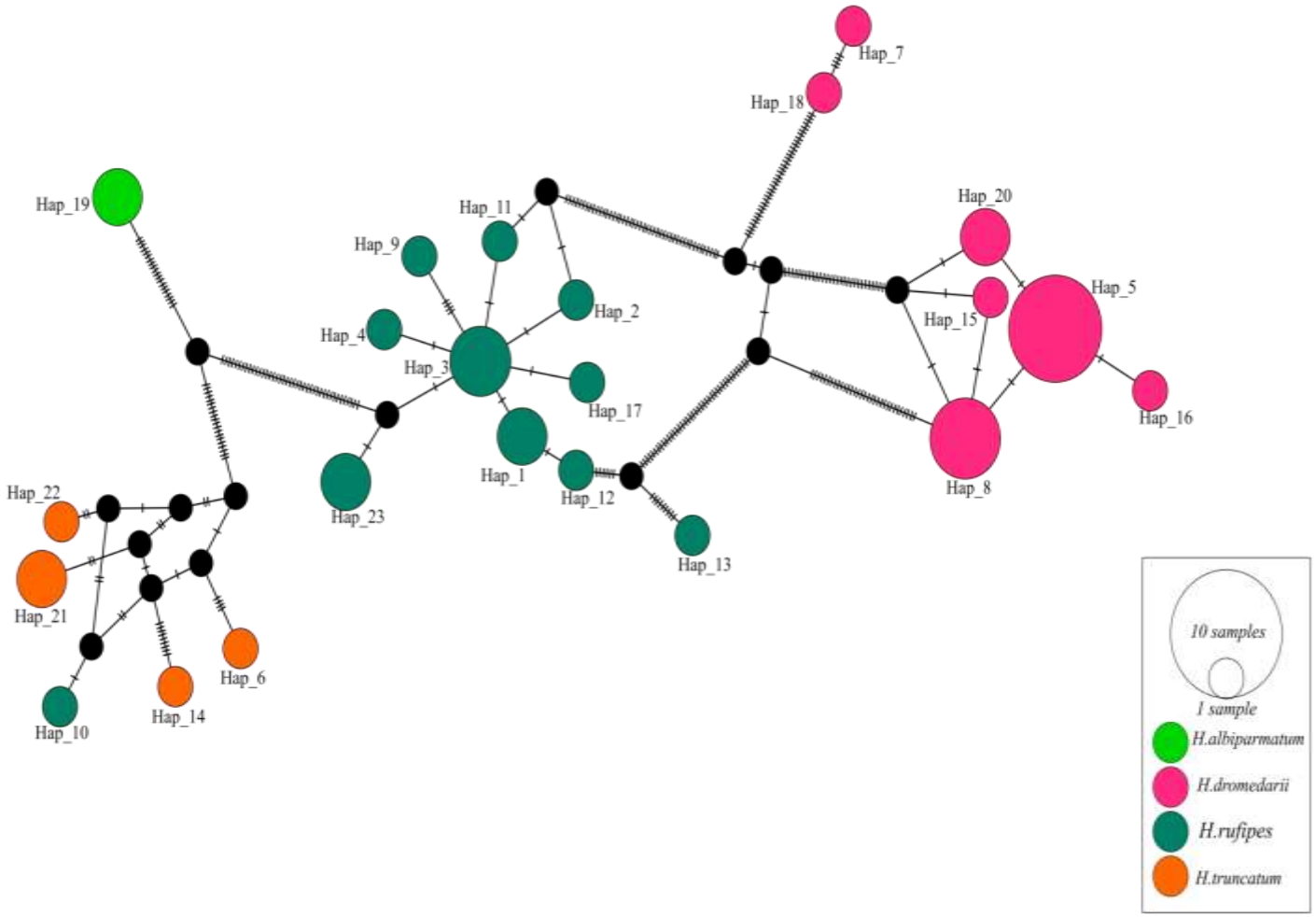

Figure 17: Median-joining CO1 haplotype network of *Hyalomma* species.

The haplotypes are shown by the circles, which show how many of each haplotype there are. Various species are represented by colours. The haplotypes' mutation places are indicated by the brief line segments. Hypothetical (unsampled) haplotypes are indicated by black circles joining sampled haplotypes.

The total number of mutations detected in the sequences was 193 with the nucleotide diversity (π) and haplotype diversity values as 0.09509±0.00499 and 0.953±0.020 respectively and the average number of nucleotide differences (k) was 58.00270. Tajima's D, Fu and Li's D and Fu and Li's F test statistics were all non-significant with values of 1.00790 ($p > 0.10$), 1.31982 ($0.10 > p > 0.05$) and 1.43958 ($p > 0.10$) respectively.

Nucleotide differences between populations (Kxy), average number of nucleotide substitutions per site between populations (Dxy), pairwise F_{ST}, inter-population nucleotide diversity (Nst), population diversity (Hs), synonymous mutation rate (Ks), genetic differentiation index based on the frequency of haplotypes (Gst) and the number of net nucleotide substitutions per site between populations (Da) are shown in Table 10. AMOVA results (Table 11) showed higher genetic variation within among species (83.05%) compared to variation within species (16.95%)

Table 10: Gene flow and genetic differentiation indices between *Hyalomma* species populations

