

Thesis Ref. No _____

**SPECIES DIVERSITY AND SEASONAL PATTERN OF TICKS AND TICK-BORNE PATHOGENS IN CATTLE OF ADA'A AND BOSET DISTRICTS,
CENTRAL OROMIA, ETHIOPIA**

MVSc Thesis



BY

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**ADDIS ABABA UNIVERSITY DEPARTMENT OF PATHOLOGY AND
PARASITOLOGY MVSc PROGRAM IN VETERINARY PARASITOLOGY**

JUNE, 2017

BISHOFTU, ETHIOPIA

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MVSc Thesis



A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis
Ababa University in partial fulfillment of the requirements for the degree of Master of
Veterinary Science in Veterinary Parasitology

By

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June, 2017
Bishoftu, Ethiopia

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DEDICATION

I dedicated this manuscript to my beloved mother, Ayelech Botale, and my father Naramo Langana, and my wife, Shibire Tekele, who sacrificed a lot to bring me up to this level, nursing me with affection, and for their dedicated partnership in success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my bonafide work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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ACKNOWLEDGMENTS

It is my great pleasure to express my sincere and deepest gratitude to my advisors Dr. Bersissa Kumsa, Dr. Teshale Sori, Dr. Dinka Ayana and Prof. Frans Jongejan for their thorough and unreserved comments, guidance, professional assistance and valuable criticisms, devotion of their time and overall support. In fact this is an excellent opportunity for me to express my deepest pleasure to Dr. Bersissa's exemplary professional quality, constructive comments and material support throughout my research work.

I would like to acknowledge Bill and Melinda Gates Foundation Parasite Mapping Project, Ethiopia, with collaboration of Clinvet International Bloemfontein Laboratory, South Africa for financial, logistic and Laboratory service support that helped me to undertake this MSc research.

My sincere thanks go to the technicians of Parasitology Laboratory of PAPA, CVMA, Addis Ababa University for their technical support in the Laboratory, and I also acknowledge cattle owners for their willingness in providing me their cattle during my research work.

I would like to thank the College of Veterinary Medicine and Agriculture (CVMA) for providing library and internet services during writingup of this MSc thesis work.

I never forget the hardships and happiness we have shared with my friends and colleagues and also I would like to thank my friends for their support during my study period.

Last but not the least; I am also obliged to my family for their enormous love and patience throughout my absence.

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
CSA	Central Statistics Authority
DNA	Deoxyribo nuclic acid
FTA	Flinders Technology Associates
masl	Meter above sea level
OIE	World Animal Health Organization
PAs	Peasant Associations
PCR	Polymerase Chain Reaction
rRNA	ribosomal RNA
spp	Species
TBD	Tick-Borne Disease
USD	United State Dollar

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ABSTRACT

Ticks and tick-borne pathogens are the major health constraints of cattle productivity and contribute to significant economic losses in Ethiopia. A cross-sectional study was conducted from August 2016 through May 2017 to determine the species diversity and seasonal pattern of ticks on cattle during the rainy, dry and short rainy seasons and occurrence of tick-borne pathogens in the highland PAs of Ada'a and Boset districts, central Oromia, Ethiopia. Ticks were identified to species level by using morphological identification keys under a stereomicroscope. In addition, molecular tools were employed to assess the presence of tick-borne pathogens in blood samples collected from cattle. During the study period, a total of 720 cattle were examined for presence of ticks. The study revealed that 659 (91.5%) of cattle were infested by one or more ticks during the study period. A total of 7430 (5220 males and 2210 females) adults and 794 immatures (46 larvae and 748 nymphs) stages of ixodid ticks that belong to a total of eleven species were collected from cattle during the study period. During the rainy period nine species of ticks belonging to three genera (*Amblyomma*, *Rhipicephalus* and *Hyalomma*) and subgenus *Rhipicephalus* (*Boophilus*) were identified, whereas ten and nine species of ixodid ticks were recorded during the dry season and short rainy season, respectively. In Ada'a district, a total of 3612 ticks were collected in various seasons. From this 907, 987 and 1718 adults and immature ticks were collected and identified in rainy, dry and short rainy seasons, respectively. Nine adult tick species and immature stages of genera (*Amblyomma* and *Rhipicephalus*) and subgenus *Rhipicephalus* (*Boophilus*) were identified. In Boset district, out of 4612 adults and immature ticks collected, 1235, 621 and 2756 were collected during the rainy, dry and short rainy seasons, respectively. Nine adult tick species and immature stages of genera (*Amblyomma* and *Rhipicephalus*) were identified. In the highland (Ada'a), *Amblyomma variegatum*, *Rhipicephalus* (*Boophilus*) *decoloratus*, *Hyalomma rufipes*, *Hyalomma truncatum* and *Rhipicephalus evertsi* were the five most commonly encountered tick species on cattle. In the lowland (Boset) district *Rhipicephalus pulchellus*, *Amblyomma gemma*, *Hyalomma rufipes* and *Hyalomma truncatum* are widely distributed tick species. *Amblyomma variegatum* (59.6%) and *Rhipicephalus pulchellus* (77.8%) were the most abundant tick species in Ada'a (highland) and Boset (lowland) agroecology, respectively.

Furthermore, a total of 240 blood samples (120 in Ada'a and 120 in Boset) were examined by real time PCR to detect the DNA of *Babesia bigemina*, *Ehrlichia ruminantium*, *Anaplasma centrale*, *Anaplasma marginale* and *Trypanosoma theileri* in the blood of cattle. Result of the study showed that a total of 182 (75.8%) blood samples were positive for at least one of the aforementioned tick-borne pathogens. Molecular result revealed that *Anaplasma marginale* (63.4%) was the most prevalent tick-borne pathogens followed by *Babesia bigemina* (20%) and *Ehrlichia ruminantium* (10.5%). *Anaplasma centrale* (4.2%) and *Trypanosoma theileri* (1.7%) were the least in Boset and Ada'a districts, respectively. In conclusion, findings of this study suggest that ticks and tick-borne pathogens were the most important problems of cattle of the study areas. Therefore, the increasing threat of ticks and tick-borne pathogens of cattle warrants urgent strategic application of acaricides and creation of awareness among livestock owners to prevent and control tick infestation and tick-borne pathogens of cattle.

Keywords: *Ada'a, Boset, Cattle, Ethiopia, Seasonal pattern, Ticks, Tick-borne pathogens.*

1. INTRODUCTION

Ethiopia has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zones that are suitable to host a very huge animal population (Mekasha *et al.*, 2014). The country has the largest livestock population in Africa with the estimated domestic animal number of 57.83 million cattle, 28 million sheep, 28.6 million goats, 1.23 million camels, 60.5 million poultry, 2.1 million horses, 0.4 million mules and 7.88 million donkeys (CSA, 2016). The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Metaferia *et al.*, 2011; Leta and Mesele, 2014). Livestock mainly cattle in Ethiopia represent the pillar of the economy and plays vital roles in generating income to farmers, ensuring food security, contributing to asset, and social, cultural and environmental values, (Solomon, 2005; Metaferia *et al.*, 2011).

Despite high livestock population and existing favorable environmental conditions, the current livestock output of the country is far below the expected potential. The most important constraints to the livestock productions and productivity are widespread and high prevalence of endemic diseases (Solomon, 2005). Ectoparasites are responsible for decreased production and reproduction of these animals as well as leather quality deterioration, down grading and rejection of skins (Wall and Shearer, 2001). Ectoparasites mainly ticks are greatly associated with health problems of man and his animals and contribute to important economic losses in Ethiopia (Kumsa *et al.*, 2016). It directly affects the socio-economic development of resource limited farming community by affecting health and productivity of animals in Ethiopia (Mokonnen *et al.*, 2001; Bekele, 2002; Yacob *et al.*, 2008; Kumsa *et al.*, 2012).

Ticks are obligate blood feeding ectoparasites of vertebrates particularly mammals, birds and reptiles throughout the world (Wall and Shearer, 2001). All ticks spend most of their life cycle away from their hosts, hiding either in soil and vegetation or in the nests of their hosts (Latif and Walker, 2004). The blood from mammals, birds, reptiles and amphibians is the only critically important source of nutrition for growth, development of organs for male, female, larvae and nymphs of ticks (Walker *et al.*,

2014). They belong to the phylum Arthropod; class Arachnid, and order Acari (Wall and Shearer, 2001). To date, a total of 896 tick species have been described worldwide. Ticks are grouped into three families as Ixodidae (hard ticks) with 702 officially recognized species, Argasidae (soft ticks) comprising 193 species and Nuttalliellidae with a single species (Guglielmone *et al.*, 2010). Ixodid ticks pass via four stages in their development; eggs, 6- legged larva, 8-legged nymph and adult (Minjauw and McLeod, 2003). They are categorized into one-host; two-host or three-host life cycles according to the number of host required to complete their lifecycle (Walker *et al.*, 2014).

In Ethiopia, ticks are very common and widely distributed in all agro-ecological zones of the country (Pegram *et al.*, 1981; Kumsa *et al.*, 2013). They are prominent parasites and competent vectors of pathogens that affect both humans and animals (Estrada-Peña, 2015) and are usually considered to have more veterinary significance because they negatively affect the health and productivity of domestic animals as a consequence of direct parasitism and disease transmission (Latif and Walker, 2004). They are responsible for severe economic losses both through the direct effects associated with their blood sucking behavior (Jongejan and Uilenberg, 2004; Kumsa *et al.*, 2015a) and also indirectly act as reservoirs and vectors for a wide range of human and animal pathogens worldwide and thus inject pathogens such as viruses, bacteria, protozoa and toxins in to their hosts (Jongejan and Uilenberg, 2004).

The main genera of ticks found in Ethiopia are *Amblyomma*, *Rhipicephalus*, *Hyalomma* and *Haemaphysalis* and a subgenus *Rhipicephalus* (*Boophilus*) (Mokonnen *et al.*, 2007). Previous studies documented the presence of more than 50 species of ticks in the country including genus *Amblyomma* (8 spp.), subgenus *Boophilus*, (2 spp.), *Haemaphysalis* (4 spp.), *Hyalomma* (9 spp.), *Rhipicephalus* (15 spp.), *Ixodes* (1 spp.), *Argas* (1 spp.) and *Ornithodoros* (2 spp.) which are reported to have great veterinary and medical importance in Ethiopia (Pegram *et al.*, 1981). Several studies have been conducted in different parts of Ethiopia on ticks infesting cattle (Pegram *et al.*, 2004; Yacob *et al.*, 2008; Kumsa *et al.*, 2016).

Earlier data show that approximately 80% of cattle populations of the world are at risk of tick infestation and tick-borne diseases (Kumsa and Mekonnen, 2011). In Ethiopia anaplasmosis, babesiosis, cowdriosis and theileriosis have been reported as major tick-

borne diseases affecting domestic animals (Mokonnen, 2001; Tomassone *et al.*, 2012; Teshale *et al.*, 2016). Among tick-borne pathogens of zoonotic importance spotted fever *Rickettsia* spp., *Borrelia* spp., *Coxiella burnetii*, and *Bartonella* spp. have been documented from Ethiopia (Kumsa *et al.*, 2015a, b).

The seasonal variations within a bioclimatic zone may favor or hinder the development or activity of a tick species during certain periods (Latif and Walker, 2004). Dry environmental conditions are a serious danger to ticks, particularly to the questing larvae which are very susceptible to drying out fatally (Walker *et al.*, 2014). Also the start and end of the rainy season may influence the different phases of the life cycle. For instance, *Amblyomma variegatum* and *Rhipicephalus (Boophilus) decoloratus* are considered as the most prevalent and predominant tick species in higher rainfall season in Ethiopia (Pegram *et al.*, 1981; Ayalew *et al.*, 2014). Other species are more restricted to a specific habitat where their specific hosts are present and where climatic conditions allow survival and reproduction (Mokonnen *et al.*, 2007).

Recent reports show worldwide growing health risks associated with ticks and tick-borne diseases in both humans and animals (Dantas-Torres *et al.*, 2012). Tick-borne pathogens have nowadays received greater importance and thus there is increasing necessity for accurate detection and identification of these pathogens and the role of ticks as vectors of pathogens (Teshale *et al.*, 2016). However, there is lack of precise and up-to-date comprehensive information on species diversity and seasonal dynamics of ticks and accurate identities of tick-borne pathogens of cattle in Ada'a and Boset districts, central Oromia, Ethiopia.

Therefore, the major objectives of this study were:

- ✚ To determine species diversity and seasonal pattern of ticks infesting cattle in Ada'a and Boset districts
- ✚ To assess the presence of tick-borne pathogens of cattle in Ada'a and Boset districts using molecular methods.

2. LITERATURE REVIEW

2.1. Ticks Taxonomy

Ticks belong to the phylum (Arthropoda), class (Arachnida), sub class (Acari) and Order (Parasitiformes) (Torr *et al.*, 2003). Within the Parasitiformes, ticks belong to the suborder Ixodida, which contains a single super family, the Ixodoidea, which is divided into three families, Argasidae (soft ticks) and Ixodidae (hard ticks), and the third family Nuttalliellidae, with a single species (Rodriguez-Vivas *et al.*, 2004). To date, a total of 896 tick species have been described, divided among the Ixodidae (702 spp., 14 genera), Argasidae (193 spp. the number of genera is controversial and currently under discussion), and Nuttalliellidae (one species) (Barker & Murrell, 2008; Guglielmone *et al.*, 2010). In the most commonly used classification of ticks, the family Ixodidae comprises 2 major groups, the Prostriata and the Metastricata (Figure1). Two subfamilies are currently recognized in the Argasidae: the Argasinae and the Ornithodorinae (Parola and Raoult, 2001).

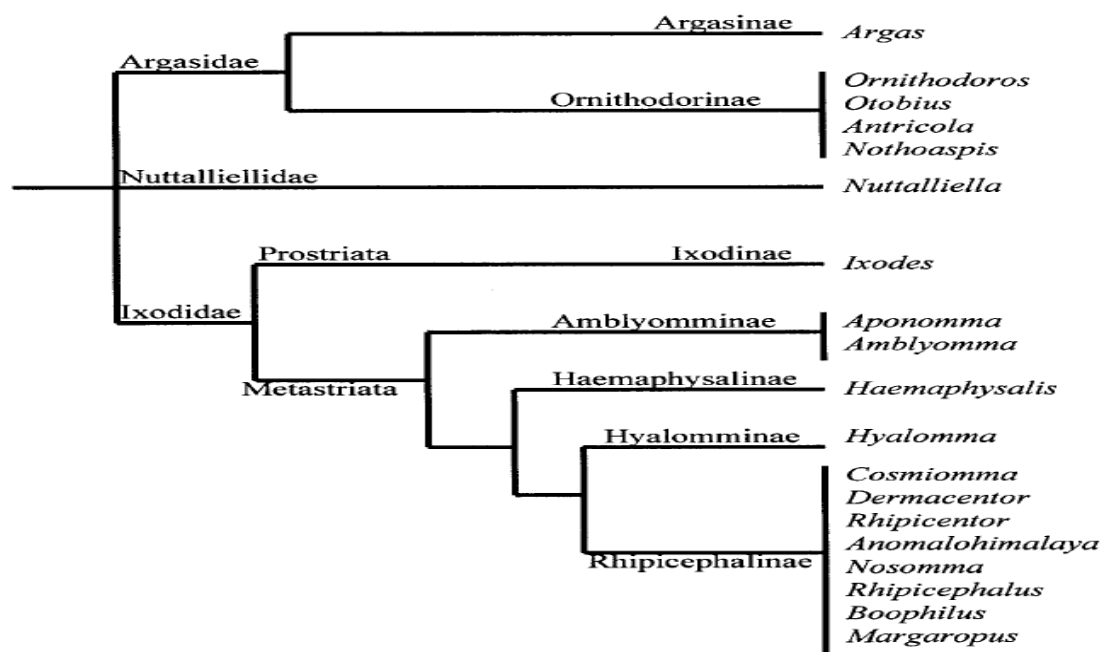


Figure 1: The traditional Hoogstraal classification of ticks (Parola and Raoult, 2001)

2.2. Ticks Morphology

2.2.1. Family Ixodidae (hard ticks)

The Family of Ixodid varies in shape and species. Ticks belong to the super order of Acarina, which have certain characteristics that distinguish them from other arachnids such as spiders. Ticks have a rounded body, without a clear boundary between the anterior and posterior parts. The body is divided into a capitulum (gnathosoma) and the rest of the body (idiosoma). Ticks have six pairs of appendages including the chelicerae, pedipalps, and four pairs of locomotors appendages (Wall and Shearer, 2001). Tick morphology consists of two primary regions, the mouthparts (*capitulum*) and the body (*idiosoma*). The mouthparts (*capitulum*) have three specialized structures called palps, chelicerae and a hypostome that are attached to a base called the basis capituli. These structures function together and allow ticks to penetrate the host skin and extract a blood meal from the tissues below (Figure 2). The body (*idiosoma*) of ticks is typically not hardened to a great extent (Walker *et al.*, 2014).

In hard ticks, most of the exterior cuticle is soft and has many internal folds that look like grooves on the surface of the body. The uniform, rectangular folds located on the rounded posterior end of hard ticks are called festoons (Latif and Walker, 2004). Male and female hard ticks can be distinguished from one another by the size of the scutum. In males, the scutum is large and covers the entire upper surface of the body, whereas in females the scutum is much smaller and covers less than half of the upper body surface (Figure 2), thus allowing the body to greatly expand during engorgement with host blood. One pair of eyes is normally located near the front corners of the scutum in hard ticks (Houseman, 2013).

Coloured patterns occur on some ticks as a pigment (enamel) in the outer body wall. It occurs on the scutum, conscutum or legs. The presence of pigment is known as enamelling or ornamentation and such ticks may be called ornate. The colour is usually ivory white but in *Amblyomma* ticks it is often orange and green. However, this colour has limited use for identification of ticks. Even with the colourful *Amblyomma* ticks it is the pattern made by the light coloured enamel against the dark uncoloured body wall that is more important than the actual colour of the enamel (Walker *et al.*, 2014).

