

Thesis Ref. No _____

**ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND
AGRICULTURE
DEPARTMENT OF PARASITOLOGY AND PATHOLOGY**



**TICKS OF RUMINANTS AND EQUINES IN ANILEMO DISTRICT, HADYIA ZONE,
SNNPRS: SPECIES DIVERSITY, EFFICACY AND USAGE OF ACARICIDES**

MVSC THESIS

BY

MULUGETA MELESE ULSIDO

ADVISOR: DR. BERSISSA KUMSA (DVM, MVSc, PhD, ASSOC. PROF.)

JUNE, 2019

BISHOFTU, ETHIOPIA

**TICKS OF RUMINANTS AND EQUINES IN ANILEMO DISTRICT, HADYIA ZONE,
SNNPRS: SPECIES DIVERSITY, EFFICACY AND USAGE OF ACARICIDES**

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

MULUGETA MELESE ULSIDO

ADVISOR: DR. BERSISSA KUMSA (DVM, MVSc, PhD, Assoc. Prof)

JUNE, 2019

BISHOFTU, ETHIOPIA

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Pathology and Parasitology

**TICKS OF RUMINANTS AND EQUINES IN ANILEMO DISTRICT, HADYIA ZONE,
SNNPRS: SPECIES DIVERSITY, EFFICACY AND USAGE OF ACARICIDES**

Submitted by: Mulugeta Melese	_____	20-06-2019
Name of Student	Signature	Date

Approved for submittal to thesis assessment committee

1. Bersissa Kumsa (DVM, MSc, PhD, Assoc. Professor)	_____	_____
Major Advisor	Signature	Date
2. Prof. Yacob Hailu (DVM, MSc, PhD, Professor)	_____	_____
Department Chairperson	Signature	Date

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Pathology and Parasitology

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by Mulugeta Melese Ulsido, entitled: “**Ticks of Ruminants And Equines in Anilemo District, Hadiya Zone, SNNPRS: Species Diversity, Efficacy and Usage of Acaricides**” and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Parasitology.

_____	_____	_____
Chairman	Signature	Date
Dr.Zewdu Seyoum (Associate Professor)	_____	_____
External examiner	Signature	Date
Prof. Yacob Hailu (DVM, MSc, PhD, Professor)	_____	_____
Internal examiner	Signature	Date
Dr. Bersissa Kumsa (DVM, MSc, PHD, Assoc. Pro)	_____	_____
Advisor	Signature	Date
Prof. Yacob Hailu (DVM, MSc, PhD, Professor)	_____	_____
Department Chairperson	Signature	Date

DEDICATION

I dedicated this manuscript to my beloved mother, Meskele Erbalo, my father, Melese Ulsido and my brother Degefe Melese, who forewent a lot to bring me up to this level, nurturing me with love, and for their enthusiastic corporation 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 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.

Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Mulugeta Melese Ulsido

Signature: _____

College of Veterinary Medicine, Bishoftu, Ethiopia

Date of Submission: June 20, 2019

ACKNOWLEDGEMENTS

Throughout the writing of this thesis, I have received a great deal of support and assistance. First of all I would like to thank and praise the Almighty God for the eternal activities and the strength he gives me in every moment of my life.

I would like to express my deepest respect and most sincere gratitude to my advisor Dr. Bersissa Kumsa whose expertise was invaluable in the formulating of the research topic and methodology in particular. He has helped me in tick identification process both practically and theoretically on top of giving me relevant literatures, some preservatives and equipment, and for his guidance and encouragement at all stages of this work. His constructive criticism and comments from the beginning till the end of this work is highly appreciated.

I also thank the Addis Ababa university college of veterinary medicine and agriculture, Parasitology laboratory workers for their facilitating working environment in the laboratory. I would also like to thank the staff members in Anilemo district, animal and fishery resource department especially, animal health work process experts for their assistance during tick collection and questionnaire survey in the study area.

Finally, I would like to thank my family; my parents, my brothers and sisters for supporting me spiritually throughout writing this thesis and my life in general. I also thank my friends who have encouraged me to accomplish this work. Last, but not the least for my best friend, Behredine Jemal, who lives abroad the country, for his unreserved support both morally and materially from the very beginning till the end of the study period.