POPULATION 1	POPULATION 2	Hs	Ks	Kxy	Gst	Nst	Fst	Dxy	Da
H. rufipes	*H. dromedarii*	0.876	19.427	77.556	0.048	0.751	0.746^{NS}	0.127	0.094
H. rufipes	*H. truncatum*	0.958	19.521	65.266	0.040	0.758	0.752^{NS}	0.106	0.080
H. rufipes	*H. albiparmatum*	0.971	20.201	64.600	0.171	0.822	0.822^{NS}	0.105	0.087
H. dromedarii	*H. truncatum*	0.811	14.784	94.352	0.078	0.871	0.863^{NS}	0.154	0.133
H. dromedarii	*H. albiparmatum*	0.794	14.644	93.941	0.198	0.915	0.912^{NS}	0.154	0.140
H. truncatum	*H. albiparmatum*	0.900	6.714	45.200	0.276	0.900	0.896^{NS}	0.074	0.066

Note: F_{ST}: Wright's F-statistics, pairwise genetic distance; Hs: population diversity; Ks: synonymous mutation rate; Kxy: Average proportion of nucleotide differences between populations; Dxy: The average number of nucleotide substitutions per site between populations; Da: The number of net nucleotide substitutions per site between populations; Gst: Genetic differentiation index based on the frequency of haplotypes; ^{NS}: not significant ($p < 0.05$).

Table 11: AMOVA analysis of population diversity of *Hyalomma* species based on CO1 gene sequences

Source	df	Sum of squares	Mean squares	% variation	Fixation index
Among species	3	75314.657	1901.295	83.05	0.83050^{NS}
Within species	35	13581.702	388.049	16.95	
Total	38	88896.359	2289.344		

CHAPTER FIVE
DISCUSSION

Camels play a significant multi-purpose role in the livelihood of the people of northern Kenya and the Kenyan economy. In rural households, camels provide milk, meat, blood, transport, and performing traditional rites such as dowry payment and burial ceremonies (Gitao *et al.*, 2021). Nationally, they contribute approximately KES 3 billion annually (Yazan and Oliver, 2015). However, a disease outbreak affecting camels has been reported in northern Kenya since 2015. Acute Camel Death Syndrome (ACDS) is an unknown disease that is affecting camel herds in northern Kenya. It is characterized by abrupt onset, fatigue, severe respiratory distress, sporadic nervous signs, and nearly 100% mortality in adult animals if antibiotic treatment is not received (Gitonga, 2016). The disease threatens over three million camels which support a camel meat and milk industry worth approximately US$11 million annually and the livelihoods of millions of herders in northern Kenya. However, clinical and post-mortem findings from the 2016 outbreak indicated the presence of several disease conditions including heartwater, a tick-borne infection, haemorrhagic septicaemia/pasteurellosis (Qarir) a bacterial infection, and trypanosomiasis/Surra (Dukan). This formed the rationale of the study which aimed at assessing the tick species infesting camels as well as the population genetic diversity of *Hyalomma* species to determine the presence of hybridisation.

From the findings, a total of 1517 ticks were identified, constituting three genera with decreasing relative abundance *Rhipicephalus* (58.27%), *Hyalomma* (32.03%), and *Amblyomma* (9.70%). This observation was per the findings of Gebremeskel *et al*. (2022) and Kiros *et al*. (2014) from Tigray and Borana areas in Ethiopia. Specifically, nine species were identified, with *Rhipicephalus pulchellus*, also known as the zebra tick, being the most abundant while *Hyalomma albiparmatum* was the least abundant. All the nine species identified were collected during the dry and wet seasons of 2015 with a slightly higher load observed during the wet season than the dry season. This finding agrees with the report of Mapholi *et al*. (2022) and Alanazi *et al.* (2020). This can be explained by the fact that high humidity facilitates the growth and survival of ticks at all their different life stages (Nuttall, 2022). The male-to-female ratio was also high, with the male ticks of all species outnumbering the females. This is generally in agreement with the report of Belete and Mekuria, (2023) and Onyiche *et al.*, 2022 from various domestic animals. The outnumbering of the male ticks is because female ticks once fully

engorged, drop off the host to oviposit while males remain on the host for several months and continue feeding and mating.

In the present study, *Rhipicephalus pulchellus* was the most abundant tick species, with a percentage occurrence of 48.71%. This agrees with the findings of Feyera *et al.* (2017), Silatsa *et al*. (2019), and Getange *et al.* (2021), who reported a prevalence of 50%, 46.8%, and 48.6%, respectively. However, a higher number of *Rhipicephalus puchellus* was collected during the dry season than in the wet season. This disagrees with the report of Walker *et al*. (2005), which described the seasonal data from northern Somalia and eastern Ethiopia on this tick, showing that it was most active during the wet season. This can be explained because it is a tick of savannah, steppe, and desert climatic regions (Walker *et al*., 2003). The tick is known to occur at altitudes below 2000m with an annual rainfall of 100mm-800mm (Walker *et al*., 2005). *Rhipicephalus pulchellus* is one of the commonest ticks in North-East Africa to the rift valley and is commonly found in areas with high numbers of cattle (Masiga *et al*., 2022), as is the case at the Athi River abattoir.