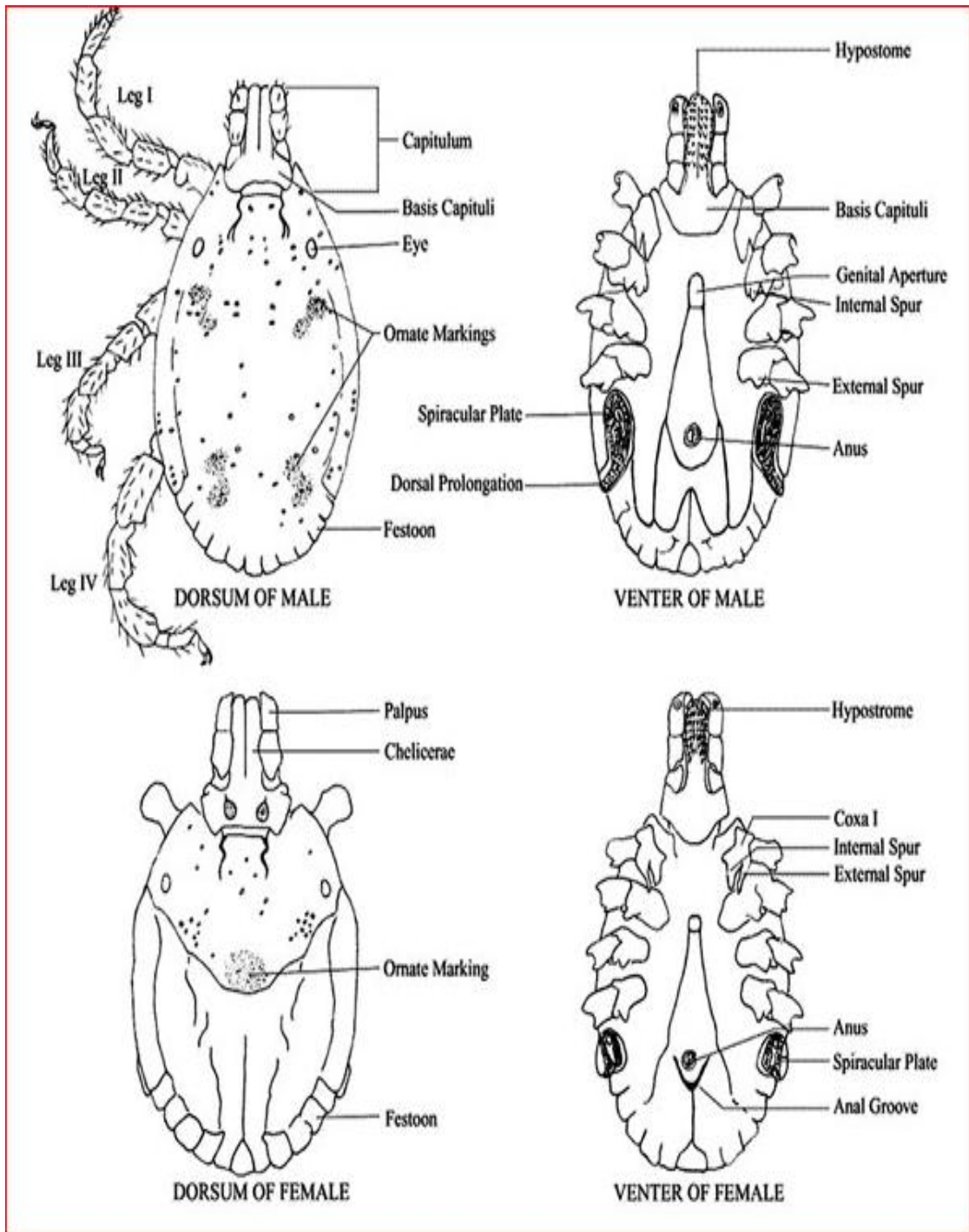


Figure 2: Key morphological features of male and female hard ticks on the top (dorsum) and bottom (ventral) of their bodies (Houseman, 2013).

2.2.2. Family Argasidae (soft ticks)

The second families of ticks are Argasidae (soft tick); having a leathery and unsclerotised body with a texture surface which in unfed ticks which may be characteristically marked with folds or grooves (Figure 3). The integument is inornated; the palps appear somewhat leg like with the third and fourth segments are equal in size. The gnathosoma is located ventrally and is not visible from the dorsal view in nymphs and adults (Walker *et al.*, 2014). Soft ticks rarely possess a scutum, but they have small, smooth, disk-shaped structures that provide sites for internal muscle attachment. These smooth areas are arranged in various patterns. One pair of eyes is normally located near the front corners of the scutum in hard ticks and is found underneath in soft ticks (Houseman, 2013).

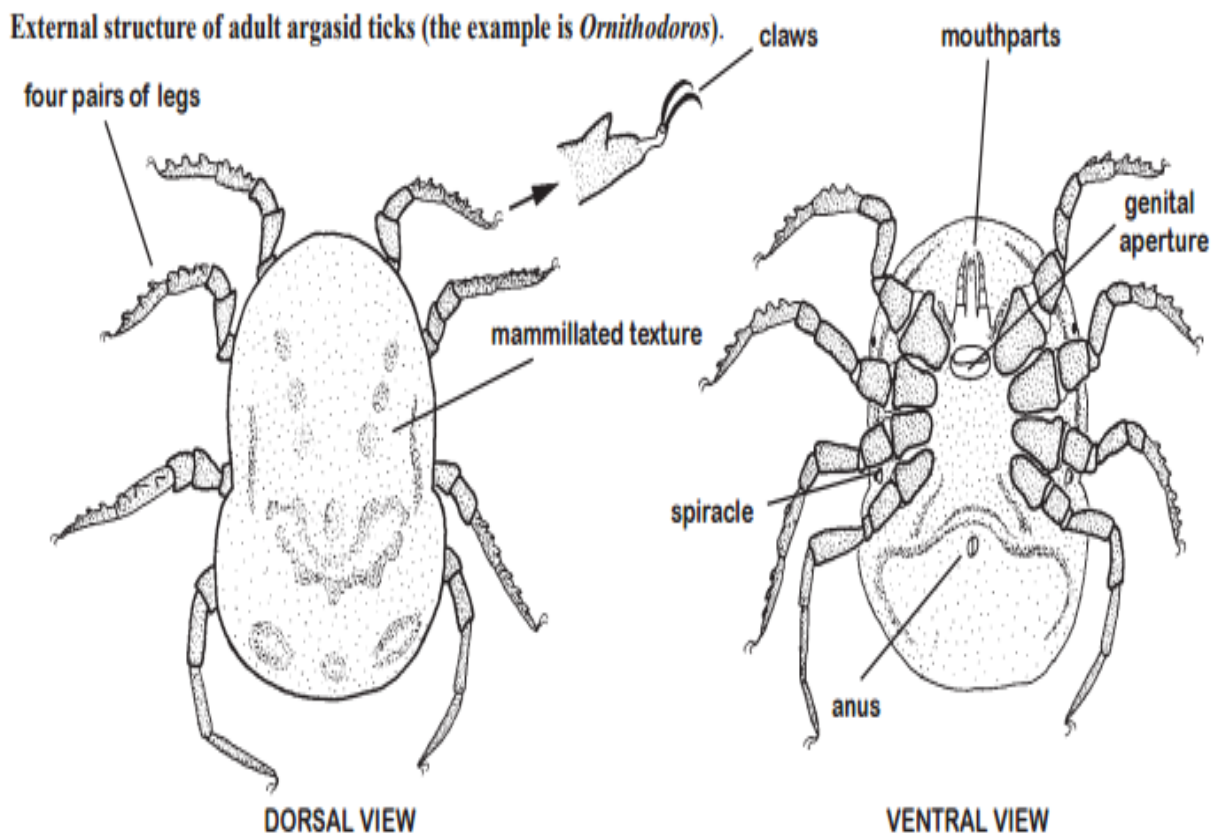


Figure 3: Morphological features of soft ticks on the top (dorsum) and bottom (ventral) of their bodies (Walker *et al.*, 2014).

2.3. Developmental Strategies of Ticks

2.3.1. Mating

Adult soft ticks mate while off of the host, but adult hard ticks mating takes place on the host species (Jongejan and Uilenberg, 2004), except with *Ixodes* where it may also occur when the ticks are still on the vegetation. Blood feeding is required by males for their sperm to mature, but they do not engorge as much as females and immature stages do. Males take small quantities of blood as they wait for females to attach nearby where they are able to detect them (Houseman, 2013). They transfer a sac of sperm (spermatheca) into the female ticks. The females mate only once, before they are ready to engorge fully with blood. When they finally engorge they detach from the host and have enough sperm stored to fertilize all their eggs. Female hard ticks lay many eggs (2,000 to 20,000) in a single batch (Figure 4) (Charles and Robinson, 2006)

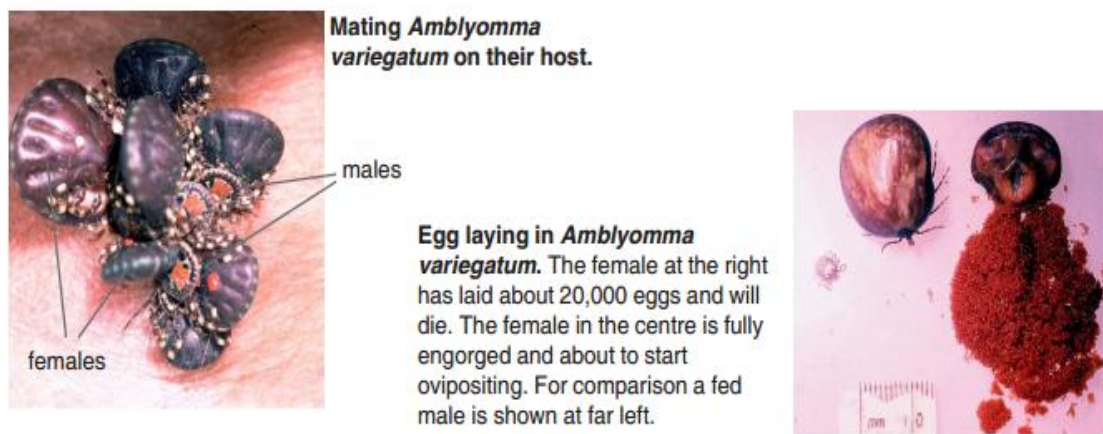


Figure 4: Mating and egg laying behavior of hard ticks (Walker *et al.*, 2014).

2.3.2. Life cycles

The completion of the lifecycle of Ixodid ticks requires four stages in their development; eggs, 6- legged larva, 8-legged nymph and adult (Minjauw and McLeod, 2003). Members of the family Ixodidae are categorized into one-host; two-host or three-host life cycles (Table 1) according to the number of host required to complete their development (Walker *et al.*, 2014).

2.3.2.1. One host ticks/ monotropic cycle

All the three instars (Larva, Nymph, and Adults) feed on one host to complete their life cycles. Male and female copulate on host during feeding process (Walker *et al.*, 2014). The engorged female, then drop to the ground to lay eggs species (Jongejan and Uilenberg, 2004). Eggs are laid as one batch of large quantity and the female afterwards die a natural physiological death (Figure 5). Examples of this type are the species of *Boophilus* (Table 1) (Walker *et al.*, 2014).

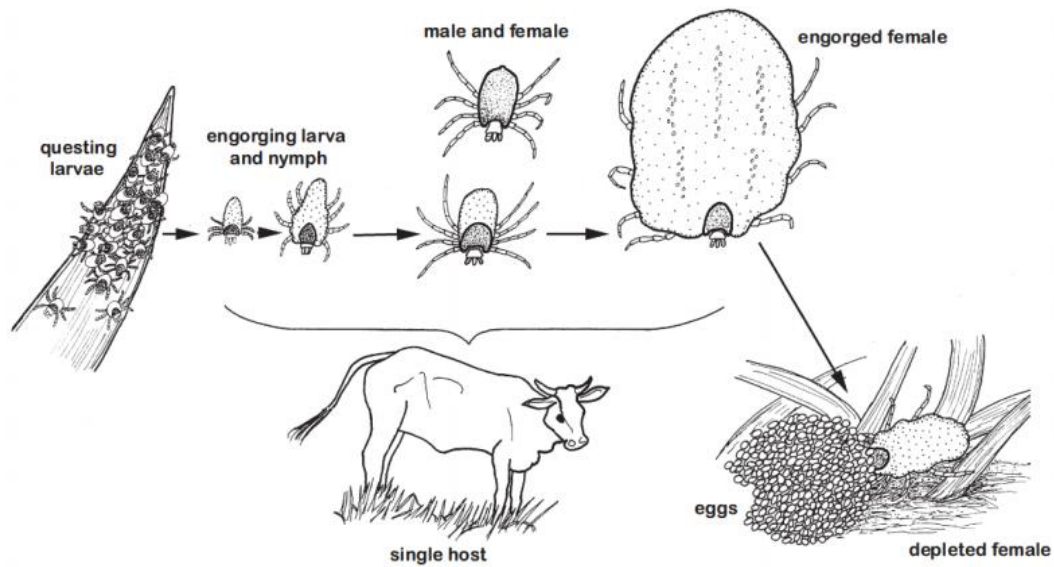


Figure 5: One-host life cycle. The example is *Rhipicephalus decoloratus* (Walker *et al.*, 2014).

2.3.2.2. Two host ticks/ ditropic cycle

The two-host life cycle is ticks attach as larvae and complete development through the nymphal stage but feed on the same individual host. The replete nymph then drops from the host, molts to the adult stage, and later seeks a second host to complete development (Figure 6). Example: The red-legged tick, *Rhipicephalus evertsi* and *Hyalomma detritum* (Table 1) (Walker *et al.*, 2014).

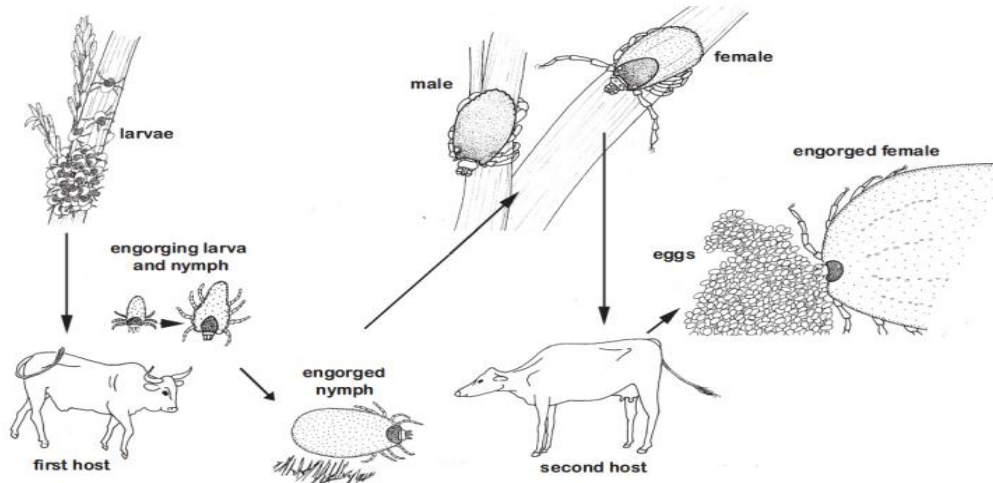


Figure 6: Two-host life cycle. The example is *Rhipicephalus bursa* (Walker *et al.*, 2014).

2.3.2.3. Three host ticks/ telotropic cycle

The larvae merge from the egg deposited on the ground, look for a host, feed on it for 3-7 days, drop off and molt after 3-4 weeks on the ground (Latif and Walker, 2004). The nymph must then find another host, get on that host, attach to that host, engorge on that host's blood, drop from that host, and molt to the adult stage (Houseman, 2013) on the ground after 2- 8 weeks. Then the adult ticks look for a third host to feed on, and for copulation with male, which takes 1-3 weeks. Finally it drops off and completes that cycle with oviposition on the ground to continue another cycle (Figure 7). Example: All species of *Amblyomma* (Table 1) (Walker *et al.*, 2014).

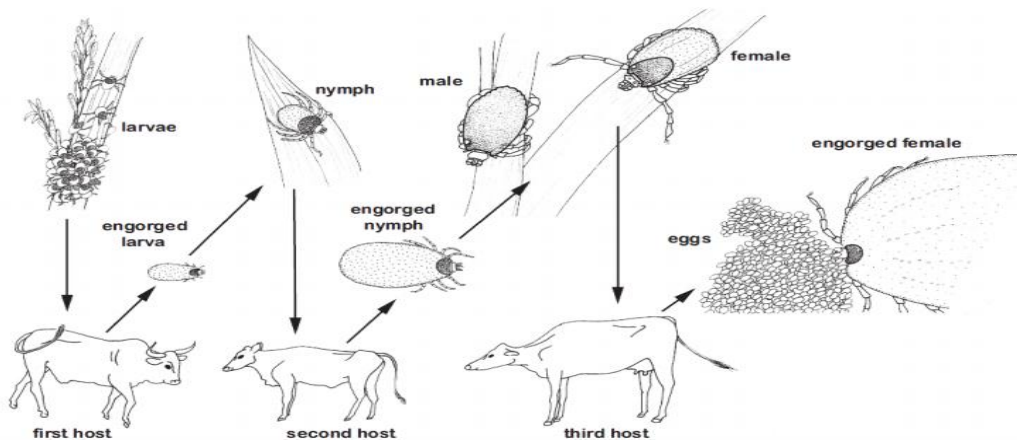


Figure 7: Three-host life cycle. The example is *Rhipicephalus appendiculatus* (Walker *et al.*, 2014)

Table 1: Categories of Ixodid ticks based on lifecycle

Tick genera	One host tick	Two host tick	Three host tick
<i>Rhiphicephalus</i> (<i>Boophilus</i>) spp.	<i>Rh. decoloratus</i> , <i>Rh.annulatus</i> , <i>Rh.</i> <i>Microplus</i>		
<i>Ambylomma</i> spp.			All species of <i>Ambylomma</i>
<i>Hyalomma</i> spp.	<i>Hyalomma</i> <i>Scupense</i>	<i>Hy. turanicum</i> , <i>Hy. Dentritum</i>	<i>Hy. truncatum</i> , <i>Hy.</i> <i>dromedarii</i> , <i>Hy.</i> <i>marginutum</i> , <i>Hy.</i> <i>excavatum</i> , <i>Hy.</i> <i>Anatolicum</i>
<i>Rhiphicephalus</i> spp.		<i>Rh. evertsi</i> , <i>Rh.</i> <i>Bursa</i>	<i>Rh. simus</i> , <i>Rh.pravus</i> , <i>Rh. pulchellus</i> , <i>Rh.appendiculatus</i>
<i>Haemaphysalis</i> spp <i>Ixodes</i> spp.			<i>Hae.punctata</i>
<i>Dermacentor</i> spp.	<i>D. albipictus</i>		Most spp.

Source: (Walker *et al.*, 2014)

2.4. Feeding Habits of Ticks

All feedings of ticks at each stage of the life cycle are parasitic. Ticks feed only on the blood (blood cells and blood plasma) and lymph of their hosts. The ticks crawl onto their host and attach to the skin. Ticks use a combination of cutting mouthparts, which penetrate the skin, and often an adhesive (cement) is secreted in the saliva to aid attachment to the skin (Walker *et al.*, 2014). At the end of the mouthparts are sharp chelicerae that scrape a hole into the dermis. This breaks the capillary blood vessels very close to the surface of the skin and the tick feeds on released blood and lymph which may accumulate at the wound. On the ventral surface of the mouthparts is the hypostome which is barbed with teeth to grip the host. A feeding tube into the tick is formed loosely between the hypostome and the sheath surrounding the chelicerae

(Figure 8). The feeding of ticks makes is important to the health of domestic animals and humans (Latif and Walker, 2004).

Feeding ticks concentrate the blood meal by removing excess water. This helps to accommodate the large blood intake (several milliliters) in the relatively small body of the engorging tick. The immature stages (larvae and nymphs) commonly feed for three to six days, while the adults may feed for as long as two weeks. Digestion of the blood may start during the first few hours after the beginning of feeding, and usually lasts for several weeks or even months. While digestion takes place, possible pathogens acquired from the hosts cross the gut wall, become incorporated in the haemolymph circulation of the tick, and invade the cells of the body tissues. After feeding, the tick will moult; after the moult is complete it takes several days for the cuticle to harden completely, and the tick will then actively quest for a new host (Estrada-Peña, 2015).

The feeding of ixodid ticks is slow because the body wall needs to grow before it can expand to take a very large blood meal. Larvae take typically 3 to 5 days to fully engorge with blood, nymphs 4 to 8 days, and females 5 to 20 days. When the ticks have fully engorged with blood they detach from the host's skin and drop to the ground. Males of most types of ticks feed but do not expand like the females. They feed enough for their reproductive organs to mature (Walker *et al.*, 2014).