Hyalomma rufipes was the second most abundant tick species with a percentage abundance of 20.17%. This result agrees with Boulanger *et al*. (2019), who recorded an abundance of 22.9% in Nigeria. A higher number of *Hyalomma rufipes* was also observed during the dry season compared to the wet season. This is per the report of Alanazi *et al.* (2020). *Hyalomma rufipes* is active during the dry season and is primarily found in desert, semi-desert, and arid areas with low rainfall (Vatansever, 2017). *Hyalomma rufipes* is the primary vector of the Crimean-Congo Haemorrhagic Fever Virus (CCHFV) in Africa, which affects humans (Walker *et al*., 2003). CCHFV is one of the underestimated tick diseases in humans despite several cases reported in slaughterhouses in the North-Eastern parts of Kenya. A study by Sang *et al*. (2011) and Chiuya *et al*. (2021), detected CCHFV from three pools of *Hyalomma rufipes* ticks collected from camels at a slaughterhouse in Garissa and Busia respectively. *Hyalomma rufipes* being the second most abundant tick in the present study, highlights the potential risk faced by the workers at the abattoir therefore, assessment of human exposure is required as well as sensitisation of the population. Transovarial and transtadial transmission of *Babesia occultans* has also been demonstrated (Fesseha *et al*, 2022). *Rickettsia aeschlimanni, Ehrlichia sp,*

Coxxiella burnetti and *Borellia burgdorferi* have also been detected in nymphs of *Hyalomma rufipes* (Toma *et al*., 2014).

Amblyomma gemma (13.28%) was the third most abundant tick species, with 133 and 68 ticks being collected in the wet and dry seasons, respectively. This finding agreed with the report of Elias *et al*. (2020), Abdullahi *et al*. (2019), and Onyiche *et al.* (2020), who reported a prevalence of 15.00%, 13.60%, and 11.35%, respectively, and is quite the opposite with the finding of Abebaw, (2004), Alanazi *et al.* (2020) and Gelelcha *et al.* (2019) who reported a prevalence of 5.79%, 4.10%, and 7.10%. *Amblyomma gemma* is known to be prevalent in many parts of Somalia, Ethiopia, Tanzania, Kenya, and eastern Uganda (Walker *et al*., 2003). It has been shown to occur in areas receiving 100-800mm annual rainfall and is widely distributed in bushland, woodland, grassland and wooded habitats in semi-arid and arid areas between the altitudes of 500-1750m receiving 350-750mm rainfall annually (Wondimu and Bayu, 2021) which is the case at the Athi River abattoir in Machakos County. According to Walker *et al*. (2003), the tick is not known to be of economic importance; however, its long mouthparts are significant in causing udder damage and are a risk factor for mastitis in camels (Elias *et al*, 2020; Onyiche *et al.*, 2020).

Hyalomma dromedarii (6.39%) was the fourth most abundant tick in this area. This finding is comparable to Elias *et al*. (2020) and Alanazi *et al.* (2020) who reported an abundance of 3.9% and 5.9%, respectively. However, this disagreed heavily with the findings of Desalegn *et al*. (2015), Kaba (2022), Abdullahi *et al.* (2019), and Getange *et al.* (2021), who reported an abundance of 20.4%, 15.36%, 15.4%, and 26.8% respectively. This difference could be due to the variation in the ecology of the study areas. *Hyalomma dromedarii* is the most utterly desert-adapted tick in Africa and has been shown to exist at altitudes of 1700m in arid ecological zones (Wondimu and Bayu, 2021). However, Walker *et al*. (2003) described this tick's distribution in regions with Mediterranean and steppe climates in addition to deserts. Dromedary camels are the primary host of *Hyalomma dromedarii;* thus, their presence is mainly in regions where camels exist (Walker *et al.,* 2003), which is not the case in our study area the possibility that the camels might have carried these ticks from their county of origin.

Hyalomma truncatum (4.61%) was among the moderately abundant tick species, with 16 and 54 ticks collected during the wet and dry seasons. This differed slightly from the findings of

Alanazi *et al.* (2020) who reported an abundance of 7.19%. *Hyalomma truncatum* is a tick adapted to dry environments and areas of little vegetation; therefore, it is widespread in arid and semi-arid regions of eastern, central, and southern Africa (Walker *et al*., 2003). This tick is found in areas of 1070-1700m altitude. Yawa *et al.* (2018) reported this species as mainly from 900-1800m altitude with some populations down to 600m in Tanzania and Kenya. Guglielmone *et al.* (2014) stated that this tick was collected from sea level to just over 2400m in altitude. It is restricted to regions of long, continuous dry seasons of over 3-7 months and between 650-1300mm mean annual rainfall, just as Machakos County.

Amblyomma lepidum had an abundance of 2.97%, with 35 and 10 ticks being collected during the wet and dry season, respectively. This finding was comparable to that of Hussen and Agonafir (2018), who had a relative prevalence of 3.02%. At the same time, it was higher than that of Feyera *et al.* (2017) who reported an abundance of 1.1%. *Amblyomma lepidum* occupies intermediate habitats between wet and dry zones (Wondimu and Bayu, 2021). It has been found in areas of 670-1500m in altitude and a mean annual rainfall of less than 250mm (Guglielmone *et al*., 2014), which is way lower than that reported by Wondimu and Bayu, (2021), which is between 750-1000mm. Therefore, the prevalence of *Amblyomma lepidum* is high during the long dry season of about 4-7 months. However, this disagreed with our findings which showed *Amblyomma lepidum* having a high abundance in the wet season. Golo *et al*. (2017) showed that *Amblyomma lepidum* had a low prevalence during the dry season with a gradual increase in numbers during the wet season. *Amblyomma lepidum* has been shown to transmit *Ehrlichia ruminantium,* which causes heartwater, and the protozoans *Theileria mutans* and *Theileria velifera,* which causes benign bovine theileriosis (Walker *et al*., 2003).

Hyalomma impeltatum had an abundance of 2.01%. This finding was almost similar to that of Onyiche *et al*. (2020), who reported an abundance of 3.00%, while it disagrees with the results of Alanazi *et al.* (2020) who reported a very low abundance of 0.10%. *Hyalomma impeltatum* closely resembles *Hyalomma dromedarii,* with the difference being the marginal lines and the punctation pattern in males (Walker *et al.,* 2003). *Hyalomma impeltatum* is a species of the Near East, North Africa, West Africa, and East Africa. It is reported to occur in areas with between 500-750mm mean annual rainfall and from 900-1500m altitude in Tanzania,

while it has also been shown to occur in areas with altitudes below 600m and less than 250mm mean annual rainfall (Kasaija *et al*., 2021).

Other species that were collected include *Rhipicephalus pravus* and *Hyalomma albiparmatum*. *Rhipicephalus pravus* was the second least abundant tick species with an abundance of 1.40%. It is a species sensitive to the length of the dry season, preferring areas with a long dry season of up to six continuous months (Kasaija *et al*., 2021; Shekede *et al*., 2021). *Hyalomma albiparmatum* was the least abundant tick in this study, with a percentage abundance of 0.42%. This tick highly resembles *Hyalomma truncatum* and has even been cited as the synonym of *Hyalomma truncatum* (Sands *et al.,* 2017). The difference between the males of *Hyalomma truncatum* and *Hyalomma albiparmatum* is an ivory-coloured parma in the central festoon (Walker *et al*., 2003). However, it is impossible to distinguish between females of *H. albiparmatum* and *Hyalomma truncatum,* which could explain the absence of *Hyalomma albiparmatum* females in this study. According to Guglielmone *et al.* (2014), this tick is confined to East Africa (Kenya and Tanzania). It has been shown to transmit *Rickettsia conorii* (Bonnet *et al*., 2023).

BLASTn searches on GenBank using the CO1 gene marker confirmed the morphological identification of eight tick species with identity percentages ranging between 96-99% while *Amblyomma gemma* showed an identity percentage of 86% with *Amblyomma hebreaum*. However, intraspecific pair-wise similarities of *Amblyomma gemma* ticks from this study compared with five *Amblyomma gemma* sequences retrieved from the BOLD database ranged between 98.1% to 99.6%. The conflict between *Amblyomma gemma* reference sequences in BOLD and GenBank could be attributed to either errors in the reference libraries or the query sequences (Pentinsaari, 2020).

DNA barcoding has been widely used to distinguish animal species using COI as the standard marker and has been proven to be very effective (Antil *et al.,* 2023). In species that possess difficulties in taxonomic resolution, DNA barcodes have been applied for routine species identification, detection of hybrid species and detection of host-specific lineages within species (Ramírez *et al.,* 2014). An ideal DNA barcode needs to be cost-effective, reliable and allow fast species identification by users having no or little experience in taxonomy (Antil *et al.,* 2023). In

population genetics studies, DNA barcodes relay the first signal to the extent of divergence therefore facilitating comparative population studies in different species (Kaur and Singh, 2020).

BOLD and GenBank are the two main public databases of DNA barcode data for animals, plants and fungi. However, these databases are faced with a myriad of challenges including “dirty data” that might arise as a result of misidentification or poor-quality sequences which might infer wrong taxa. According to Meiklejohn *et al.,* (2019), most errors reported in the GenBank were as a result of cross-contamination as most sequences for *Hexagenia limbata* were a truncated version of that for *Glossina palpalis*. It is therefore recommended to rely on and correctly identify species morphologically and only use the GenBank or BOLD as a confirmation or in extreme cases where morphology cannot be relied upon.

Molecular markers for species identification rely on homology searches against sequences in important databases. Since individual divergence levels within the same species are lower than those of closely related species, a high identity between these sequences indicates genetic similarities (Kelava *et al.,* 2023). Since mitochondrial genes have a large copy number per cell and evolve more rapidly than nuclear genes, they have been shown to offer significant advantages as molecular markers (Abouelhassan *et al.,* 2019). As a result, they can be used to analyse variations between closely related individuals within a species. Because it is flanked by universal primers that have been utilized for investigations on a variety of metazoan taxa, the 650 base pair fragment at the 5' end of the CO1 gene, also known as the "Folmer region," has been employed under the international DNA barcoding program (Rach *et al.,* 2017).

Furthermore, the mitochondrial protein-coding gene COI has other aspects which make it stand out as an appropriate molecular marker in studying evolution. COI as the terminal catalyst in the respiratory chain has been well studied at the biochemical level. This gene consists of highly variable and conserved regions with distinct rates of mutation which makes it useful for evolutionary studies (Kaur and Singh, 2020). COI is also the largest among the cytochrome oxidase subunits therefore making it the largest mitochondrial protein coding gene in the metazoan taxa which allows the amplification and sequencing of many functional nucleotides than in any other mitochondrial gene (Pentinsaari *et al.,* 2016). The third nucleotide position that codes for proteins in COI has a high rate of substitution resulting in a greater molecular evolution rate compared to other mitochondrial genes (Young and Hebert, 2015). Finally, the amino acid

sequence of COI evolves slowly which helps in resolving species at higher species levels (Hoque *et al.,* 2022). It is because of the above reasons that COI was chosen for the present study however it should be combined with other molecular markers to get better inferences due to some limitations with this gene.

Despite the wide distribution of *Hyalomma* tick species in camels and its role as a vector for a wide array of pathogens, little is known about its population genetics and diversity. In the current study, population genetic diversity and haplotype networking of *Hyalomma* spp based on the CO1 gene were assessed. In this study, no *Hyalomma* species formed a monophyletic group. Using maximum parsimony phylogenetic analysis, just one sample of *Hyalomma rufipes* was indistinguishable from *Hyalomma truncatum*. Introgressive hybridisation, or the insertion of genes from one species into the gene pool of another, may help to explain this.