Soft ticks have life cycles that vary in the number of stages and feeding bouts. They are nest parasites and are likely to feed repeatedly on the same animal or the same family group of animals within the nest. Most soft ticks do not attach to the host or enlarge greatly while feeding. Those that do attach only do so during the larval stage. Nymphs and adults feed quickly on the host while it is in the nest and then return to their resting or hiding place within the nest (Houseman, 2013).

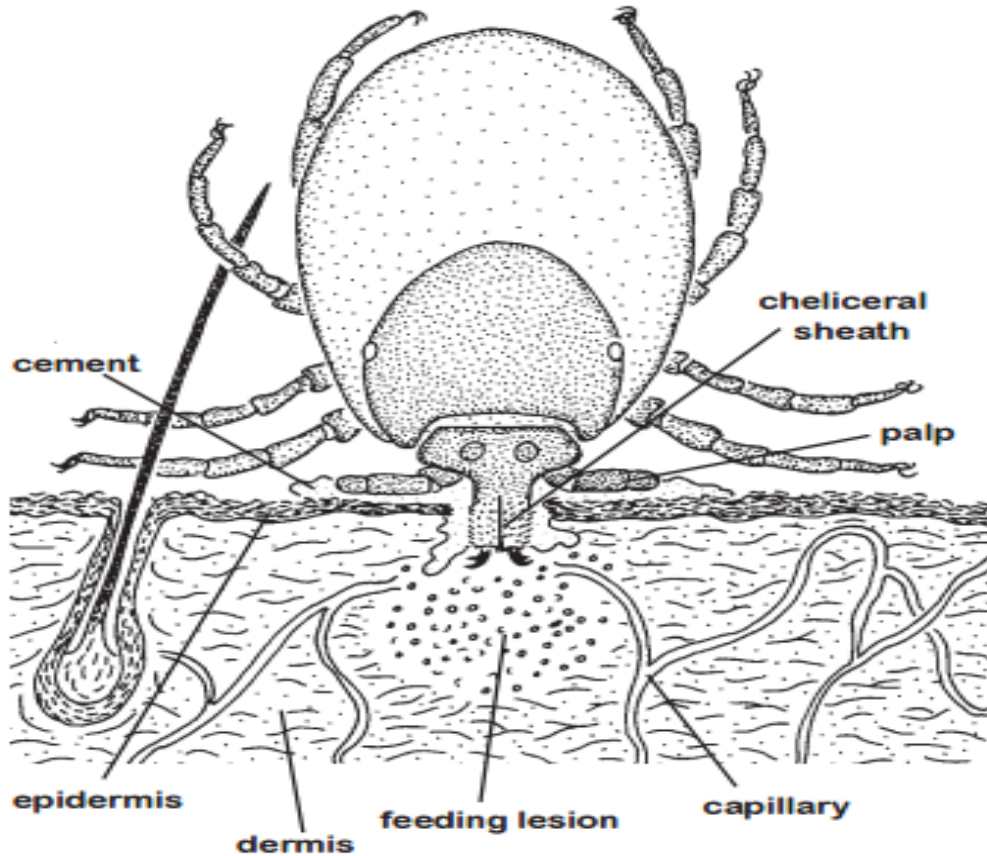


Figure 8: Feeding of an ixodid female tick at skin of host (Walker *et al.*, 2014).

2.5. Host Seeking Behavior in Ticks

All ticks spend most of their life cycle away from their hosts, hiding either in soil and vegetation or in the nests of their hosts. So they need to be able to find hosts on which to feed. Ticks find their hosts in several ways. Many ticks have the eggs and moulting stages in soil or vegetation in the environment in which their hosts graze or hunt. The ticks crawl onto vegetation and wait for their hosts to pass by. This is a type of ambush and the behavior of waiting on vegetation is called questing. Thus in the group of ixodid ticks such as *Rhipicephalus* the larvae, nymphs and adults will quest on vegetation. The ticks grab onto the hosts using their front legs and then crawl over the skin to find a suitable place to attach and feed (Latif and Walker, 2004).

Adult ticks of the genera *Amblyomma* and *Hyalomma* are active hunters; they run across the ground after nearby hosts. The general behavior of seeking hosts in an open environment is described as exophilic. Argasids ticks and many *Ixodes* species spend their entire life cycle in their host's nest and attach to their hosts there. This is called

endophilic or nidicolous behavior. A few species of ticks, such as the dog tick *Rhipicephalus sanguineus*, have adapted to living in housing built by humans and will feed on domestic animals there. This is called domestic behavior (Walker *et al.*, 2014).

The main determinant of tick host-seeking behavior can be attributed to the presence of host kairomones. Ticks utilize host kairomones to detect the proximity of hosts and to identify host type. Behavioral assays have demonstrated the attractive quality of host breath, dermal and gland pelage and urine. Carbon dioxide (CO₂), ammonia (NH₃), and acetone are all attractive components of human and animal breath. Nitrogenous wastes, ammonia and ammonium hydroxide are major components of dermal pelage and urine (McMahon and Guerin, 2002).

2.6. Ticks Distribution and their Seasonal Dynamics in Ethiopia

The distribution and abundance of tick species infesting domestic ruminants in Ethiopia vary greatly across different agro-ecological zones and seasons of the country. The distribution and seasonal dynamics of the following predominant ixodid tick species in Ethiopia is presented below one after the other.

Amblyomma variegatum occurs in areas with a wide variety of climates ranging from highland, savannah through to lowlands (Walker *et al.*, 2014) but its abundance varies greatly across different agro-climatic zones in Ethiopia. It is widespread and abundant tick parasitizing cattle in the central highlands of Ethiopia as well as in the highland areas of the eastern parts of Ethiopia (Pegram *et al.*, 1981). However, its abundance was much lower in tropical woodland and thorn-bush vegetation habitats in the Rift Valley and absent in the arid south-eastern areas with annual rainfall ranging from 400-500mm in Ethiopia (Mokonnen *et al.*, 2001). The tick requires moisture and warmth for its survival and the activity of adult ticks commences during the beginning of spring rain between March and June in Ethiopia. *Am. variegatum* is considered as the most prevalent and predominant tick species in higher rainfall areas in Ethiopia (Pegram *et al.*, 1981; Ayalew *et al.*, 2014; Kumsa *et al.*, 2016).

Amblyomma gemma adults feed on domestic animals such as cattle and camels, although large herbivores such as giraffes and buffaloes are the preferred hosts of this

tick species. *Am. gemma* has been recorded from areas with climates ranging from highland through steppe to desert (Walker *et al.*, 2014). It is mainly distributed in eastern Ethiopia, northern and southern Somalia. *Am. gemma* is a xerophilic species, prefers woodland, bushland, wooded and bushy grassland in arid and semi-arid areas. It is mainly abundant in areas with altitude of 500-1750 m and 350-750 mm rainfall. *Am. gemma* and *Rh. pulchellus* are confined to semi-arid areas and lowland tick densities are usually greater than those in the highlands (Pegram, 1981). *Am. gemma* is an important tick on cattle and camels in eastern and South-eastern parts of Ethiopia particularly in Afar, Somalia, Harar and eastern Tigray, Amhara and SNNP regional states (Mokonnen, 2007). In Ethiopia, this tick is most prevalent during the rainy season from March to October and is generally restricted to the semi-arid lowlands in the Rift Valley and in eastern areas receiving 100-800 mm annual rainfall (Pegram *et al.*, 1981).

Rhipicephalus (Boophilus) decoloratus is also known as the blue tick, because of the colour of the engorged females. Cattle are the main host. It occurs in regions with savannah and temperate climates, typically in grasslands and wooded areas used as cattle pasture (Walker *et al.*, 2014). In Ethiopia, *Rh. (Bo.) decoloratus* widely distributed tick species in different agro ecological and seasons of the country. It is the commonest and most wide spread tick in the country; collected in all administrative regions except in the Afar region (Mokonnen *et al.*, 2007). Its distribution pattern is similar to that of *Am. variegatum*. In previous records, *Rh. decoloratus* was collected between 1200-2400 m altitude and at 1000-2400 mm rainfall in both rainfall modes and was predominate in broad-leaved and coniferous forest areas (Pegram *et al.*, 1981). Several authors reported this tick from all agro-climatic zones, but it is more common in the highland and mid highland altitudes in Ethiopia.

Rhipicephalus pulchellus is also known as the zebra tick due to a pattern of stripes of white enamel on a dark brown background over their entire conscutum and its use of zebras as a favorite host. They attaches in large numbers on cattle. *Rh. pulchellus* adults appear to be most active during the rainy season. *Rh. pulchellus* is a tick of savannah, steppe and desert climatic regions. In Ethiopia, *Rh. pulchellus* has been reported as the most predominant tick species on camels in eastern Ethiopia, on small

ruminants in eastern part of Ethiopia and on cattle in Borana zone in Oromia region (Zelege and Bekele, 2004; Abunna *et al.*, 2009; Regassa, 2001).

Rhipicephalus evertsi is known as the red-legged tick due to their uniform orange colored legs and thus it is easy to differentiate from all other *Rhipicephalus* species. Its distribution includes desert, steppe, savannah and temperate climatic regions (Walker *et al.*, 2014). The native distribution of *Rh. evertsi* in Ethiopia seems to be connected with middle height dry savannas and steppes, in association with zebra and ruminant and it is widely distributed throughout Ethiopia. This tick species shows no apparent preference for particular altitude, rainfall zone or seasons. These ticks are active mainly during the summer but are present throughout the year in warm regions (Pegram *et al.*, 1981).

Rhipicephalus praetextatus is a three-host tick and adults of this species are most abundant during the rainy season (Walker *et al.*, 2014). *Rh. praetextatus* occurs in a wide range of climatic regions from highland areas of Ethiopia and Kenya, through to savannah and steppe of East Africa to the semiarid and desert climates in North East Africa and the eastern Sahara. In Ethiopia, *Rh. praetextatus* has been reported as the most predominant tick species on cattle in three districts of Afar region (Pegram *et al.*, 1981).

Hyalomma truncatum is also known as the shiny *Hyalomma* because of the smooth surface of the male ticks. *Hy. truncatum* is adapted to dry habitats and is commonest in desert, steppe and savannah climatic regions, but is also recorded from highland climates (Walker *et al.*, 2014). In Ethiopia, it is present in different agro-climatic zones except in the wetter South-western part of the country (Pegram *et al.*, 1981). Previous investigator in Ethiopia collected *Hy. truncatum* as one of the important tick species in central Oromia (Kumsa and mokonnen, 2011).

Hyalomma rufipes is also known as the hairy *Hyalomma*. The main hosts of the adult ticks are cattle; also sheep, goats, horses and wild ungulates are infested (Wall and Shearer, 2001). The adults are most numerous during the early part of the wet season and the immature stages during the dry season. *Hy. rufipes* is widely distributed in much of Africa and has been recorded from every climatic region from desert to rain forest (Walker *et al.*, 2014). In Ethiopia, it is widely distributed throughout the

country between altitudes 500 and 3500 m, except in the south-west where the climate is wet most of the year (Pegram *et al.*, 1981). Important collections were made from cattle in central Oromia (Ayalew *et al.*, 2014) and in eastern parts of Ethiopia in (Tafesse, 1996). Common tick species which are found in Ethiopia are presented in summary table below (Table 2).

Table 2: Summary table in distribution of the most predominant and economically important tick species of cattle in different parts of Ethiopia

Tick Species	District/Part of Country	Prev. %	Reference
<i>Amblyomma</i>	Somalia	4.2	Abebe <i>et al.</i> , 2010
<i>variegatum</i>	Northwest, Ethiopia	13.4	Alemu, 2014
	Western Ethiopia	17.2	Kumsa <i>et al.</i> , 2013
	Southren, Ethiopia	20.82	Shifarew, 2005
<i>Amblyomma</i>	Central Oromia	90	Pegram, 1981
<i>cohaerens</i>	BoranaProvince	1	Regassa, 2001
	Western Ethiopia	66.3	Kumsa <i>et al.</i> , 2013
<i>Amblyomma</i>	Somali Ethiopia	9.4	Tomassone <i>et al.</i> , 2012
<i>gemma</i>	Borana zone	5	Regassa, 2001
<i>Rhipicephalus</i>	Western Ethiopia	38.2	Kumsa <i>et al.</i> , 2013
<i>(Boophilus)</i>	Northern Ethiopia	23.9	Yacob <i>et al.</i> , 2008
<i>decoloratus</i>	Southern Ethiopia	46.57	Wogayehu <i>et al.</i> , 2016
<i>Rhipicephalus</i>	Western Ethiopia	25.8	Kumsa <i>et al.</i> , 2013
<i>evertsi</i>	North shewa	20.6	Tadesse and Sultan, 2014
	Northwest Ethiopia	11.51	Alemu <i>et al.</i> , 2014
<i>Rhipicephalus</i>	Somali Ethiopia	40.1	Tomassone <i>et al.</i> , 2012
<i>pulchellus</i>	Adami Tulu	0.9	Ayalew <i>et al.</i> , 2014
<i>Rhipicephalus</i>	Adami Tulu	19.6	Ayalew <i>et al.</i> , 2014
<i>praetextatus</i>	Western Ethiopia	0.4	Kumsa <i>et al.</i> , 2013
<i>Hyalomma rufipes</i>	Ada'a	17.8	Ayalew <i>et al.</i> , 2014
	Somali Ethiopia	13.3	Tomassone <i>et al.</i> , 2012
<i>Hyalomma</i>	Adami Tulu	25.1	Ayalew <i>et al.</i> , 2014
<i>truncatum</i>			

2.7. Challenges to Ticks Distribution and Abundance

The distribution of ticks (and their hosts) at the local level, can be influenced by a variety of factors. Microclimate is dependent on a variety of biotic and abiotic factors, such as the amount of vegetation present (shade and leaf litter provide lower temperatures but higher humidity) and the type of soil (water retention and potential interstitial refuges) (Schwarz *et al.*, 2009).

The microclimate: is composed of the collection of micro-scale climatic conditions measured in defined areas, for example, near the ground surface, including temperature, wind speed, degree of exposure, saturation deficit (SD) and soil moisture. Variations in microclimate can increase or decrease the survival chances and the developmental rate of a tick, induce changes in population growth, behavior, susceptibility to pathogens, incubation period of pathogens, seasonality of activity and pathogen transmission (Schwarz *et al.*, 2009). Tick questing activity is strongly affected by temperature. The climatic variations are the main factors which determine the ecological and the geographical distribution of ticks. Species of ticks are adapted to a certain range of temperature and moisture. Some ticks occur only in warm regions with a fair degree of humidity, while ticks in temperate areas become most active during summer season. Changes in the climatic situation may considerably cause changes in the geographical distribution of ticks (Gilbert *et al.*, 2014).

A tick's habitat: is composed of the variety of living and non-living things in the space in which it lives. Ticks are adapted to two contrasting components of their habitat: the physical environment and their host. When ticks are moulting and then questing in the physical habitat they are in danger of drying out and starving. The larvae are most susceptible because of they have a high surface area relative to their small volume. They are also exposed to predators such as rodents, birds, reptiles and ants, and to pathogens such as fungi. These adverse factors limit the type of habitats that a species will be found (Latif and Walker, 2004; Walker *et al.*, 2014).

Seasonal dynamics: It is within a bioclimatic zone will favor or hinder the development or activity of a tick species during certain periods. In tropical climates, the dominant factor is rainfall. The start and end of the rainy season influence the different phases of the life cycle. Parasitism is reduced during the dry months and

increases sharply within days following the first major winter rainfall. The population remains stable for a few weeks, and then slowly diminishes (Gashaw, 2004).

The behavior of hosts: is an important factor in the distribution and dispersal of ticks. The movement of hosts through the landscape facilitates dispersion, including invasion into new regions (Estrada-Peña, 2013). The chance of being picked up by a suitable host is increased by high population densities and diverse communities of hosts, which might be a reason for the predominant association of many species of hard tick larvae and nymphs with small mammals (Kiffner *et al.*, 2011).

Attachment Site: Site specificity is one of the populations limiting system that operate through the restriction of tick species to certain parts of the host body. The ticks grab on to the hosts using their front legs and then crawl over the skin to find a suitable place to attach and feed (Walker *et al.*, 2014). Depending on the tick, site preference on the host depends on the accessibility for attachment, to get blood and protection to overcome the environment damage that inhibits its existence and grooming activity of the host (Latif and Walker, 2004).

Acquired resistance against ticks: tick infestation and the development of pathogens in both ticks and their vertebrate hosts are mediated by species-specific molecular mechanisms (De la Fuente *et al.*, 2015), but are also modified by the host individual's age, sex and immune status (Szep and Moller, 1999). Tick feeding induces host immune regulatory and effector pathways involving antibodies, complement, antigen-presenting cells, T lymphocytes, and other bioactive molecules. Acquired resistance impairs tick engorgement, ova production, viability and blocked moulting and the death of engorging ticks (Wikel, 1996).

2.8. Economic Impact of Ticks

In Ethiopia, ticks and tick-borne diseases cause considerable losses to the livestock economy, ranking third among the major parasitic diseases, after trypanosomosis and endoparasitism (Pegram *et al.*, 1981).

2.8.1. Direct Impact

Feeding by large numbers of ticks causes reduction live weight, unrest, tick worry irritation, severe dermatitis, anemia, and serious physical damages, while the direct injury also reduce the quality of hides due to tick bites, loss of blood due to the feeding of ticks. These parasites generate direct effects in cattle in terms of milk production and reduce weight gain (Peter *et al.*, 2005). Tick infestation is associated with huge economic losses and at a conservative estimate, one million USD is lost annually only through rejection of downgraded hides and skins attributed to tick damage in Ethiopia (Gashaw, 2005).

2.8.2. Indirect Impact

In addition to sucking large volume of blood, ticks also act as reservoirs and vectors for a wide range of human and animal pathogens worldwide and thus inject pathogens such as viruses, bacteria, protozoa and toxins in to their hosts. Some of the most important tick-borne diseases are East Coast Fever, Redwater, anaplasmosis and heartwater. Many other fatal and benign babesiosis and theileriosis are also transmitted by various tick species (Jongejan and Uilenberg, 2004). The estimated annual global costs associated with ticks and tick-transmitted pathogens in cattle amounted to between US\$ 13.9 billion and US\$ 18.7 billion (De Castro *et al.*, 1997). An annual cost of 3 million Birr (US\$ 1.5 million) to purchase acaricides was estimated in 1989 (Newson, 1991). When other losses such as deaths, reduced growth rate and reduced milk production are added, economic losses due to ticks and tick-borne diseases are highly significant.

2.9. Control Methods of Ticks

2.9.1. Chemical control method

The main weapon for controlling ticks at present is the use of chemical. Control of tick infestation through the use of acaricides is one of the methods that can be used to reduce the tick-borne diseases. A wide range of acaricides, including arsenical, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids are being used for controlling ticks on livestock (De Meneghi *et al.*, 2016). The performance of an acaricide in the control of ticks depends not only on the activity of

a product, but on the quality and quantity of active ingredient deposited on cattle or delivered internally (Walker, 2011). Application of chemicals Various methods including dipping, spraying, ear tagging or pour on, have been used to apply chemicals to protect livestock against ticks. Direct application of acaricides to animals is the most popular method of controlling ticks on livestock. Applications of acaricide to tick-infested cattle via dipping or sprayer can be equally effective under ideal conditions with proper handling of equipments without injuring animals and subsequent dilution of a product (George *et al.*, 2008; De Meneghi *et al.*, 2016). Acaricide application is still the main tick control method in Ethiopia.