According to Rees *et al*. (2003), males of *Hyalomma dromedarii* and *Hyalomma rufipes* and the females of *Hyalomma trancatum* have been shown to hybridise producing putative hybrids. These hybrids are indistinguishable from the female (*Hyalomma truncatum*) in terms of the CO1 gene since CO1 is maternally inherited. The maximum parsimony method was used to build the *Hyalomma* species phylogenetic tree since it determines evolutionary relationships among different species based on the least changes that might have brought the present traits (Hekimoglu and Ozer, 2017). However, phylogenetic analysis is poorly descriptive of variations found in gene sequences therefore it was only used to correctly identify the *Hyalomma* species in their respective clades.

To resolve this, we used the haplotype network analysis which is more reliable even in members of the same species since it detects single nucleotide variations between sequences (Dumaidi *et al.,* 2020). Out of 23 haplotypes, Hap_3 and Hap_5 for *Hyalomma rufipes* and *Hyalomma dromedarii* respectively were widespread indicating the possibility of ancestral sequences as compared to the other haplotypes. The presence of different haplotypes as shown might favour interbreeding and subsequently increase genetic recombination (Wang *et al.,* 2019). Hap_3 was also unique in that single haplotypes around it formed a stellate shape which indicates low sequence divergence and high levels of unique mutations hinting at rapid population expansion.

Molecular diversity indices demonstrated overall low nucleotide diversity and a high haplotype diversity which is a signature of population expansion (Zhao *et al*., 2021). Despite many haplotypes (23 out of 40 sequences), some differed from each other marginally while Hap_7 and Hap_18 of *Hyalomma dromedarii* differed significantly from the rest indicating that species in these two haplotypes have been accumulating mutations over time. The genetic differentiation indices between species were high (0.746-0.912) indicating high genetic differentiation between the species. Genetic differentiation is based on Wright's F-statistic which permits analysis and measurement of genetic distance between sub-populations using the concept that the sub-populations which are not inter-breeding will have diverse allele frequencies compared to those in the total population (Matthee, 2020). Fst values between *H. rufipes* and *H. dromedarii* and between *H. rufipes* and *H. truncatum* were comparable and quite lower than between *H. rufipes* and *H. alpibarmatum, H.dromedarii* and *H. truncatum* and *H. dromedarii* and *H. albiparmatum.* This shows that there might be a degree of similarity between *H. rufipes* and *H. dromedarii* and *H. rufipes* and *H. truncatum* with *H. rufipes* possibly driving the gene flow between these species however more studies need to be undertaken using a large sample size to confirm this hypothesis. AMOVA results coincided with Fst as it showed a higher percentage variation among species but a low percentage variation within species.

CHAPTER SIX
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Camels are vital to the livelihoods of people in northern Kenya, providing milk, meat, and transport, while also contributing approximately KES 3 billion annually to the national economy. However, since 2015, an outbreak of Acute Camel Death Syndrome (ACDS) has posed a serious threat to camel populations, leading to nearly 100% mortality without treatment. The study investigated tick infestations on camels, revealing 1,517 ticks from three genera, with *Rhipicephalus* (58.27%) being the most abundant, followed by *Hyalomma* (32.03%) and *Amblyomma* (9.70%). Rhipicephalus pulchellus was the predominant species, with its abundance varying by season, contrary to earlier reports. *Hyalomma rufipes*, the second most abundant tick, poses a risk due to its role as a vector for Crimean-Congo Hemorrhagic Fever Virus.

DNA barcoding confirmed species identification, revealing high identity percentages (96-99%) for most species. Genetic analysis using the CO1 gene showed low nucleotide diversity but high haplotype diversity among *Hyalomma* species, indicating potential interbreeding and genetic expansion. Phylogenetic analysis suggested introgressive hybridization, particularly among *Hyalomma rufipes* and *Hyalomma truncatum*. The study highlights the ecological and health implications of tick infestations in camels, underscoring the need for monitoring and control.

Conclusions

This study shows a wide array of tick species infesting camels from three different genera (*Rhipicephalus, Hyalomma* and *Amblyomma*) with *Rhipicephalus pulchellus* the most abundant tick while *Hyalomma albiparmatum* was the least abundant. Molecular identification using COI gene sequences obtained from 15 representative samples was consistent with the morphology and confirmed the tick diversity.

Molecular diversity indices of *Hyalomma* species showed overall low nucleotide diversity and high haplotype diversity while genetic differentiation indices between species were high. Wright's F statistics showed some degree of similarity between three species of *Hyalomma* ticks with *Hyalomma rufipes* hypothetically assumed to drive the gene flow.

Future, present, and previous efforts are impacted by the existence of morphologically undetectable hybrids. The necessity of the present morphological evaluations, which should be accompanied by genomic validation, has been brought to light by this work. It is advised to use genomic techniques to identify phenotypic features that more reliably distinguish between species and hybrids to achieve higher accuracy in species identifications. Rapid climate change is bringing together animals that might not have otherwise interacted as they adjust to dwindling habitats. A parasite's host range, diseases, and hybridisation between two closely related species can all be further expanded through hybridisation, making newly developed host species more vulnerable to parasitic infection and disease than those that coevolved with the parasite.

The detection of hybridisation between *Hyalomma rufipes* and *Hyalomma truncatum* from this study raises several questions; How widespread is hybridisation in *Hyalomma* species? Are *Hyalomma* hybrids fertile? If yes, what are the fitness consequences of hybridisation? Does hybridisation affect the role of these species as disease vectors? Are other species of *Hyalomma* involved? Does hybridisation occur in other genera of ticks and how widespread is it? Is hybridisation linked to host use? These and further challenges await.