2.9.2. Ecological and traditional control method

Ecological control method is used for habitat and host linked treatment. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks. Vegetation management, such as the removal of shrubs and undergrowth that provide suitable habitat for rodent hosts can be successful on a small scale but is costly and time-consuming over larger areas and destroys the habitat of a wide variety of other species, including those beneficial to humans (Walker *et al.*, 2014). Replacement of natural vegetation, cropping, and soil cultivation are integrated methods that enable pasture improvement and tick eradication. The traditional methods of ticks control include, grass burning, hand removal of the ticks, extracts of leaves and cattle dusting with ash are largely used in Africa (Dipeolu *et al.*, 1992). Uses of ethno-veterinary remedies probably reduce tick-burdens while maintaining endemic stability to tick-borne diseases. In western Ethiopia juices of crushed leaves of *Phytolaca dodecandra*, *Vernonia amygdalina*, and crushed seed of *Lepidium sativum* mixed with fresh cattle faeces, were used to control ticks (Regassa, 2000).

2.9.3. Biological control method

Entomopathogens are group of organisms that attack ticks and insects. It can be macro- or microorganisms that affect arthropods (Samish and Alekseev, 2001). The biological agents, which potentially include predators like rodents, birds, ants, spiders, lizards and beetles as well as Parasitoids (destroy the host: the wasp lay the eggs in the engorged ticks and larvae eats the tick and emerges as adult to attack another tick) and parasites (Nematodes and fungus) are attack soil living stages of the ticks are effective

and depending on the conditions, these predators can consume a large number of ticks (Latif and Walker, 2004). Yet, having such effective importance the development of a biological tick control methods has been neglected as compared to the control of plant pests or dipterous insects harmful to men and animals (Samish and Alekseev, 2001). Until this is known, it is not possible to recommend such alternatives to producers for adoption and practical use in the field in Ethiopia.

2.9.4. *Anti-tick vaccine*

The development of the first effective vaccine against *B. microplus* was a great advance in the fight against a serious pest that interferes with world food production. The two commercially available vaccines (TickGARD Plus®, Hoechst, Australia and Gavac™, Heber Biotec, Cuba) are now on the market in a limited number of countries (Willadsen, 2006). Both vaccines are based on hidden antigens from the gut of the tick that once inoculated into cattle, induce the production of antibodies which, when ingested by the tick, result in damage to the gut, slightly reduced survival, reduced egg production and reduced hatchability of eggs (Guerrero *et al.*, 2012). Consequently, tick populations decline with time, although there is little direct mortality effect on ticks. Tick vaccines reduce the number of engorging female ticks, their weight and reproductive capacity, meaning that the greatest vaccination effect is seen as a reduced larval infestation in the subsequent generation (Merino *et al.*, 2013).

2.9.5. *Genetic control method*

The host's genetic variation in response to tick infestation may be used to improve animal production. Selecting animal genotypes that are resistant to tick infestation may reduce the costs of controlling ticks. Immunological responses to tick infestations have been suggested as one of the determinants of the genotypes' resistance or susceptibility to tick infestation (Marufu *et al.*, 2013). Genetically determined resistance of the host to ticks has been demonstrated and *Bos indicus* breeds in general have much higher resistance than European *Bos taurus* breed cattle. Various breeds of cattle differ in their response to tick infestations. *Bos indicus* pure breeds and crossbreeds were reported to be more innately resistant than *Bos Taurus* breeds. However, over 50 tick species are known to exist in the in Ethiopia. The effects of ticks on indigenous cattle compared to exotic breeds shown to be minimal (Morel,

1980; Mekonnen *et al.*, 2001). In Ethiopia, Boran and Horo breeds are known tick resistant cattle breeds (de Castro, 1991).

2.10. Major Tick-borne Diseases of Cattle in Ethiopia

Ticks and tick-borne pathogens have evolved together, resulting in a complex relationship in which the pathogen's life cycle is perfectly coordinated with the tick's feeding cycle, and the tick can harbor high pathogen levels without affecting its biology. Tick-borne diseases (TBDs) continue to emerge and/or spread, and pose an increasing threat to human and animal health (De la Fuente *et al.*, 2015).

2.10.1. Babesiosis

Bovine babesiosis is a hemoparasitic disease caused by protozoa of the genus *Babesia* (Phylum: Apicomplexa), which infects mainly ruminants (Melendez, 2000). In cattle the disease also is known by such names as bovine babesiosis, piroplasmosis, Texas fever, red-water, tick fever, and tristeza (Zaugg, 2009). It is associated with *B. bigemina* and *B. bovis* is the most important disease of tropical and subtropical. Both species are transmitted transovarially by *Boophilus* ticks, but only tick larvae transmit *B. bovis*, whereas nymphs and adults transmit *B. bigemina* (Radostits *et al.*, 2008). Several studies have been conducted in different region in Ethiopia, including abundance, distribution and prevalence of bovine Babesiosis with over all prevalence of 1.5% in Benishangul Gumuz Regional State, Western Ethiopia out of which two species of *Babesia* comprising of *B. bovis* (1.24%) and *B. bigemina* (0.248%) were identified (Bihonegn *et al.*, 2015).

2.10.2. Theileriosis

Theileriosis is a hemoparasitic disease caused by protozoa of the genus *Theileria* (Apicomplexa) (Allison and Meinkoth, 2010). Theileriosis is caused by *Theileria* spp. in cattle, goats, sheep and wild and captive ungulates (Radostits *et al.*, 2008). The parasites are transmitted by tick such as *Rhipicephalus* and *Hyalomma* species. These parasites undergo repeated merogony in the lymphocytes ultimately releasing small merozoites, which invade the red cells to become piroplasms. Theileriosis have a variety of tick vectors which cause infections ranged from clinically inapparent to rapidly fatal (Taylor *et al.*, 2007). Recently *Theileria* (*T. velifera*, *T. mutans*, *T. ovis*,

T. separata, *T. annulata*) infection in domestic ruminants is reported in northern Ethiopia (Gebrekidan *et al.*, 2014).

2.10.3. Cowdriosis (*Heartwater*)

Bovine ehrlichiosis (also known as heartwater or cowdriosis) is an infectious and tick-borne disease of ruminants caused by the rickettsial organism, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) and transmitted by ticks of the genus *Amblyomma* through stage to stage transmission, particularly *Am. variegatum* which is widespread in Ethiopia (Mekonnen *et al.*, 2001). The disease is endemic in sub-Saharan African countries and it has a serious negative impact on livestock productivity, with high morbidity and mortality rates (up to 90%) in susceptible ruminants. European breeds are generally more susceptible than more susceptible than indigenous African breeds (OIE, 2011).

2.10.4. Anaplasmosis (*gall sickness*)

Anaplasmosis is a vector-borne infectious blood disease in cattle caused by the bacterial parasites, *Anaplasma marginale* and *A. centraele*. It occurs primarily in warm tropical and subtropical areas. Anaplasmosis is not contagious; numerous species of tick vectors (*Boophilus*, *Rhipicephalus* and *Hyalomma*) can transmit *Anaplasma* species (Taylor *et al.*, 2007). It can also be transmitted via contaminated surgical instruments, biting flies and mosquitoes. The intracellular parasites destroy the red blood cells. It causes anaemia, fever, weight loss, breathlessness, uncoordinated movements, abortion and death. It is transmitted by the tick through transovarian and stage to stage transmission (de la Fuente *et al.*, 2005)

3. MATERIALS AND METHODS

3.1. Study Areas

The study was conducted in two districts of Central Oromia, Ethiopia; namely Ada'a and Boset districts (Figure 9).

Ada'a district is located 47 km South East of Addis Ababa, covering a land area of 1750 km² on east of Addis Ababa, and lies between longitudes 38°51' to 39°04' East and latitudes 8°46' to 8°59' North. It has a maximum and minimum altitude of 2300 meters above sea level and 1500 meters above sea level respectively. This site has maximum and minimum annual rainfall of 1,200 mm and 800 mm respectively. It experiences bimodal patterns of rainfall with the main rainy season extending from June to September. It has the minimum and maximum temperatures of 13 and 26.5°C. A short rainy season occurs between March and May with an average rainfall of about 800mm. The highest temperature is recorded in May. The mean relative humidity is 61.3% (NMSA, 2015). It has a livestock population of 186,234 cattle, 45,678 sheep, 7,432 goats, 5,009 horses, 1,200 mules, and 25,654 donkeys (CSA, 2013).

Boset district is a part of the rift valley in East Shoa Zone, Oromia Regional State. It is bordered on the south by the Arsi zone, on the west by the Awash River which separates it from Adama, on the north by the Amhara region and on the east by Fentale. It has a maximum and minimum altitude of 1,400 meters above sea level and 1,000 meters above sea level respectively. It has maximum and minimum annual rainfall of 1,200 mm and 550 mm, respectively. It has the minimum and maximum temperatures of 16.6 and 31°C respectively (NMSA, 2015). It has a mixed crop–livestock farming system (CSA, 2013).

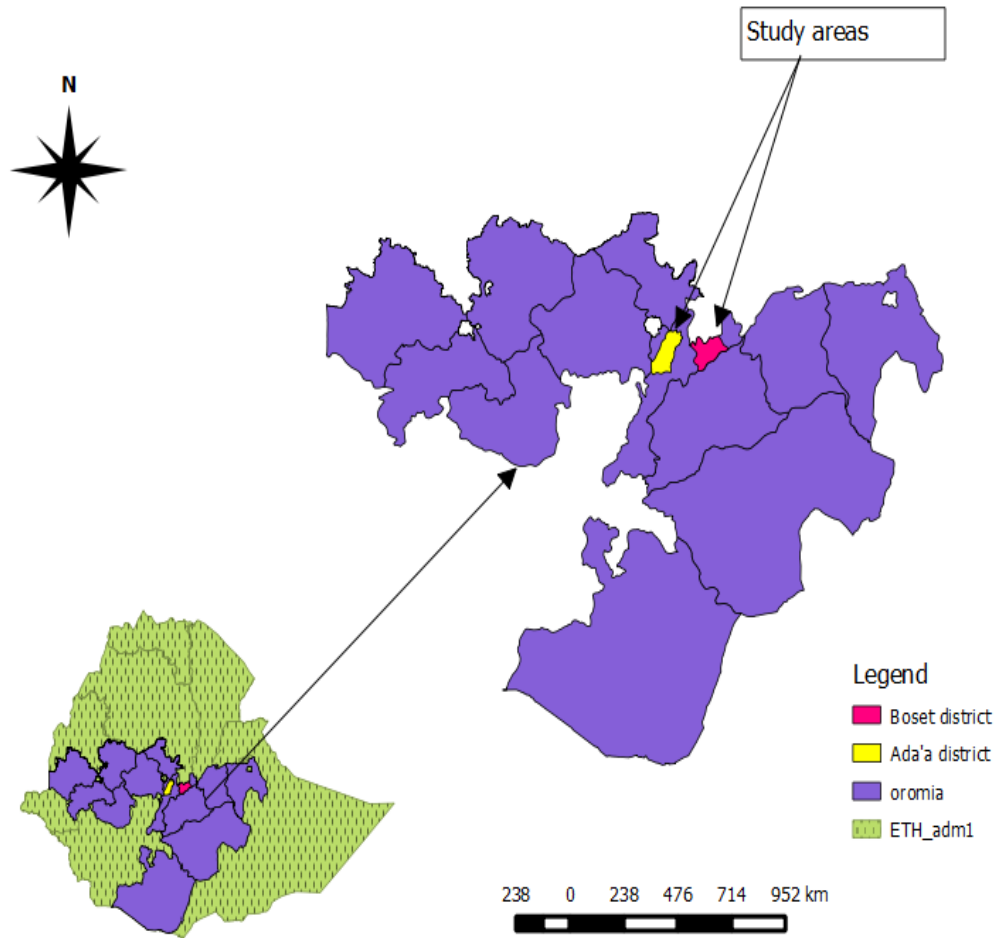


Figure 9: Map of Ethiopia showing the current study areas

3.2. Study Design and Animals

The cross-sectional study was conducted from August 2016 through May 2017 on 720 cattle to determine species diversity and seasonal pattern of ticks infesting cattle during the rainy, dry and short rainy periods and to assess the presence of tick-borne pathogens in blood of cattle in Ada'a and Boset districts, central Oromia, Ethiopia. The animals that were included in this study were those cattle which were not treated with any treatments for a month prior to sample collection time. Information about agroecology and time of visit (season) were recorded (Appendix 1). For seasonal dynamics of ticks, sample collections were done from 120 cattle in each agroecological zone (Ada'a and Boset) during rainy (August), dry (December) and short rainy (April) season.

3.3. Sampling Methods

The study sites were selected by purposive sampling technique for comparison of two agroecological zones: Ada'a (2200 - 2300 masl) as a highland and Boset (1200 - 1300 masl) as lowland in Ada'a and Boset districts, respectively based on GPS (Android new version 4.2.0 of GPS Essential) data record and accessibility. The study cattle were selected by purposive sampling based on the study animal's inclusion criteria: (i) age between 1 year and 3 years (ii) not been treated with any treatment for the last month to determine seasonal pattern and species diversity of ticks and to assess the presence of tick-borne pathogens in study cattle. The total number of cattle for ticks study was 720 cattle among which 120 cattle were sampled from each agro-ecological zone in each study seasons. For tick-borne pathogens 120 cattle were sampled once from each district.

3.4. Study Methods

3.4. 1. Tick collection and identification

Tick collection was done during its parasitic phase from the cattle for species identification. The selected cattle were casted down and restrained appropriately then, the skin of each selected cattle were inspected for the presence or absence of ticks. All visible ticks (larvae, nymph and adults) were manually collected by using forceps from half regions of the animals' body and care was taken to avoid decapitulation (Walker *et al.*, 2014). For analysis the half body tick counts were doubled to obtain whole body tick mean burden per animal. The collected ticks from predilection sites (Appendix 2) were counted into separate bottle, labeled and preserved in sampling bottles containing 70% ethanol from each study cattle from both agro-ecological zones during rainy, dry and short rainy seasons. The ticks were then transported to the Parasitology Laboratory of the College of Veterinary Medicine and Agriculture of Addis Ababa University located at Bishoftu, for identification.

Ticks were identified to the species level according to their morphological key structures such as shape of scutum, leg colour, scutum ornamentation, body grooves, punctuations, basis capitulum, coxae and ventral plates (Appendix 3). During tick identification in the laboratory the sample were put on petridish and adult ticks were

identified to species level whereas larvae and nymphs were identified to genus level under a stereomicroscope using the standard identification keys of Pegram *et al.* (1987); Houseman (2013) and Walker *et al.* (2014). For each tick, photographs of the dorsal view of larvae, nymph and adult male and female ticks were captured and depicted on Figure 10 in the result section.

3.4.2. Blood sample collection

Blood samples were collected from the middle ear vein of 240 cattle using a capillary tube for molecular detection of tick borne pathogens. For this purpose, cattle were casted and immobilized. The middle ear vein was pricked with a blood lancet and blood was collected with a glass capillary tube. Blood samples were applied onto Whatman® classic FTA cards, air-dried and labelled appropriately and packed in foil pouches with a silica gel, prior to DNA analysis (Muhanguzi *et al.*, 2014). FTA ® classic cards provide a safe, secure and reliable method for the room temperature, collection, transport and storage of DNA and then it was sent to the molecular laboratory of Clinvet International Bloemfontein laboratory, South Africa for molecular analysis.

3.4.3. Molecular detection of tick-borne pathogens

3.4.3.1. Extraction and purification of DNA

DNA was extracted and eluted from FTA® classic card sample discs according to a manufacturer's protocol described as follows: Briefly, each FTA® classic card was placed on a supporting base (Whatman BioScience Ltd) and stored in dark boxes containing silica gel in order to avoid damages due to the growth of microorganism and sun light. DNA was extracted from 2 x 5mm diameter punches using the MagMAX™ DNA Multi-Sample Ultra Kit. DNA was extracted from 92 samples per run, including extraction controls for each run using a KingFisher 96-flex instrument. And then DNA was eluted in theoretical 100 µl, but evaporative losses and retention to beads resulted in eluted DNA in approx. 75 µl volumes. Detection sensitivities were performed using 2 sets of independent serial dilutions and performing each standard curve for each template in triplicate and able to detect ≥ 10 copies of input DNA per 5 µl input volume for each PCR under single-plex and multiplex conditions.

The multiplex runs were performed as listed below: Multiplex 1: *B. bovis*, *A. centrale* and *T. parva*, Multiplex 2: *B. bigemina*, *A. marginale* and *T. theileri* and Single plex: *E. ruminantium*.

3.4.3.2. DNA amplification and detection of tick-borne pathogens

Extracted DNA was used as template for the above mentioned multiplex reaction setups (using a Hamilton Nimbus robotic liquid handling system – also used for standard curve preparations). Each PCR contained positive control samples (representing 10 copies of each target per reaction), negative control (bovine DNA from a Clinvet donor animal) and extraction control (to validate absence of any detectable template in the extraction kits). A total of 5 µl template DNA was used in a 15 µl final PCR reaction. Species-specific real time PCR was performed in molecular laboratory of Clinvet International Bloemfontein laboratory, South Africa as the following author's protocols. Quantitative pCS20 real-time PCR TaqMan probe assay was the most sensitive, rapid and can be performed within 2h it is an effective assay for epidemiological surveillance and monitoring of infected animals for *E. ruminantium* (Steyn *et al.*, 2008). Duplex real-time PCR for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale* (Decaro *et al.*, 2008) and Taqman-Based Real-Time PCR Assays for detection of *Babesia bovis* and *Babesia bigemina* and *T. parva* (Kim *et al.*, 2007; Papli *et al.*, 2010). Multiplex 1 was performed first to ensure that no template derived inhibition were observed (using the IAC), then followed by the other 2 PCRs. qPCRs were performed using the QuantStudio6 qPCR system and analyzed using the QuantStudio Real-Time PCR Software v1.2. Samples exhibiting the correct amplification profiles (shape and Ct crossing) were considered as positive, whereas the rest were considered as neegative.

3.5. Data Analysis

The data (Animal identity code, ticks count, seasons and districts) collected and identified result (ticks species and tick-borne pathogens) from the study cattle and study areas were entered in to Microsoft Excel spread sheets and the data was coded appropriately and analyzed using STATA version 13.0 statistical software (StataCorp., 2013). The data was summarized by descriptive statistics and displayed by tables and graphs. Poisson regression was used to analyse the number (count) of

ticks on animals as a function of different explanatory variables (seasons and agroecology). Furthermore, Negative binomial regression was applied by fitting the model after Poisson regression to overcome over dispersion. Logistic regression was employed to analyse the degree of association of binary outcome (presence or absence of tick and tick-borne infection) as a function of various explanatory factors. Odds ratio and chi square tests were used to quantify the association among the factors with the presence of tick infestation and tick-borne infection. For all statistical analysis a statistical significance level of $p < 0.05$ was considered.

3.6. Ethical Statement

Ethical approval for the collection of ticks and blood sample from cattle with ethical manner obtained from the Animal Research Ethics and Review committee (Certificate Ref. No: VM/ERC/12/06/2017) from College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia. Sample collection from cattle was not opposing the welfare of the animals.

4. RESULTS

4.1. Ticks Infesting Cattle

4.1.1. Species diversity and relative abundance

Of the total 720 cattle examined 659 (91.5%) of them were infested with one or more ticks. Overall, eleven species belonging to three ixodid tick genera (*Amblyomma*, *Rhipicephalus* and *Hyalomma*) and subgenus *Rhipicephalus* (*Boophilus*) were identified (Table 3). A total of 7430 (5220 males and 2210 females) adult and 794 immature (46 larvae and 748 nymphs) stages of ixodid ticks were collected during the wet (2142), dry (1608) and short rainy (4474) seasons from cattle in Ada'a and Boset districts. Nine (9), ten (10) and nine (9) tick species were collected during rainy, dry and short rainy seasons respectively, in both districts. Findings of the current study indicated that ticks were present on cattle during all seasons of the study period.

Overall, *Amblyomma* (76%) was the most predominant genus in Ada'a district followed by subgenus *Rhipicephalus* (*Boophilus*) (10%), genus *Hyalomma* (8.1%) and genus *Rhipicephalus* (5.9%) in decreasing order. In Boset district *Rhipicephalus* (82.7%) was the most predominant and widespread tick species followed by genera of *Amblyomma* (11.2%), *Hyalomma* (5.8%) and the subgenus *Rhipicephalus* (*Boophilus*) (0.3%). The present study revealed that the nymphal stage (56.1%) of the genus *Amblyomma* was significantly ($p < 0.05$) higher during the dry season than all the other seasons (rainy and short rainy) in Ada'a district. In Boset district, *Rhipicephalus* nymphs were collected at higher proportion during the rainy season than both dry and short rainy seasons (Table 4). *Amblyomma* nymphs were mostly collected in dry periods, while adults were more abundant during the short rainy and rainy seasons.

Table 3: Overall frequencies, percentages and Poisson regression analysis significant value of genera of ticks collected from Ada'a and Boset districts

Tick genera	District		Over all Count (%)	p-value
	Ada'a Count (%)	Boset Count (%)		
<i>Amblyomma</i>	2752 (76)	513 (11.2)	3265 (39.5)	0.000
<i>Rhipicephalus</i>	215 (5.9)	3815 (82.7)	4030 (49)	0.000
<i>Hyalomma</i>	293 (8.1)	259 (5.8)	562 (6.9)	0.312
<i>Subgenus Rhipicephalus</i> (<i>Boophilus</i>)	362 (10)	15 (0.3)	377 (4.6)	0.000
Total ticks	3622 (100)	4512 (100)	8224 (100)	0.000

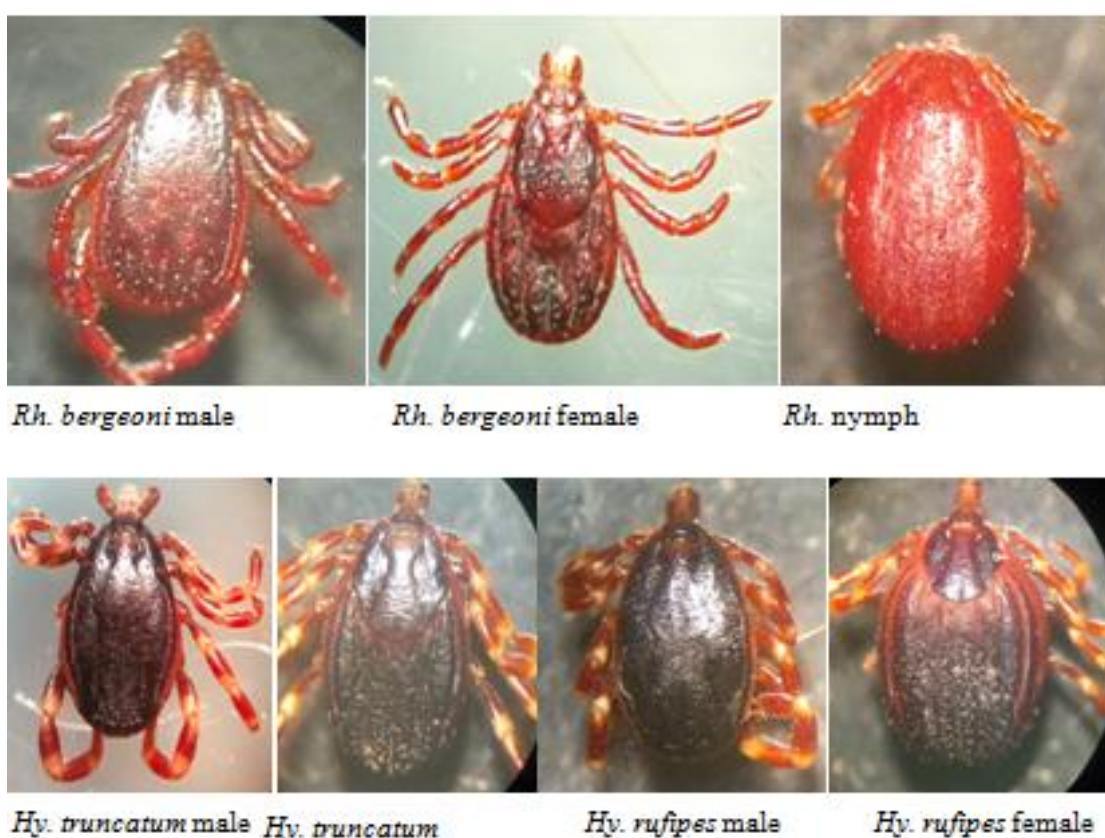


Figure 10: Photographs of ixodid ticks collected from Ada'a and Boset districts
(Taken by Misgana Naramo from August 2016 to May 2016)

Figure 10: (Continued)

Pictures of tick species



Rh. (Bo.) decoloratus male

Rh. (Bo.) decoloratus

Rh. (Bo.) nymph



Rh. (Bo.) larvae

Am. gemma male

Am. gemma female

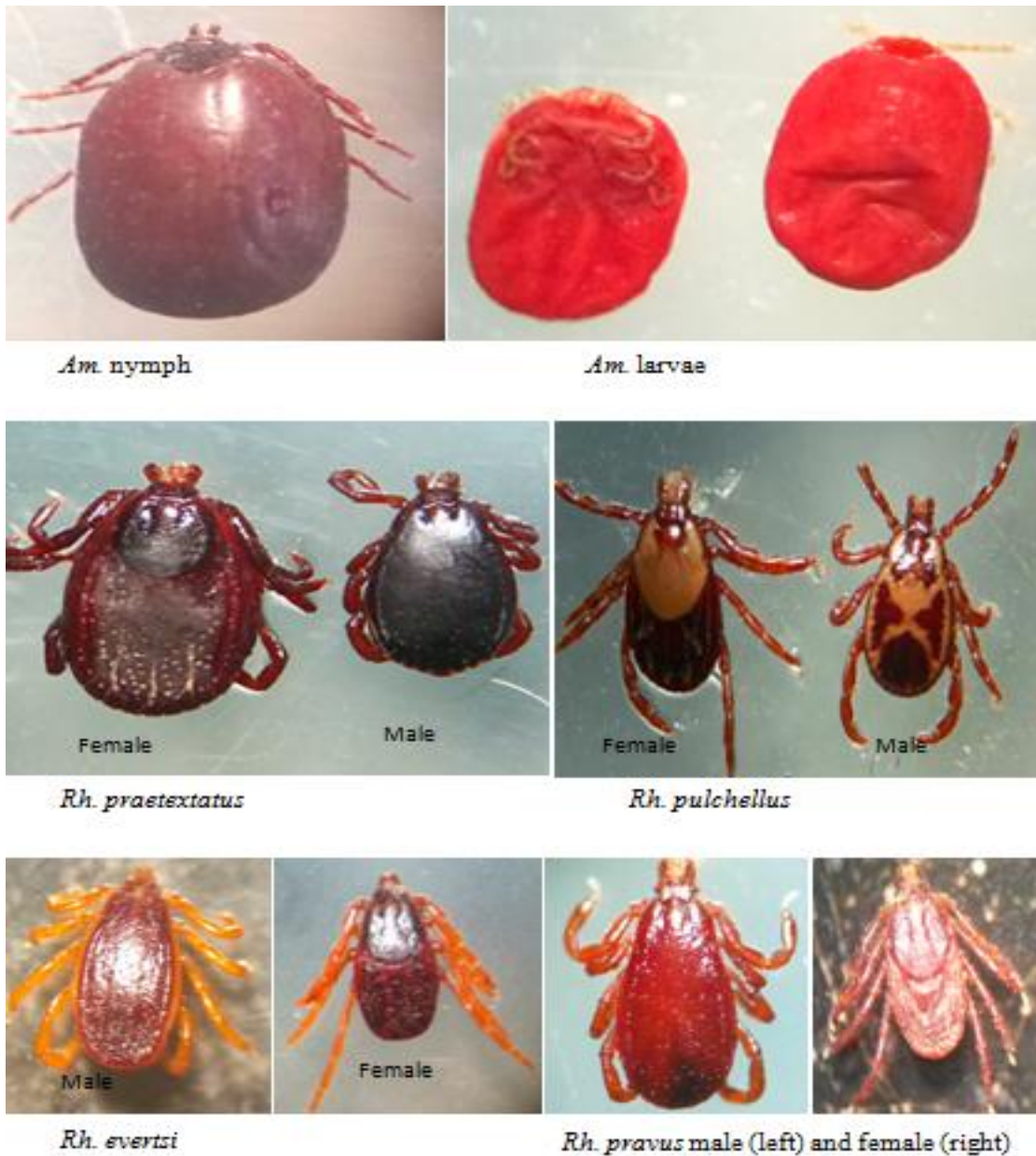


Am. variegatum male

Am. variegatum female

Am. cohaerens male

Figure 10: (Continued)



In Ada'a district, out of 360 examined cattle, 320 (88.8%) of them were infested by one or more ticks and an overall of 3612 ticks were collected during all the study seasons. Of these ticks, 907, 987 and 1718 adult and immature ticks were collected during the rainy, dry and short rainy seasons, respectively. Nine (9) adult tick species and immature stages of the genera (*Amblyomma*, and *Rhipicephalus*) and subgenus *Boophilus* were identified. The current study showed that *Amblyomma variegatum* (55.4%), *Hyalomma truncatum* (14.2%), *Rhipicephalus (Boophilus) decoloratus* (13%), *Rhipicephalus praetextatus* (5.1%), *Boophilus* nymph (5%), *Hyalomma*

rufipes (3.7%), *Rhipicephalus bergeoni* (2.2%) and *Rhipicephalus evertsi* (1.2%), *Amblyomma nymph* (0.1%) and *Rhipicephalus numph* (0.1%) were collected during the rainy season. On other hand during the dry season, *Amblyomma nymph* (56.1%), *Hyalomma truncatum* (9.4%), *Rhipicephalus (Boophilus) decoloratus* (6.8%), *Rhipicephalus praetextatus* (5.9%), *Rhipicephalus (Boophilus) nymph* (4.7%), *Hyalomma rufipes* (3.6%), *Amblyomma variegatum* (3.1%), *Amblyomma larvae* (2.9%), *Rhipicephalus bergeoni* (2.5%) and *Rhipicephalus evertsi* (1.8%), *Rhipicephalus (Boophilus) larvae* (1.7%) and *Amblyomma cohearens* (0.1%) were collected. During the short rainy season, *Amblyomma variegatum* (94.3%), *Rhipicephalus (Boophilus) decoloratus* (3.5%), *Rhipicephalus evertsi* (1.2%), *Amblyomma nymph* (0.3%), *Hyalomma truncatum* (9.4%), *Rhipicephalus (Boophilus) nymph* (0.5%) *Rhipicephalus pulchellus* (0.2%) and *Amblyomma cohearens* (0.1%) were collected.

In Boset district, out of 360 examined cattle, 339 (94.2%) of them were infested by one or more ticks and an overall of 4612 ticks were collected during all the study seasons. Of these collections, 1235, 621 and 2756 adult and immature ticks were recorded during the rainy, dry and short rainy seasons, respectively. Nine (9) adult tick species and immature stages of genera (*Amblyomma*, and *Rhipicephalus*) and subgenus *Rhipicephalus (Boophilus)* were identified. The present study indicated that *Rhipicephalus pulchellus* (80.1%), *Amblyomma gemma* (7.9%), *Hyalomma truncatum* (3.6%), *Rhipicephalus nymph* (3.2%), *Rhipicephalus evertsi* (2%), *Hyalomma rufipes* (1%), *Boophilus nymph* (1%), *Rhipicephalus pravus* (0.5%), *Rhipicephalus (Boophilus) decoloratus* (0.3%), *Amblyomma variegatum* (0.2%) and *Amblyomma nymph* (0.2%) were collected during the rainy season. Likewise, during the dry season, *Rhipicephalus pulchellus* (54.2%), *Amblyomma gemma* (15.4%), *Hyalomma rufipes* (9.2%), *Hyalomma truncatum* (8.6%), *Rhipicephalus evertsi* (7.6%) *Rhipicephalus nymph* (3.1%) *Rhipicephalus praetextatus* (1.5%) and *Rhipicephalus pravus* (0.2%) were collected. During the short rainy season *Rhipicephalus pulchellus* (81.9%), *Amblyomma gemma* (11.3%), *Hyalomma rufipes* (2.4%), *Rhipicephalus evertsi* (1.9%), *Hyalomma truncatum* (1.3%), *Rhipicephalus praetextatus* (0.5%), *Rhipicephalus nymph* (0.2%) and *Rhipicephalus pravus* (0.2%) were collected (Table 4).

Table 4: Diversity, count and percentages of cattle ticks during the rainy, dry and short rainy seasons in Ada'a and Boset districts

Ticks species	Ada'a				Boset				Overall Count (%)
	Rainy	Dry	Short rainy	Total	Rainy	Dry	Short rainy	Total	
	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	
<i>Am. variegatum</i>	502 (55.4)	31 (3.1)	1620 (94.3)	2153 (59.6)	3 (0.2)	0	3 (0.1)	6 (0.13)	2159 (26.3)
<i>Am. gemma</i>	0	0	0	0	98 (7.9)	95 (15.4)	310 (11.3)	5.3 (10.9)	503(6.1)
<i>Am. cohaerens</i>	0	1 (0.1)	1 (0.06)	2 (0.02)	0	0	0	0	2 (0.02)
<i>Am. larvae</i>	0	29 (2.9)	0	29 (0.8)	0	0	0	0	29 (0.4)
<i>Am. nymph</i>	1 (0.1)	552 (56.1)	5 (0.3)	558 (15.4)	2 (0.2)	1 (0.2)	1 (0.04)	4 (0.09)	562 (6.8)
<i>Hy. truncatum</i>	129 (14.2)	93 (9.4)	1 (0.06)	223 (6.2)	45 (3.6)	53 (8.6)	37 (1.3)	135 (2.9)	358 (4.4)
<i>Hy. rufipes</i>	34 (3.7)	36 (3.6)	0	70 (1.9)	12 (1)	57 (9.2)	65 (2.4)	134 (2.9)	204 (2.5)
<i>Rh. praetextatus</i>	46 (5.1)	58 (5.9)	0	104 (2.9)	0	9 (1.5)	13 (0.5)	22 (0.5)	126 (1.5)
<i>Rh. pulchellus</i>	0	4 (0.4)	3 (0.18)	7 (0.2)	989 (80.1)	336 (54.2)	2265 (81.9)	3590 (77.8)	3597 (43.7)
<i>Rh. evertsi</i>	11 (1.2)	18 (1.8)	20 (1.16)	49 (1.4)	25 (2)	47 (7.6)	53 (1.9)	125 (2.7)	174 (2.1)
<i>Rh. bergeoni</i>	20 (2.2)	25 (2.5)	0	45 (1.2)	0	0	0	0	45 (0.5)
<i>Rh. pravus</i>	0	0	0	0	6 (0.5)	1 (0.2)	6 (0.2)	13 (0.3)	13 (0.16)
<i>Rh. nymph</i>	1 (0.1)	9 (0.9)	0	10 (0.3)	40 (3.2)	19 (3.1)	6 (0.2)	65 (1.4)	75 (0.9)
<i>Rh. (Bo). decoloratus</i>	118 (13)	67 (6.8)	60 (3.5)	245 (6.8)	4 (0.3)	0	0	4 (0.09)	249 (3.02)
<i>Rh. (Bo). larvae</i>	0	17 (1.7)	0	17 (0.5)	0	0	0	0	17 (0.2)
<i>Rh. (Bo). nymph</i>	45 (5)	47 (4.7)	8 (0.46)	100 (2.8)	11 (1)	0	0	11 (0.3)	111(1.3)
Total	907 (25.1)	987 (27.3)	1718(47.6)	3612 (43.9)	1235 (26.8)	621 (13.5)	2756 (59.7)	4612 (56.1)	8224(100)

4.1.2. Distribution of tick species in highland and lowland Agroecological zones

This study indicated that *Am. variegatum* (59.6%) was the most predominant tick species followed by *Rh. (Bo). decoloratus* (6.8%) and *Hy. truncatum* (6.2%) in highland PAs of Ada’ a district (Figure 11). Significantly ($p < 0.00$) higher number of *Am. variegatum* and *Rh. (Bo.) decoloratus* were recorded in highland PAs of Ada’a district than lowland Boset district (Table 5). However, in lowland agroecology *Rh. pulchellus* (77.8%) was the most prevalent and abundant tick species followed by *Am. gemma* (10.9%), *Hy. truncatum* (2.9%) and *Hy. rufipes* (2.9%) in Boset district. *Am. gemma* and *Rh. pravus* were recorded only in lowland agroecology in Boset district (Figure 11). *Rh. bergeoni* and *Am. cohearens* (Table 4) were recorded only from the highland PAs of Ada’a district.