Recommendations

Based on the outcomes of this study, the following recommendations are put forward:

1. A comprehensive tick surveillance program in camel-rearing counties to monitor tick populations and their dynamics should be established. Regular assessments can help identify seasonal patterns and potential risks associated with tick-borne diseases, enabling timely interventions.
2. Researchers should actively confirm molecular identification through morphological analysis before utilizing sequences from biological databases. This approach minimizes reliance on potentially inaccurate sequences, ensuring reliable species identification and enhancing the validity of subsequent research and ecological assessments.
3. Further additional studies focusing on the genetic diversity and hybridization among tick species, particularly within the *Hyalomma* genus should be done. Understanding the implications of interspecific hybridization can inform management strategies and aid in predicting the spread of tick-borne pathogens, ultimately contributing to improved public health outcomes.

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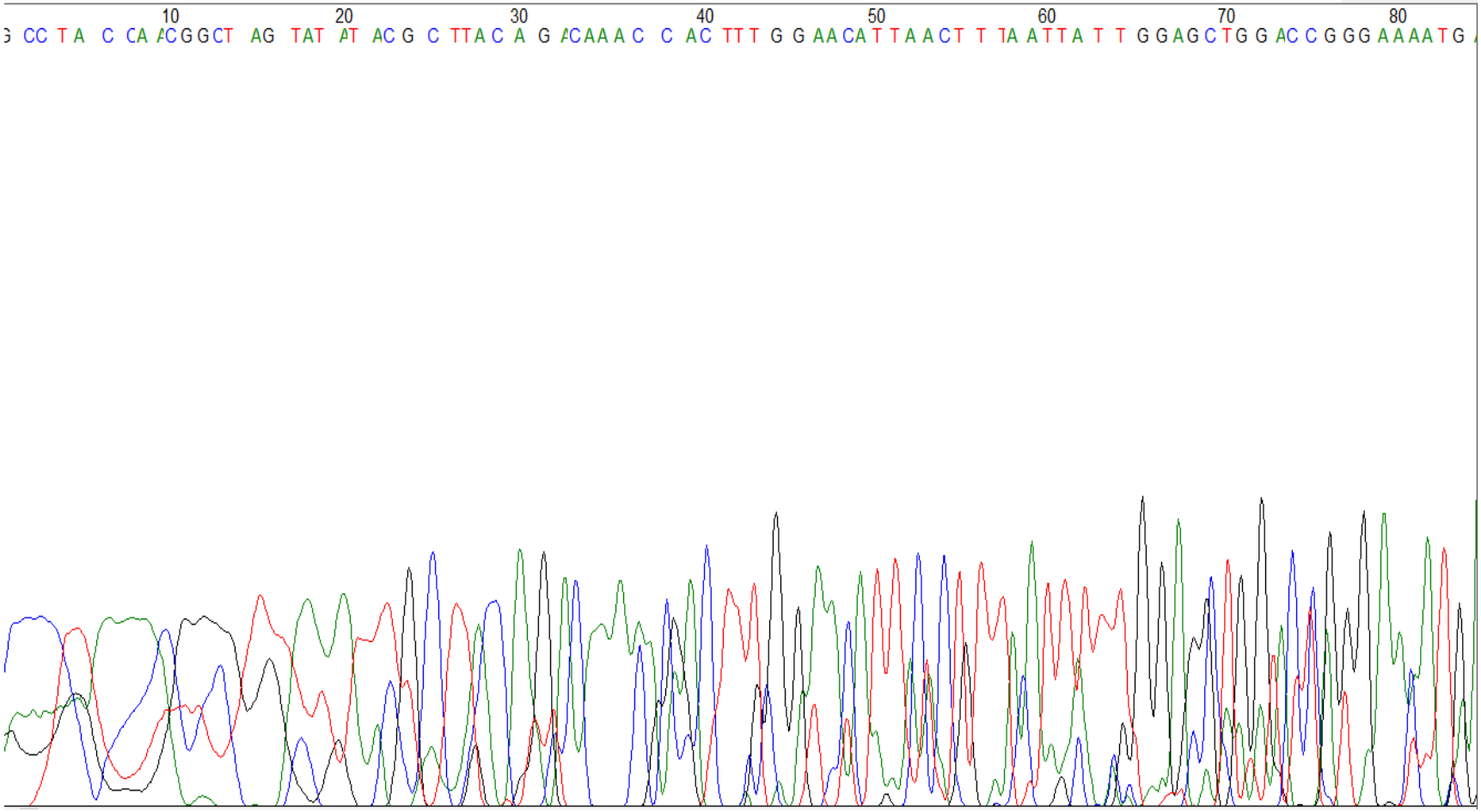
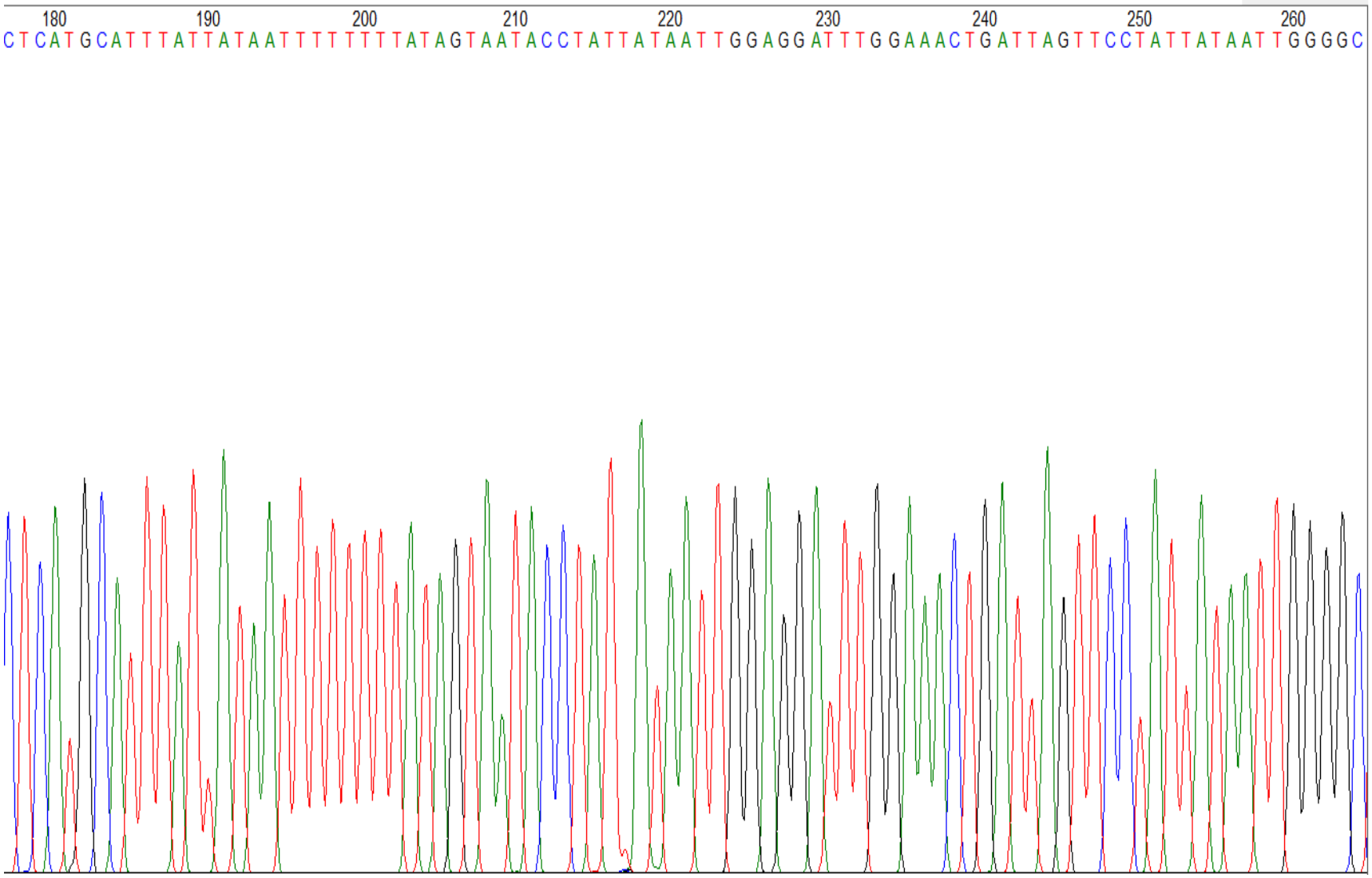
APPENDICES

Appendix I: Low quality 5’ end of a CO1 sequence chromatogram showing unclear nucleotide peaks



Appendix II: High quality 5’ end of a CO1 sequence chromatogram showing clear nucleotide peaks



Appendix III: A section of the multiple alignment of the CO1 fragment

Appendix IV: Estimates of Evolutionary Divergence between Sequences.

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Tajima-Nei model. This analysis involved 51 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 892 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. ATH 150 Hyalomma albiparmatum																			
2. ATH 232 Hyalomma albiparmatum	0.00																		
3. ATH 214 Hyalomma dromedarii	0.17	0.17																	
4. ATH 212 Hyalomma dromedarii	0.18	0.18	0.12																
5. ATH 1 Hyalomma dromedarii	0.18	0.18	0.12	0.00															
6. ATH 295 Hyalomma truncatum	0.08	0.08	0.17	0.17	0.17														
7. ATH 319 Hyalomma truncatum	0.08	0.08	0.17	0.17	0.17	0.00													
8. ATH 303 Hyalomma truncatum	0.08	0.08	0.16	0.18	0.17	0.01	0.01												
9. ATH 54 Hyalomma rufipes	0.11	0.11	0.16	0.15	0.15	0.12	0.12	0.12											
10. ATH 300 Hyalomma rufipes	0.11	0.11	0.16	0.15	0.15	0.12	0.12	0.12	0.00										
11. ATH 159 Hyalomma rufipes	0.12	0.12	0.16	0.15	0.16	0.13	0.12	0.12	0.01	0.01									
12. Rhipicephalus pulchellus (Outgroup)	0.24	0.24	0.21	0.21	0.21	0.25	0.24	0.24	0.21	0.21	0.21								
13. Hyalomma truncatum (Genbank)	0.08	0.08	0.17	0.17	0.18	0.03	0.03	0.03	0.12	0.12	0.12	0.23							
14. Hyalomma rufipes (Genbank)	0.12	0.12	0.17	0.16	0.16	0.13	0.13	0.13	0.01	0.01	0.00	0.20	0.12						
15. Hyalomma dromedarii (Genbank)	0.18	0.19	0.13	0.00	0.01	0.18	0.18	0.18	0.16	0.16	0.15	0.20	0.17	0.14					
16. Hyalomma aegyptium (Genbank)	0.14	0.14	0.14	0.15	0.15	0.14	0.14	0.14	0.11	0.11	0.11	0.22	0.14	0.11	0.15				
17. ATH 386 Hyalomma dromedarii	0.18	0.18	0.01	0.13	0.14	0.18	0.18	0.18	0.16	0.16	0.17	0.21	0.17	0.17	0.14	0.14			
18. ATH 335 Hyalomma dromedarii	0.18	0.19	0.13	0.00	0.01	0.18	0.17	0.18	0.16	0.16	0.15	0.20	0.17	0.14	0.00	0.15	0.14		
19. ATH 285 Hyalomma rufipes	0.12	0.12	0.16	0.15	0.16	0.12	0.12	0.12	0.01	0.01	0.01	0.21	0.12	0.01	0.15	0.11	0.16	0.15	
20. ATH 104 Hyalomma rufipes*	0.08	0.08	0.17	0.18	0.18	0.02	0.01	0.01	0.13	0.13	0.12	0.24	0.02	0.13	0.18	0.14	0.18	0.18	0.1
21. ATH 297 Hyalomma dromedarii	0.18	0.19	0.13	0.00	0.01	0.18	0.18	0.18	0.16	0.16	0.15	0.20	0.17	0.14	0.00	0.15	0.14	0.00	0.1
22. ATH 291 Hyalomma dromedarii	0.18	0.19	0.13	0.00	0.01	0.18	0.18	0.18	0.16	0.16	0.15	0.20	0.17	0.14	0.00	0.15	0.14	0.00	0.1
23. ATH 150 Hyalomma rufipes	0.12	0.12	0.16	0.16	0.16	0.13	0.13	0.12	0.01	0.01	0.00	0.21	0.13	0.00	0.15	0.12	0.17	0.15	0.0
24. ATH 28 Hyalomma rufipes	0.12	0.12	0.16	0.16	0.16	0.13	0.12	0.12	0.01	0.01	0.00	0.21	0.12	0.00	0.15	0.11	0.17	0.15	0.0
25. ATH 116 Hyalomma rufipes	0.12	0.12	0.17	0.15	0.15	0.13	0.13	0.13	0.01	0.01	0.00	0.20	0.12	0.01	0.14	0.11	0.17	0.14	0.0
26. ATH 305 Hyalomma rufipes	0.13	0.13	0.16	0.16	0.16	0.14	0.14	0.14	0.03	0.03	0.03	0.21	0.13	0.03	0.15	0.11	0.16	0.15	0.0
27. ATH 196 Hyalomma dromedarii	0.18	0.19	0.13	0.00	0.01	0.18	0.18	0.18	0.16	0.16	0.15	0.20	0.17	0.15	0.00	0.15	0.14	0.00	0.1
28. ATH 51 Hyalomma truncatum	0.08	0.09	0.18	0.19	0.19	0.03	0.02	0.03	0.13	0.13	0.12	0.23	0.02	0.12	0.18	0.14	0.19	0.18	0.1

Appendix V: Genetic differentiation estimates table

Population	**Number of sequences**	**Number of segregating sites (S)**	**Number of haplotypes**	**Haplotype diversity (Hd)**	**Average number of differences (K)**	**Nucleotide diversity (π)**	**Nucleotide diversity with Jukes and Cantor correction**
Hyalomma rufipes	15	126	12	0.97143	22.89524	0.03753	0.04058
Hyalomma dromedarii	17	74	7	0.79412	16.36765	0.02683	0.02904
Hyalomma truncatum	5	21	4	0.90000	9.40000	0.01541	0.01560
Hyalomma albiparmatum	2.	0	1	0.00000	0.00000	0.00000	0.00000
TOTAL	**39**	**163**	**23**	**0.94602**	**58.02969**	**0.09513**	

Appendix VI: Summary of numbers of camels sampled in 2015 prior to slaughter at Athi River, showing the five regions of origin for camels sampled at Athi River, animal numbers, sex and median age(interquartile range [IQR]).

Sampling Site	**Origin of Camels**	**Animals Sampled (male/females)**	**Median Age in Years (IQR)**
Athi River	Isiolo	35(22/13)	6(5)
	Wajir	51(25/26)	10(3.5)
	West Pokot	5(1/4)	11(2)
	Baringo	8(4/4)	5(1.75)
	Marsabit	131 (44/87)	10(6.25)
	Total	**230(96/134)**	**10(5)**

Appendix VII: Ethical approval letter

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

P.O. Box 30197,
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Dr. Esther Kanduma
University of Nairobi
Dept of Biochemistry

REF: FVM BAUEC/2019/200

07/03/2019

Dear Dr. Kanduma,

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

The role of heartwater (E. rimunantium) and other tick-borne infections in Acute Camel Death Syndrome.

We refer to your ethical clearance letter dated 28th February 2019.

We have reviewed your proposal and are satisfied that the proposed handling and management of the animals meets acceptable standards for animal welfare.

We have also noted that registered veterinary surgeons will supervise the work. We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

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

Appendix VIII: Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **238088**

Date of Issue: **16/March/2022**

RESEARCH LICENSE

This is to Certify that Mr.. Elvis Daniel Kairu Njoroge of Egerton University, has been licensed to conduct research in Machakos on the topic: MOLECULAR CHARACTERISATION OF TICKS AND TICK-BORNE PATHOGENS OF ONE-HUMPED CAMELS (Camelus dromedarius) FROM CAMEL REARING COUNTIES IN KENYA for the period ending : 16/March/2023.

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