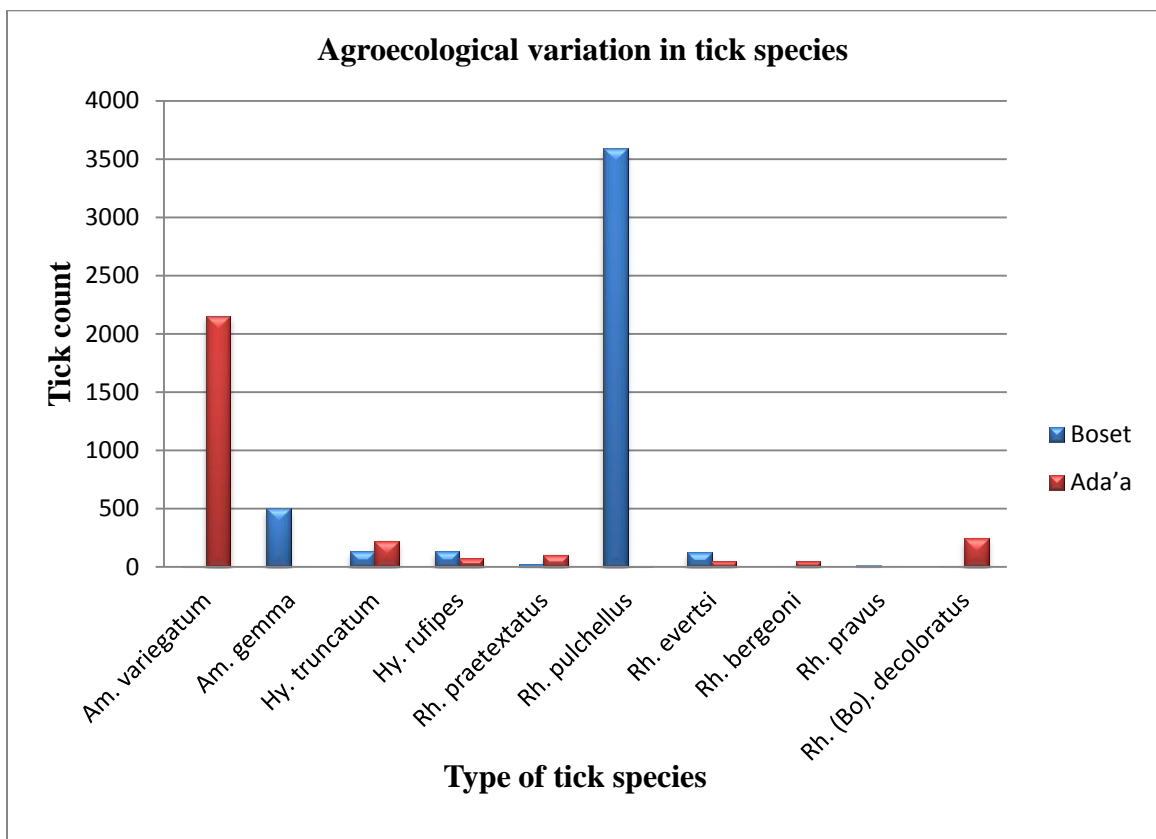


Figure 11: Overall distribution of adult tick species in Ada’a and Boset districts

Logistic regression analysis revealed that the overall risk of tick infestation in cattle of lowland in Boset district was 2.02 times higher than in cattle in highland PAs of Ada’a

district. Similarly, infestation of cattle by *Am. variegatum* and *Rh. (Bo.) decoloratus* in highland PAs of Ada'a district was 90.77 and 90.62 times higher than in the lowland Boset district, respectively. However, the probability of infestation of cattle by *Rh. pulchellus* in lowland Boset district was 674 times higher than infestation in highland PAs of Ada'a district. In addition, the risk of cattle infested by *Hy. rufipes* in lowland Boset district (OR=1.51) was 1.55 times higher than cattle infested in highland PAs of Ada'a district (Table 5).

Table 5: Comparison of risk of infestation by tick species in cattle of highland of Ada'a and lowland Boset districts using logistic regression analysis

Tick species	District	OR	SE	CI	P-value
<i>Am. variegatum</i>	Ada'a	90.77	42.01	36.6 - 224.8	0.000
	Boset *				
<i>Rh. (Bo.) decoloratus</i>	Ada'a	90.62	65.05	22.2 - 370.0	0.000
	Boset *				
<i>Hy. truncatum</i>	Ada'a	1.71	0.381	1.12 - 2.65	0.017
	Boset *				
<i>Hy. rufipes</i>	Boset	1.51	0.33	0.97 - 2.32	0.065
	Ada'a*				
<i>Rh. praetextatus</i>	Ada'a	4.57	1.87	2.06 - 10.2	0.000
	Boset *				
<i>Rh. pulchellus</i>	Boset	674.00	356	239 - 1900	0.000
	Ada'a*				
<i>Rh. evertsi</i>	Boset	2.72	0.66	1.69 - 4.37	0.000
	Ada'a *				
Overall	Boset	2.02	0.56	1.16 - 3.49	0.012
	Ada'a *				

* =Reference category, SE =Standard error, OR= Odds ratio, CI= Confidence interval

4.1.3. Seasonal dynamics of ticks in highland and lowland agroecologies

The study revealed that highest rate of overall infestation by ticks occurs during the short rainy season than both the rainy and dry seasons. *Rhipicephalus* and *Amblyomma* genera of ticks were more abundant than the others tick genera during the short rainy season than both the rainy and dry seasons. *Hyalomma* and *Rhipicephalus (Boophilus)* ticks were the least collected during all the study seasons (Figure 12).

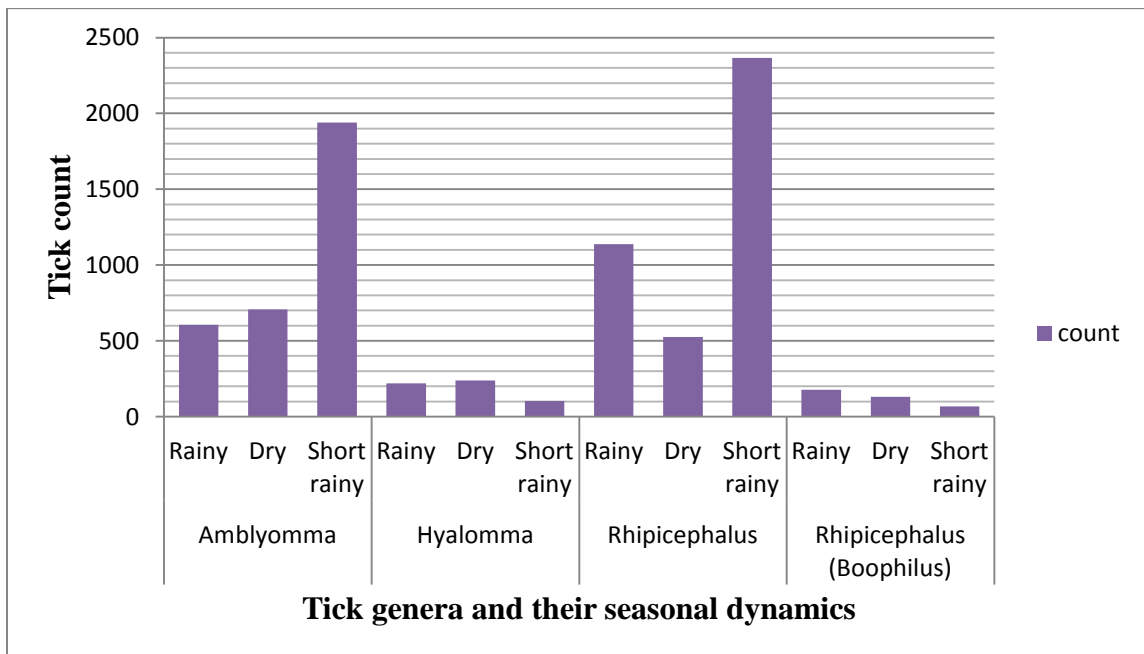


Figure 12: Overall count of tick genera during different seasons of the the study period

Higher *Rh. pulchellus* and *Am. variegatum* were observed during the short rainy and rainy season. The number of *Am. variegatum* reduced very significantly during the dry season, whereas *Amblyomma* nymph was highly abundant during the dry season. *Rh. (Bo.) decoloratus* was higher during the rainy season than both the dry and short rainy seasons. *Hy. truncatum* was higher during the rainy and short rainy season than dry season. Both *Hy. rufipes* and *Rh. evertsi* showed no much difference among different study season (Figure 13).

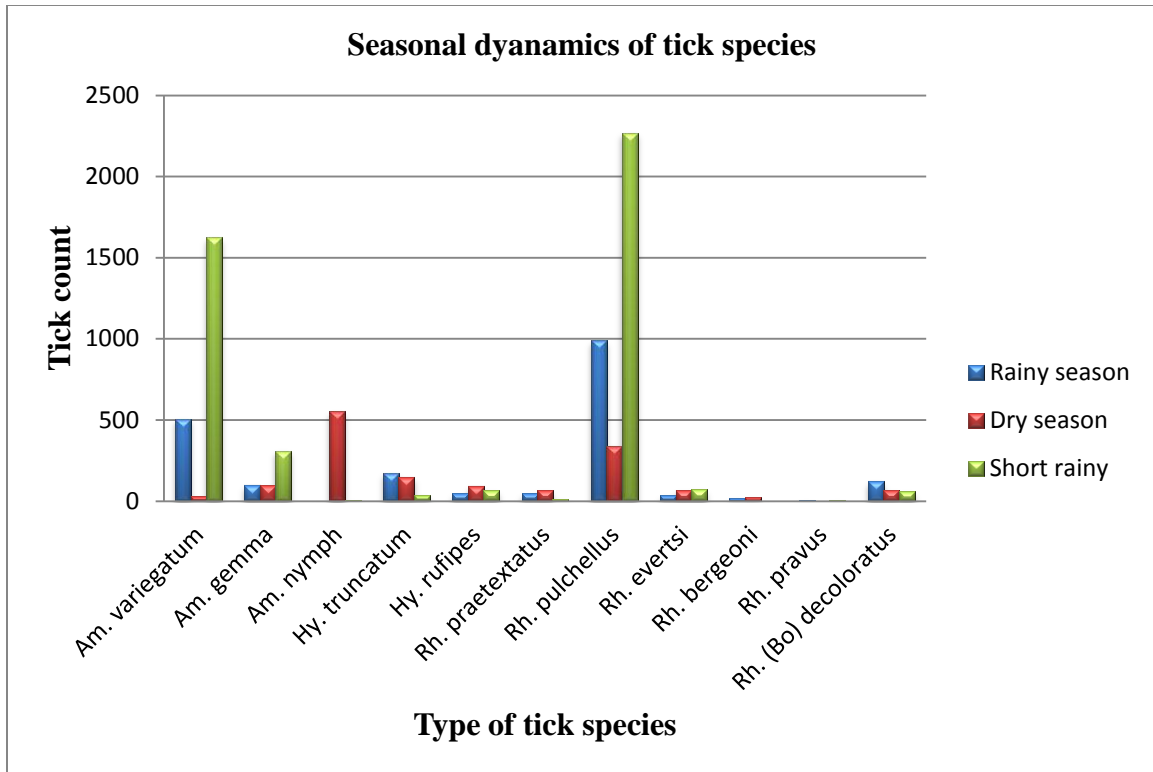


Figure 13: Overall seasonal occurrence of tick species during different season of the study period

Differences were observed between various seasons in study cattle in terms of tick counts per animal. Significantly ($p < 0.000$) higher tick load were recorded during the short rainy (26.52 ± 1.06) and the rainy seasons (19.04 ± 1.36) than in the dry seasons (14.94 ± 1.06). Statistically significant ($p < 0.000$) higher mean number of *Am. variegatum* (33.06 ± 3.46) ticks per animal was recorded during the short rainy season than during both the rainy (12.2 ± 0.58) and dry (2.82 ± 0.48) seasons. Significantly (12.64 ± 2.66 , $p = 0.000$) higher tick load of *Am. gemma* was observed during the short rainy than both during rainy (6.32 ± 0.90) and dry (7.02 ± 0.94) seasons. Significantly ($p = 0.000$) higher mean of *Rh. (Bo.) decoloratus* was recorded during the rainy season (6.22 ± 2.68) than the dry season (3.68 ± 0.24) (Table 6). Statistical significant variations ($p > 0.05$) was never observed in the mean of *Hy. truncatum*, *Rh. evertsi* and *Rh. praetextatus* among different study seasons. Statistically significant ($p < 0.000$) higher tick counts per animal of *Rh. pulchellus* was recorded during the short rainy season (39.52 ± 2.70) than both the rainy (17.05 ± 1.34) and dry (7.62 ± 0.58) seasons (Table 6).

Table 6: Overall mean tick burden (Mean±SE) during different seasons analyzed by Negative binomial regression significance value

Tick species	Season	Mean ± SE	CI	p-value
<i>Am. variegatum</i>	Rainy	12.2 ± 0.58	9.9 - 14.50	*
	Dry	2.82 ± 0.48	1.80 - 3.82	0.000
	Short rainy	33.06 ± 3.46	26.14-39.96	0.000
<i>Am. gemma</i>	Rainy	6.32 ± 0.90	4.44 - 8.18	*
	Dry	7.02 ± 0.94	3.10- 8.98	0.457
	Short rainy	12.64 ± 2.66	7.30-18.00	0.001
<i>Rh. (Bo.) decoloratus</i>	Rainy	6.22 ± 2.68	0.70 - 11.72	*
	Dry	3.68 ± 0.24	3.18 - 4.16	0.000
<i>Hy. truncatum</i>	Short rainy	4.14 ± 0.48	3.20-5.00	0.613
	Rainy	11.6 ± 2.62	6.08 - 17.16	*
	Dry	10.4 ± 1.24	7.82 - 12.92	0.189
<i>Hy. rufipes</i>	Short rainy	4.92 ± 0.76	3.26 – 6.60	0.227
	Rainy	4.18 ± 0.25	3.36- 7.08	*
	Dry	8.36 ± 0.55	5.67 – 11.93	0.203
<i>Rh. pulchellus</i>	Short rainy	4.28 ± 1.00	3.20 – 7.34	0.009
	Rainy	17.05 ± 1.34	14.36 - 19.74	*
	Dry	7.62 ± 0.58	6.48 - 8.78	0.000
<i>Rh. praetextatus</i>	Short rainy	39.52 ± 2.70	34.14- 44.90	0.000
	Rainy	3.84 ± 0.56	2.68 - 4.96	*
	Dry	6.86 ± 1.36	4.96 - 9.80	0.885
<i>Rh. evertsi</i>	Short rainy	3.70 ± 0.80	1.72 – 5.68	0.920
	Rainy	6.86 ± 1.16	3.48 - 10.06	*
	Dry	6.08 ± 1.14	2.12 – 5.08	0.733
<i>Rh. bergeoni</i>	Short rainy	8.44 ± 1.50	5.18 – 15.73	0.139
	Rainy	4.44 ± 0.86	2.44 - 6.44	*
	Dry	3.12 ± 0.52	2.02- 4.22	0.240
<i>Rh. pravus</i>	Rainy	2.40 ± 0.40	1.28 - 3.50	*
	Short rainy	2.28 ± 0.28	1.58-2.98	0.752
Overall	Rainy	19.04 ± 1.36	16.94-21.12	*
	Dry	14.94 ± 1.06	12.80 -17.06	0.000
	Short rainy	26.52 ± 1.06	23.82-29.22	0.000

*= Reference category, SE =Standard error, OR= Odds ratio, CI= Confidence interval

Current study disclosed that statistically significant differences in the risk of infestation of cattle by *Am. variegatum* during the short rainy (7.20) and rainy season (5.33) times higher than infestation during the dry season. Similarly, infestation of cattle by *Am. gemma* was 2.02 and 1.17 times higher during the short rainy and rainy seasons, respectively, than during the dry season. *Hy. truncatum* and *Rh. praetextatus* were statistically significant between short rainy and dry season, *Am. variegatum*, *Hy. rufipes* and *Rh. pulchellus* were had significant difference in all study seasons. *Rh. (Bo.) decoloratus* was significantly higher during rainy season (OR=2.87, P=0.000) than dry season. The odds of cattle infested by *Rh. (Bo.) decoloratus* were 2.87 times higher in rainy season than dry season. In case of *Rh. evertsi* there was significant difference in dry and rainy seasons (Table 7).

Table 7: Overall presence of tick species infesting cattle during different seasons of the study period analyzed by using Logistic regression

Tick species	Season	OR	SE	CI	p-value
<i>Am. variegatum</i>	Dry *				
	Short rainy	7.20	1.86	4.33 – 11.97	0.000
	Rainy	5.33	1.39	3.20 - 8.91	0.000
<i>Am. gemma</i>	Dry *				
	Short rainy	2.02	0.53	1.22 3.36	0.007
	Rainy	1.17	0.33	0.67 - 2.03	0.576
<i>Rh. (Bo.) decoloratus</i>	Dry *				
	Short rainy	1.04	0.29	0.59 - 1.81	0.888
	Rainy	2.87	0.71	1.77 - 4.66	0.000
<i>Hy. truncatum</i>	Dry*				
	Short rainy	0.23	0.07	.13 .413	0.000
	Rainy	1.05	0.22	.69 - 1.59	0.831
<i>Hy. rufipes</i>	Dry*				
	Short rainy	0.48	0.13	0.28 - 0.82	0.007
	Rainy	0.57	0.15	0.34 - 0.95	0.032
<i>Rh. pulchellus</i>	Dry*				
	Short rainy	1.45	0.27	1.01 - 2.09	0.043
	Rainy	1.55	0.28	1.05 - 2.16	0.027
<i>Rh. praetextatus</i>	Dry*				
	Short rainy	0.18	0.08	0.08 - 0.42	0.000
	Rainy	0.67	0.19	0.38 - 1.17	0.163
<i>Rh. evertsi</i>	Dry*				
	Short rainy	0.87	0.23	0.53 - 1.45	0.605
	Rainy	0.55	0.16	0.36 - 0.97	0.039
<i>Rh. bergeoni</i>	Dry*				
	Rainy	0.55	0.23	0.24 - 1.26	0.156
<i>Rh. pravus</i>	Dry *				
	Short rainy	6.13	6.64	.73 - 51.29	0.094
	Rainy	5.08	5.59	0.59 - 43.85	0.139

*= Reference category, SE =Standard error, OR= Odds ratio, CI= Confidence interval

4.1.4. Sex ratio

Higher number of males in genera of *Amblyomma*, *Rhipicephalus*, and *Hyalomma* were recorded in both study sites during all seasons of the study period. However, higher number of females than males of the subgenus *Rh. (Bo.) decoloratus* collected in both study sites during all seasons of the study period (Table 8). The male to female ratio of *Am. variegatum*, *Rh. pulchellus*, *Hy. rufipes*, *Am. gemma*, *Rh. evertsi*, *Rh. praetextatus* and *Rh. bergeoni* were 13.7:1, 1.5:1, 3.8:1, 2.7:1, 2.7:1, 1.5:1 and 1.8:1 respectively. In *Rh. (Bo.) decoloratus* (0.07:1) male to female sex ratio was recorded (Table 8).

Table 8: Overall male/female sex ratio of tick species collected during all the seasons of the study period in Ada'a and Boset districts

Tick species	Sex ratio			Overall
	M	F	Total count	M/F ratio
<i>Am. variegatum</i>	2011	147	2159	13.7: 1
<i>Am. gemma</i>	367	136	503	2.7: 1
<i>Am. cohaerens</i>	2	0	2	0
<i>Rh. (Bo.) decoloratus</i>	17	232	249	0.07:1
<i>Hy. truncatum</i>	255	103	358	2.5:1
<i>Hy. rufipes</i>	162	42	204	3.8:1
<i>Rh. evertsi</i>	127	47	174	2.7:1
<i>Rh. praetextatus</i>	75	51	126	1.5:1
<i>Rh. pulchellus</i>	2167	1431	3597	1.5:1
<i>Rh. bergeoni</i>	29	16	45	1.8:1
<i>Rh. pravus</i>	8	5	13	1.6:1
Total	5220	2210	7430	2.4:1

4.1.5. Attachment sites

The present study disclosed that the preferred attachment sites of ixodid ticks infesting cattle vary among different genera of ticks. Stronger preferences for attachment areas were noted in some genera of ticks as compared to the other genera of ticks. This study

suggested that *Amblyomma* species has got greater preference for ventral parts such as udder/scrotum, dewlap/brisket, perineum, legs and shoulder to tail base of cattle in descending order. Tick species that belong to the genus *Hyalomma* showed greater preference for tail and anal region, ventral parts such as legs, perineum and brisket of their host cattle. Likewise, member of the genus *Rhipicephalus* species seems to had got greater preferences for the ears, tail and anal area, head, neck and shoulder to tail base/belly/back regions of their host. The subgenus *Rh. (Bo.) decoloratus* looks to had got greater preferences for the attachment sites such as head and neck, shoulder to tail base, and ears of their host cattle (Figure 14). These preferences for attachment sites of ticks were noted during all the study seasons in both districts.

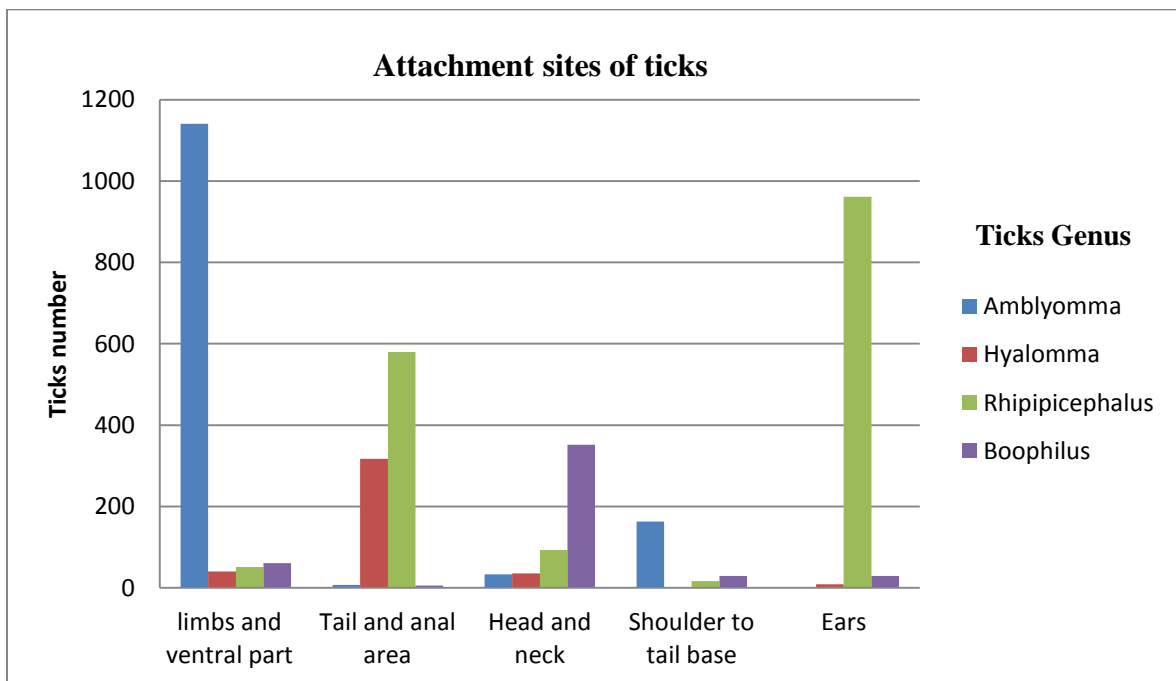


Figure 14: Overall preference for attachment sites on cattle by different genera of ticks during the study period

4.2. Tick-borne Pathogens of Cattle

Of the total of 240 blood samples of cattle (120 from Ada'a and 120 from Boset) examined for the presence of tick-borne pathogens, an overall of 75.8% (182/240) of cattle were infected by one or more tick-borne pathogens. *Babesia bigemina*, *Anaplasma marginale*, *Anaplasma centrale*, *Ehrlichia ruminantium* and *Trypanosoma theileri* DNA were detected. *Babesia bovis* and *Theileria parva* weren't detected in blood samples collected from both districts.

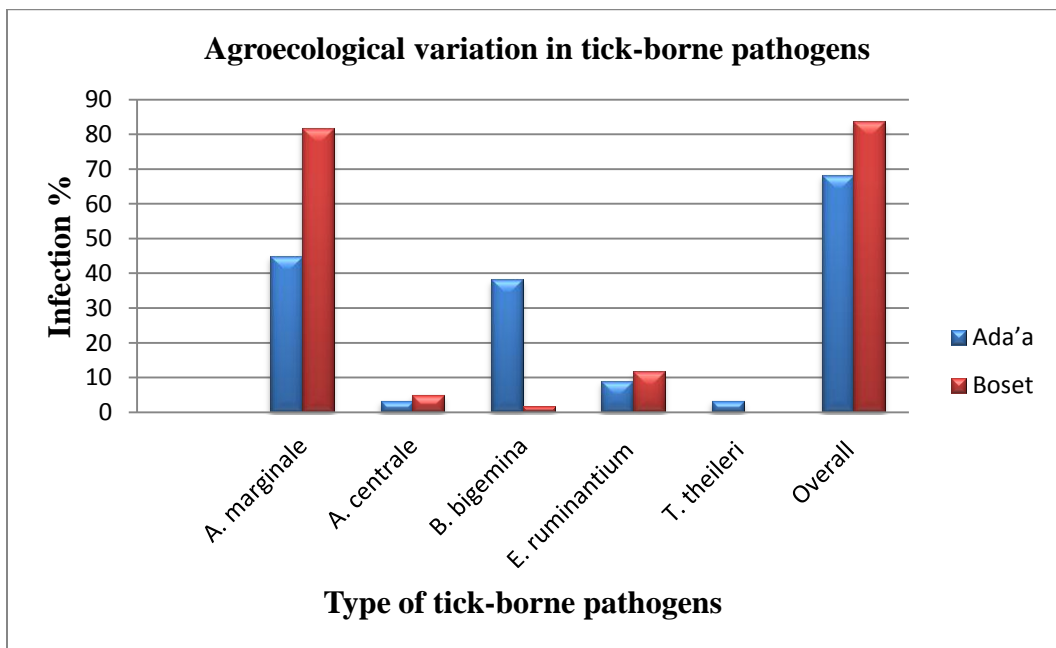


Figure 15: Tick-borne pathogens detected in blood of cattle in both districts

In Ada'a district, 68.3% (82/120) of the tested blood samples were positive for the DNA of one or more tick-borne pathogens. Accordingly, 46 (38.3%) of *B. bigemina*, 54 (45%) of *A. marginale*, 4 (3.3%) of *A. centrale*, 11 (9.1%) *E. ruminantium* and 4 (3.3%) *Trypanosoma theileri* DNA were detected in blood samples of cattle of the district. Likewise, in Boset district, a total of 100 (83.3%) blood samples were positive for at least one tick-borne pathogens. Out of the total infected cattle 98 (82%) *A. marginale*, 14 (12%) *E. ruminantium*, 6 (5%) *A. centrale* and 2 (2%) *B. bigemina* were detected in blood samples of cattle of Boset district. *A. marginale* was the most prevalent tick-borne pathogen followed by *B. bigemina* and *E. ruminantium* and *A. centrale* in both districts.

Statistically significantly (P=0.000) higher *B. bigemina* percentages in Ada'a than Boset district whereas higher *A. marginale* in Boset district than Ada'a district were observed (Table 9).

Table 9: Species of tick-borne pathogens, their relative frequencies and measure of association in cattle of Ada'a and Boset districts, Ethiopia

Tick-borne pathogens	Ada'a (n=120)	Boset (n=120)	Total (n=240)	X ²	p-value
	Positive (%)	Positive (%)	Positive (%)		
<i>A. marginale</i>	54 (45)	98 (81.7)	152 (63.4)	39.73	0.000
<i>A. centrale</i>	4 (3.3)	6 (5)	10 (4.2)	0.417	0.518
<i>B. bigemina</i>	46 (38.3)	2 (1.6)	48 (20)	50.41	0.000
<i>E. ruminantium</i>	11 (9.1)	14 (11.7)	25 (10.5)	0.40	0.527
<i>T. theileri</i>	4 (3.3)	0	4 (1.7)	4.067	0.044
Overall	82 (68.3)	100 (83.8)	182 (75.8)	7.3664	0.007

X² = Pearson chi² %= Percentages

The present study showed that cattle in Boset district were 2.32 times more likely to be positive for DNA of tick-borne pathogens than cattle in Ada'a district. Cattle in Boset district were 5.44 times more likely to be positive for DNA of *A. marginale* than cattle in Ada'a (p<0.001) district. Similarly, cattle in Ada'a had 36.68 times higher chance of positive for DNA of *B. bigemina* than cattle in Boset district (Table 10).

Table 10: Logistic regression analysis of tick-borne pathogens in Ada'a and Boset districts

Pathogens	District	Odds ratio	SE	CI	p-value
<i>B. bigemina</i>	Boset ¹				
	Ada'a	36.68	27.04	8.64 - 155.6	0.000
<i>A. marginale</i>	Ada'a ¹				
	Boset	5.44	1.63	3.0 - 9.78	0.000
<i>E. ruminantium</i>	Ada'a ¹				
	Boset	1.31	0.56	0.57 - 3.013	0.526
<i>A. centrale</i>	Ada'a ¹				
	Boset	1.53	1.00	0.42 - 5.55	0.521
Over all infection	Ada'a ¹				
	Boset	2.32	0.73	1.26 - 4.28	0.007

¹= Reference category, SE= Standard error, CI= Confidence interval

The present study showed that cattle infested with greater number of *Amblyomma* ticks were found comprise higher proportion of animals positive for DNA of *E. ruminantium* in both Ada'a and Boset districts (Figure 16).

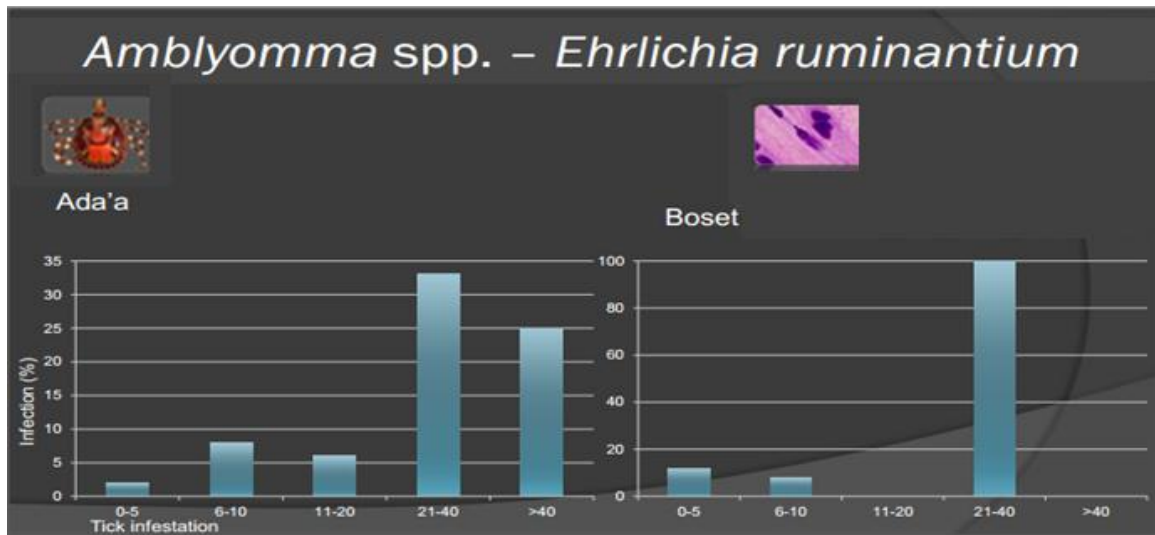


Figure 16: The correlation of *E. ruminantium* DNA positivity with degree of *Amblyomma* ticks infestation

5. DISCUSSION

5.1. Ticks

5.1.1. Tick distribution and their seasonal dynamics

Ticks and tick-borne pathogens are the major obstacles to cattle production and known to have great medical and veterinary importance worldwide (De la Fuente *et al.*, 2015) including Ethiopia (Kumsa *et al.*, 2015a). Different tick species are widely distributed in Ethiopia and previous researchers reported the distribution and abundance of tick species in different parts of the country (Solomon *et al.*, 2001; Yacob *et al.*, 2008; Tomasone *et al.*, 2012; Kumsa *et al.*, 2015a, 2016).

The current work disclosed that ixodid ticks are still widespread and most significant external parasites of cattle in Ada'a and Boset districts in Ethiopia. The observation of an overall prevalence of 91.5% tick infestation in cattle in the present study suggest that ticks still pose great problems in cattle in Ethiopia. This finding is inline with the earlier report of 89.58% tick infestation in cattle in and around Gambella town (Gudina *et al.*, 2016). Similarly, Tomassone *et al.* (2012) reported 81.5% of tick infestation in cattle in Somali Region, Ethiopia. Likewise, in other country Ndhlovu *et al.* (2009) reported 81.5% in Matabeleland South, Zimbabwe. Walker *et al.*, (2014) suggested that in area where the humidity is low, ticks resist desiccation by spending shorter periods questing for hosts and they also enter diapause at unfavorable time of the year. In this study the finding of tick infestation of cattle in lowland Boset district at greater risk of 2.018 times higher than in highland (Ada'a) was most probably attributed to the fact that lowland area with high temperature and humidity are more favorable for tick reproduction and survival than in highland altitude as has been suggested before by Kumsa *et al.* (2012). This finding agrees with several previous studies in Ethiopia (Pegram *et al.*, 1981; Mokonnen *et al.*, 2007). The observation of higher risk of tick infestation in lowland Boset agroecology is most probably attributed to the vast and more availability of grazing land in the lowland than in highland district.

The finding of *Am. variegatum* as the most prevalent and abundant tick species (59.6 %) in Ada'a district is inline with several previous studies in Ethiopias (Pegram *et al.*, 1981; Kumsa *et al.*, 2012; Ayalew *et al.*, 2014). These authors suggested that this tick species is the most prevalent and predominant in higher rainfall areas in Ethiopia. Also other African countries reported *Am. variegatum* as most common cattle tick in Nigeria (Obadiah and Shekaro, 2012) and in Tanzania (Kerario *et al.*, 2017). Most previous investigators reported that the distribution of *Am. variegatum* concides with that of *Rh. (Bo.) decoloratus*. The predominance and highest prevalence of *Am. variegatum* in Ada'a district is taken by *Am. gemma* in Boset district. This observation agrees with the previous report of Pegram *et al.* (1981) who suggested *Am. gemma* is most widely distributed and abundance cattle tick in areas with woodland and thorn bush vegetation habitats in the Rift valley in Ethiopia.

The finding of *Am. variegatum* significantly ($p < 0.05$) higher number of tick load during the short rainy (33.06 ± 3.46) and rainy season (12.2 ± 0.58) than dry season (2.82 ± 0.48) in the present study agrees with the previous studies of Gashaw (2005) in south west Ethiopia. This is due to the fact that *Am. variegatum* is not active throughout the year except during the favourable conditions during the wet season in highland areas of Ethiopia (Morel, 1980). It has been also suggested that the activity of adult *Am. variegatum* commences when spring rains begin between March and June in Ethiopia (Pegram *et al.*, 1981; Gashaw, 2005). This variation in dry season might be due to the fact that adult *Amblyomma* enter diapause at unfavorable time of the year for their survival as has been reported previously (Pegram *et al.*, 1981). *Am. variegatum* has great economic importance, because it is an efficient vector of *E. ruminatum*, the organism causing cowdrosis (heart water) and *A. marginale* (Mokonnen *et al.*, 2001; Teshale *et al.*, 2015). Furthermore, this tick species also serve as vector for emerging and remerging human and animals pathogenic bacteria including spotted fever group *Rickettsia*, *Coxiella burnetii*, and new *Borrelia* spp. (Kumsa *et al.*, 2015a,b). In the present study nymph of *Amblyomma* ticks were higher during the dry season (56.1%). This is inline with previous report of higher proportion of immature *Amblyomma* ticks during the dry season by Wogayehu *et al.* (2016).

The observation of *Rh. (Bo.) decoloratus* as the second most abundant tick species in Ada'a district in the present study agrees with the previous findings of Kassa and Yalew, (2012), Wasihun and Doda (2013), Gedilu *et al.* (2014) and Bedaso *et al.* (2014) who reported *Rh. (Bo.) decoloratus* as the most abundant tick species. This is most probably attributed to the fact that *Rh. (Bo.) decoloratus* favours wetter highlands and sub-highlands receiving annual rainfall of more than 800 mm (Pegram *et al.*, 1981). In addition, *Rh. (Bo.) decoloratus* was reported as the second most abundant tick species (Asrate and Yalew, (2012). The current observation is also consistent with previous report of *Rh. (Bo.) decoloratus* as the second most abundant tick in Gambia (Mattioli *et al.*, 1997). Significantly ($p=0.000$) higher mean of *Rh. (Bo.) decoloratus* was recorded during the rainy season (6.22 ± 2.68) than the dry season (3.68 ± 0.24). The difference is most probably attributed to the fact that its great susceptibility to percentage losses of body water at low humidity as suggested by Gashew (2005).

The findings of *Rh. pulchellus* (77.8%) as the predominant tick species in Boset district in the present study, this is in agreement with several previous studies from Borena province (Regassa, 2001) and from West Hararghe Zone, East – Ethiopia (Abebe *et al.*, 2017). This is most probably attributed to the fact that Boset district with lowland altitude and agroecology is the most favorable for survival, development and reproduction of this tick species. Conversely, this is in contrast to the previous report of least abundance of this tick species from Western Amhara (Nigatu and Teshome, 2012) and from Haramaya, Eastern Ethiopia (Kassa and Yalew, 2012). This difference is attributed to nature of this tick species that favours semi arid and low land areas in Ethiopia (Pegram *et al.* 1981). *Am. gemma* and *Rh. pulchellus* are confined to semi-arid areas (Pegram *et al.*, 1981) and the lowland tick densities are usually greater than those in the highlands. In the present study, the findings of *Rh. pulchellus* was significantly higher total count and mean tick burden per animal during the short rainy season (39.52 ± 2.70) and rainy season (17.05 ± 1.34) than dry season (7.62 ± 0.58) is inline with previous observation by Ayana *et al.*, (2013) in Borana Pastoral Area, Ethiopia. Higher prevalence rate and intensity of infestation of ticks during the rainy season than during the dry season is reported in support of present observation (Solomon *et al.*, 2003) in western Shoa zone, Ethiopia.

The observation of *Am. gemma* as the second dominant tick species in Boset district is in agreement with the previous report of Regassa (2001), Abunna *et al.* (2009) and Tomassone *et al.* (2012). This was due to the fact that the distribution of *Am. gemma* is restricted to the eastward of Rift Valley system in Ethiopia (Pegram *et al.*, 1981) and agroecological similarities.

5.1.2. Sex ratio of ticks

The current study showed that, the numbers of ticks collected from cattle were dominated by males of the genera *Amblyomma*, *Rhipicephalus*, and *Hyalomma*. However, in the subgenus *Rhipicephalus (Boophilus) decoloratus* tick collection was dominated by females. Similar findings were reported on ticks collected from cattle in southern Ethiopia (Wogayehu *et al.*, 2016), in south western Ethiopia (Chali *et al.*, 2017), Eastern Ethiopia (Kassa and Yalew, 2012) and in Oromia, Ethiopia (Kumsa *et al.*, 2016). The male to female sex ratios recorded in the present study for *Am. variegatum*, *Am. gemma*, *Rh. evertsi*, *Hy. truncatum*, *Hy. rufipes* and *Rh. pulchellus* are in agreement with earlier works of Kaiser (1987) and Bayecha *et al.* (2012). This is most probably attributed to the fact that fully engorged female ticks' drop-off to the ground to lay eggs while males tend to remain on the host for several months later to continue feeding and mating with other females as has been observed by Solomon *et al.* (2003) and Chali *et al.* (2017). The higher number of female tick collection in the case of *Rh. (Bo.) decoloratus* in this study is most probably attributed to the small size of the male which could not be seen and hence difficult to collect. Similar report was indicated (Chali *et al.*, 2017) in and around Jimma, southwest, Ethiopia.

5.1.3. Attachment sites of ticks

The current study suggested that all *Amblyomma* tick species has similar preference for ventral parts such as udder/scrotum, dewlap/brisket, perineum which is in line with the previous observation of Wasihun and Doda (2013) in southern, Ethiopia. Members of the genus *Hyalomma* showed similar preferences for the tail and anal region, ventral parts such as legs, perineum and brisket of their hosts which is consistent with previous report by Ayalew *et al.* (2014) in Central Oromia, Ethiopia. Members of the *Rhipicephalus* species

were encountered mainly in the ears, tail and anal area, head, neck and shoulder to tail base/belly/back regions as has been reported earlier by Kassa and Yalew (2012). The subgenus *Rh. (Bo.) decoloratus* showed preference for attachment sites such as head and neck, shoulder to tail base, and ears which is again in agreement with finding of Kariuki *et al.* (2012) from Kenya.

5.2. Tick-borne Pathogens

Tick-borne diseases (TBDs) are serious constraint to livestock production in many developing countries around the world mainly in tropical and sub tropical regions (Jongejan and Uilenberg, 2004). *B. bovis* and *T. parva* weren't detected in blood samples of cattle from both districts. The absence of these both pathogens in present finding could be due to the fact that absence of their vectors in Ethiopia.

The present study recorded an overall prevalence of 75.8% (182/240) of tick-borne pathogens in cattle of Ada'a and Boset districts. This finding disagrees with previous report of 25.7% in North-Central Nigeria by Kamani *et al.* (2010). This variation is most probably due to differences in sensitivity of diagnostic methods. The finding of 63.4% DNA of *A. marginale* in the blood of cattle was the most prevalent tick-borne pathogens of cattle of study areas. This observation is in agreement with the previous report of 45.2% *A. marginale* in blood samples of cattle in India (Singh *et al.* 2012) and 50% *A. marginale* in bovine samples (De la Fuente *et al.*, 2005). Cattle at Boset were 5.44 times more likely to be PCR positive for *A. marginale* than those cattle at Ada'a ($p < 0.001$).

The present observation of *B. bigemina* as the second prevalent (20%) tick-borne pathogens is in agreement with the previous report of 18% *B. bigemina* (Suleman, 2006). Higher percentages are recorded in cattle from Kenya 42.2% (Adjou Moumouni *et al.*, 2015) and 30.39% in India (Bhat *et al.*, 2014). Bock *et al.* (2004) suggested that the prevalence of *B. bigemina* in an area is the reflection of the distribution of its vectors, *Rhipicephalus evertsi* and *Rh. (Bo) decoloratus* both of which are present in Ada'a and Boset districts. The finding revealed that the chance of cattle in Ada'a district to become positive for DNA of *B. bigemina* is 36.68 times higher than cattle in Boset district, is

most probably attributed to the abundance of *Rh. (Bo.) decoloratus* tick species in Ada'a district.

Ehrlichia ruminantium is the third prevalent tick-borne pathogens with prevalence of 10.5% in this study. The finding is in agreement with previous report of Kifle and Sori (2014) in Abernosa Ranch, Ethiopia, whereas higher than previous report of Ibrahim *et al.* (2013) from Sudan. In this finding there was no observed significant ($p>0.05$) difference in the prevalence of *E. ruminantium* between Ada'a and Boset district. This could be due to the presence of similar distribution of *Amblyomma* species as vector of *E. ruminantium* in both districts.

6. CONCLUSION AND RECOMMENDATIONS

In the present study the high predominance and abundance 91.5% (659/720) of tick infestation and tick-borne pathogens 75.8% (182/240) in cattle of highland PAs in Ada'a district and lowland of Boset district suggest that ticks are still critically important ectoparasites. This study showed that *Am. variegatum* and *Rh. (Bo.) decoloratus* are the predominant and common tick species of cattle in highland PAs of Ada'a district, whereas *Rh. pulchellus* and *Am. gemma* are the predominant and widespread tick species in lowland Boset district. Likewise, *B. bigemina* is more important in Ada'a district while *A. mariginale* is more important in Boset district. This predominance of ticks of cattle and their associated tick-borne pathogens in the study districts deserves urgent attention at all levels to minimize the impacts on the health and productivity of cattle to improve the living standards of farmers of the study areas.

Based on above conclusion the following recommendations are forwarded:

- ✓ Strategic application of acaricides especially at the beginning of the short rainy and major rainy season might minimize the infestation of ticks.
- ✓ Creation of awareness of livestock owners on the potential effect of ticks and tick-borne pathogens is needed.
- ✓ Advanced molecular studies on ticks and tick-borne pathogens in wider parts of Ethiopia will be very helpful.

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8. APPENDICES

Appendix 1: Sample collection format

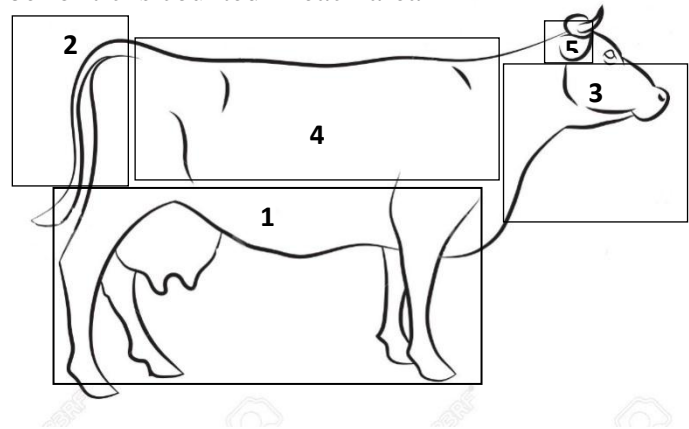
Date:	____ / ____ / ____ dd / mmm / yyyy					
season #	<input type="checkbox"/> Season 1	<input type="checkbox"/> Season 2	<input type="checkbox"/> Season 3	<input type="checkbox"/> Season 4	Department/ District	
Owner name					Country:	
GPS	Altitude					

Animal identification:

Animal ID:		-		-		-	
	Country code ¹		Department code ²		Farm number		3 digit animal number

¹First two letters of country name; ²First letter of department/District name (if the same, first two)

Appendix 2: Half body tick count, number of ticks counted in each area



- 1. Inner and outer fore-legs, hind legs and abdomen
- 2. Tail and anal area
- 3. Head and neck ,
- 4. Lateral area and dorsal area shoulders to tail base
- 5. Ears

Genera of Tick type	Number of adult ticks counted per body area				
	1	2	3	4	5
<i>Hyalomma</i>					
<i>Amblyomma</i>					
<i>Rhipicephalus</i>					
<i>Boophilus</i>					
Genus not identifiable					

Ticks counted by:	Initial:	date:
Recorded by:	Initial:	date:

Appendix 3: Ticks species identification key

<i>Amblyomma variegatum</i>	
Male	Female
<ul style="list-style-type: none"> ✚ Eyes are distinctly convex. ✚ Primary punctation sizes on conscutum are small to medium. ✚ Primary punctuation distribution on conscutum is sparse. ✚ Mesial area of enamel ornamentation on conscutum is elongate. ✚ Lateral median areas of enamel ornamentation on conscutum are absent (small areas may occur in some populations of this tick). ✚ Festoon enamelling is absent. ✚ Posteromedian stripe is narrow. ✚ Enamel colour is pink to orange. ✚ Leg colouration is with pale rings. ✚ Coxae 1 external and internal spur lengths are: external medium and internal short. 	<ul style="list-style-type: none"> ✚ Eyes are distinctly convex. ✚ Primary punctation sizes on scutum are small to medium. ✚ Primary punctuation distribution on scutum is regular. ✚ Enamel colour pink to orange. ✚ Genital aperture posterior lips have a broad U shape. ✚ Coxae 1 external and internal spur lengths are: external medium and internal short. ✚ Mesial area of enamel ornamentation on scutum is elongate (it may be indistinct in its anterior part). ✚ Lateral areas of enamel ornamentation on scutum are small. ✚ Scutum sides are straight. ✚ Scutum posterior angle is broad. ✚ Leg colouration is with pale rings.
<i>Amblyomma gemma</i>	
Male	Female
<ul style="list-style-type: none"> ✚ Eyes are slightly convex (as shown for the female). ✚ Primary punctation sizes on conscutum are small to medium. ✚ Primary punctuation distribution on conscutum is localized (between the eyes). ✚ Mesial area of enamel ornamentation on conscutum is elongate. ✚ Lateral median areas of enamel ornamentation on conscutum are large and complex. ✚ Festoon enamelling is partial (6 of 11 festoons with enamel). ✚ Posteromedian stripe is broad ✚ Enamel colour is pink to orange. ✚ Leg colouration is with pale rings. ✚ Coxae 1 external and internal spur lengths are: external medium and internal short. 	<ul style="list-style-type: none"> ✚ Eyes are slightly convex. ✚ Primary punctation sizes on scutum are small to medium. ✚ Primary punctuation distribution on scutum is localized (between the eyes). ✚ Enamel colour is pink to orange. ✚ Genital aperture posterior lips have a narrow V shape. ✚ Coxae 1 external and internal spur lengths are: external medium and internal short. ✚ Mesial area of enamel ornamentation on scutum is large and elongate. ✚ Lateral areas of enamel ornamentation on scutum are large and complex. ✚ Scutum sides are straight. ✚ Scutum posterior angle is broad. ✚ Leg colouration is with pale rings.

<i>Rhipicephalus (Boophilus) decoloratus</i>	
Male	Female
<ul style="list-style-type: none"> ✚ Cornua are distinct. ✚ Coxae 1 spurs length is short. ✚ Ventral plate spurs are distinct (3 = accessory adanal plate). ✚ Ventral plate spurs are distinct (4 = adanal plate) ✚ Caudal appendage is narrow in males. ✚ Ventral plate spurs are visible dorsally. 	<ul style="list-style-type: none"> ✚ Hypostomal teeth are in 3 + 3 columns. ✚ Palp articles 1 internal margin has a protuberance with pectinate setae. ✚ Coxae 1 spurs are distinct. ✚ Coxae 2 and 3 spurs are present. ✚ Genital aperture posterior lips have a narrow U shape. ✚ Porose areas shape is a narrow oval.
<i>Hyalomma truncatum</i>	
Male	Female
<ul style="list-style-type: none"> ✚ Cervical field's depression is not apparent. ✚ Conscutum is dark coloured (appears smooth and shiny). ✚ Lateral grooves are long (distinct grooves in posterior part, extending as lines of punctations towards eyes). ✚ Posterior ridges number two. Caudal depression is present (conspicuously deep and with large punctations). ✚ Central festoon is dark coloured. Paracentral festoons are separate anteriorly. ✚ Central festoon of <i>Hyalomma albiparmatum</i> is pale white and forms a distinct Parma.) ✚ Posteromedian groove is absent. Paramedian grooves are absent. ✚ Leg colouration is with pale rings. ✚ Punctuation size is small. Punctuation distribution is localized (restricted to marginal areas and caudal depression, central area is smooth and shiny). 	<ul style="list-style-type: none"> ✚ Scapular grooves profile is steep (grooves reach the posterior margin of scutum). ✚ Scutum is dark coloured. ✚ Scutum posterior margin is distinctly sinuous ✚ Leg colouration is with pale rings. ✚ Punctuation size is small. Punctuation distribution is localized (on cervical fields and scapulae). ✚ Genital aperture anterior groove is shallow. ✚ Genital aperture preatrial fold is concave. ✚ Genital aperture posterior lips have a broad U shape (distinctly wider than long).

<i>Hyalomma rufipes</i>	
Male	Female
<ul style="list-style-type: none"> + Cervical fields depression is not apparent. + Conscutum is dark coloured. + Lateral grooves are short. + Posterior ridges are absent. Caudal depression is absent. + Central festoon is dark coloured (form of festoons is indistinct). Paracentral festoons are separate anteriorly. + Posteromedian groove is absent. Paramedian grooves are absent. + Leg colouration is with pale rings. + Punctuation size is small. Punctuation distribution is dense. + Subanal plate alignment is with the adanal plates. + Subanal plates are distinct. + Adanal plates shape has square ends. + Spiracle areas have dense setae. 	<ul style="list-style-type: none"> + Scapular grooves profile is steep. + Scutum is dark coloured. + Scutum posterior margin is distinctly sinuous + Spiracle areas have dense setae. + Leg colouration is with pale rings. + Punctuation size is small. Punctuation distribution is dense. + Genital aperture anterior groove is deep. + Genital aperture preatrial fold is convex. + Genital aperture posterior lips have a broad V shape.
<i>Rhipicephalus evertsi</i>	
Male	Female
<ul style="list-style-type: none"> + Interstitial punctuation size is small to medium. + Interstitial punctuation distribution is dense. + Setiferous punctations are indistinct. + Coxae 1 anterior spurs are visible. + Cervical fields depression is not apparent. Cervical fieldstexture has wrinkled areas. + Eyes are very convex. + Conscutum colour is dark. + Accessory adanal plates are small (they may be absent). + Adanal plates shape is broad and curved + Caudal appendage is absent in fed males. + Spiracle plate areas have dense setae. + Posterior grooves are indistinct (represented by three flat posterior areas of wrinkled texture). + Lateral grooves type is a distinct groove. Lateral grooves texture is wrinkled. + Legs are coloured a distinctive pale orange, 	<ul style="list-style-type: none"> + Interstitial punctuation size is small to medium. Interstitial punctuation distribution is dense. + Setiferous punctations are indistinct. + Basis capituli lateral angles are blunt. + Porose areas separation is narrow. + Palp pedicels are short. + Cervical fields shape is not apparent. Cervical fields texture has wrinkled areas. + Scapular grooves profile is shallow. + Eyes are very convex. + Spiracle plate areas have dense setae. + Scutum posterior margin is slightly sinuous. + Scutum colour is dark. + Legs are coloured a distinctive pale orange, uniformly over each segment.)

<i>Rhipicephalus bergeoni</i> -large brown to redish brown tick	
Male	Female
<ul style="list-style-type: none"> ✚ Basis capituli with short, lateral angles ✚ Palp short, broad conscutum ✚ Anterior process of coxae I inconspicuous ✚ In engorged specimens body wall expanded slightly laterally and postero laterally and forming a short, blunt caudal process posteriorly ✚ Eyes marginal, almost flat, edged dorsally by a few large punctuations ✚ Cervical fields inconspicuous ✚ Legs increase slightly in size from I to IV. ✚ Ventrally spiracles short, broad, narrowing slightly only where they curve towards the dorsal surfaces ✚ Adanal plates with their posteroexternal and posterointernal margins curving in towards each other posterior to the anus; ✚ Accessory adanal plates short, pointed 	<ul style="list-style-type: none"> ✓ Basis capituli lateral angles are broad ✓ Porose areas separation is broad, slightly more than own diameter ✓ Palp pedicels are broad, somewhat flattened apically ✓ Cervical fields shape is broad, slightly depressed, their outer margins indicated by a few large setiferous punctuations ✓ Scutum usually longer than broad, posterior margin is sinuous. ✓ Ventrally genital aperture with sides of opening converging to the broadly rounded base, the genital apron depressed
<i>Rhipicephalus pulchellus</i>	
<ul style="list-style-type: none"> ✓ Interstitial punctation size in both sexes is minute to small. ✓ Interstitial punctation distribution is dense in both sexes. ✓ Setiferous punctations are distinct in both sexes. ✓ Basis capituli lateral angles are blunt. ✓ Porose areas separation is narrow. ✓ Palp pedicels are short. ✓ Cervical fields shape is not apparent in either sex. ✓ Eyes are flat in both sexes. ✓ Coxae 1 anterior spurs are visible on males. ✓ Female scutum colour is with enamel ornamentation (ivory white all over). ✓ Male conscutum colour is with enamel ornamentation (an ivory white in a pattern against a dark brown background). ✓ Posterior grooves are distinct. ✓ Caudal appendage is narrow in fed males. ✚ Lateral grooves type is punctations only. Lateral groove texture is distinctly punctate. 	

Rhipicephalus praetextatus

Female	Male
<ul style="list-style-type: none"> ✓ Basis capituli lateral angles are blunt. ✓ Porose areas separation is broad. ✓ Palp pedicels are long. ✓ Cervical fields shape is large and curved. ✓ Spiracle plate areas have sparse setae (not shown) ✓ Scutum posterior margin is slightly sinuous ✓ Scutum colour is dark. ✓ Interstitial punctation size is minute to small. ✓ Interstitial punctation distribution is sparse (these punctations are indistinct so the scutum appears smooth and shiny). ✓ Setiferous punctations are distinct. ✓ Scapular grooves profile is shallow. ✓ Cervical fields texture has no wrinkles. ✓ Eyes are slightly convex. ✓ Genital aperture posterior lips have a truncated V shape (the hyaline border, arrowed, is large). 	<ul style="list-style-type: none"> ✚ Interstitial punctation size is minute to small. ✚ Interstitial punctation distribution is sparse (these punctations are indistinct so the scutum appears smooth and shiny). ✚ Setiferous punctations are distinct. ✚ Coxae 1 anterior spurs are not visible. ✚ Cervical fields depression is not apparent. Cervical fields texture has no wrinkles. ✚ Eyes are slightly convex (as shown for the female). ✚ Conscutum colour is dark. ✚ Accessory adanal plates are large. ✚ Adanal plates shape is broad and curved. ✚ Caudal appendage is broad in fed males. ✚ Spiracle plate areas have sparse setae. ✚ Posterior grooves are absent (very shallow grooves may be visible) ✚ Lateral grooves type is an indistinct groove (the grooves enclose one festoon on each side). Lateral grooves texture is distinctly punctate (the column of punctations in this position is more prominent than the groove).

<i>Rhipicephalus praeus</i>	
Female	Male
<ul style="list-style-type: none"> ✓ Basis capituli lateral angles are blunt. ✓ Porose areas separation is broad. ✓ Palp pedicels are short. ✓ Cervical fields shape is small (and narrow). ✓ Spiracle plate areas have sparse setae (not shown). ✓ Scutum posterior margin is distinctly sinuous ✓ Scutum colour is dark. ✓ Interstitial punctation size is minute to small. ✓ Interstitial punctation distribution is dense. ✓ Setiferous punctations are distinct. ✓ Scapular grooves profile is shallow. ✓ Cervical field's texture has wrinkled areas. ✓ Eyes are distinctly convex (and in shallow orbits). ✓ Genital aperture posterior lips have a narrow V shape. 	<ul style="list-style-type: none"> ✗ Interstitial punctation size is minute to small. ✗ Interstitial punctation distribution is dense. ✗ Setiferous punctations are distinct. ✗ Coxae 1 anterior spurs are visible dorsally. ✗ Cervical fields depression is apparent (forming a narrow cervical field). Cervical fields texture has wrinkled areas. ✗ Eyes are distinctly convex (and in shallow orbits, as shown for the female). ✗ Conscutum colour is dark. ✗ Accessory plates are small (may be absent) ✗ Adanal plates shape is narrow and trapezoid (in some specimens the shape is a broader trapezoid than illustrated here). ✗ Spiracle plate areas have sparse setae. ✗ Caudal appendage is narrow in fed males. ✗ Posterior grooves are distinct (deep and with rough texture). ✗ Lateral grooves type is a distinct groove (they extend almost to the eyes). Lateral grooves texture is wrinkled.

Source: (Pegram *et al.*, 1987; Houseman, 2013; Walker *et al.*, 2014).