TABLE OF CONTENTS

CONTENTS	PAGES
STATEMENT OF AUTHOR.....	I
ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS	III
LIST OF TABLES	V
LIST OF FIGURES	VI
LIST OF ANNEXES	VII
LIST OF ABBREVIATIONS.....	VIII
1. INTRODUCTION	1
2.1. Ecology of Ticks.....	4
2.2. Taxonomy of Ticks	4
2.3. Morphology of Ticks	5
2.4. Epidemiology of Ticks.....	7
2.4.1. <i>Habitat</i>	<i>7</i>
2.4.2. <i>Host finding.....</i>	<i>7</i>
2.4.3. <i>Seasonal occurrence</i>	<i>9</i>
2.4.4. <i>Geographical distribution.....</i>	<i>9</i>
2.5. Life Cycle of Ticks	10
2.5.1. <i>Life cycle of one host ticks</i>	<i>11</i>
2.5.2. <i>Life cycle two host ticks</i>	<i>11</i>
2.5.3. <i>Life cycle of three host ticks.....</i>	<i>11</i>
2.6. Pathogenic Role of Ticks.....	12
2.7. Ticks and Their Distribution in Ethiopia.....	13
2.8. Diagnosis of Tick Infestation	18
2.8.1. <i>Collection of Ticks from Hosts</i>	<i>18</i>
2.8.2. <i>Collection of Ticks from Vegetation and other Environments.....</i>	<i>19</i>
2.8.3. <i>Preservation of Tick Samples</i>	<i>19</i>
2.8.4. <i>Transportation of Ticks.....</i>	<i>20</i>
2.8.5. <i>Laboratory Identification of Ticks</i>	<i>21</i>

2.9. Control Measures for Ticks	24
2.9.1. <i>Chemical tick control</i>	24
2.9.2. <i>Ecological control</i>	26
2.9.3. <i>Genetic control</i>	26
2.9.4. <i>Ethno-Veterinary Control of Tick</i>	26
2.9.5. <i>Biological control method</i>	27
2.9.6. <i>Immunization (anti-tick vaccine)</i>	27
3. MATERIALS AND METHODS	28
3.1. Study Area	28
3.2. Study Population	29
3.3. Study Design	30
3.3.1. <i>Sampling methods</i>	31
3.3.2. <i>Tick collection and identification</i>	31
3.3.3. <i>Questionnaire survey</i>	32
3.3.4. <i>In vitro acaricide efficacy evaluation</i>	33
4. RESULTS	35
4.1. Ticks Infesting Ruminants and Equines	35
4.1.1. <i>Species diversity and relative abundance</i>	35
4.1.2. <i>Risk factors associated with major tick infestation</i>	41
4.1.3. <i>Sex ratio</i>	47
4.2. Questionnaire Survey	47
4.3. Invitro Efficacy of acaricides	54
5. DISCUSSION	57
5.1. Tick Infestation and Associated Risk Factors	57
5.2. Sex Ratio of Ticks	59
5.3. Invitro Acaricide Efficacy	60
5.4. Questionnaire Survey	60
6. CONCLUSION AND RECOMMENDATIONS	62
7. REFERENCES	63
8. ANNEXES	73

LIST OF TABLES

Table 1. Prevalence of tick species in different study area in Ethiopia with respective authors.....	17
Table 2. Ways of application and concentration of acaricides used for tick control in Ethiopia.....	25
Table 3: Overall count, percentage and relative abundance of tick species identified during the study period in Anilemo district	36
Table 4: Total count and percentage of tick species collected from each species of domestic animals during the study period	37
Table 5: Association of different risk factors with <i>Amblyomma variegatum</i> infestation by using Negative binomial regression analysis	42
Table 6: Association of different risk factors with <i>Rh. (Bo.) decoloratus</i> infestation of the study animals by using Negative binomial regression analysis	44
Table 7: Association of different risk factors with <i>Amblyomma cohaerens</i> infestation by using Negative binomial regression analysis.	46
Table 8: Overall male/female sex ratio of ticks collected during the study period in Anilemo district	47
Table 9: Summary of the outcome of the questionnaire on the knowledge and awareness about ticks	48
Table 10: Outcome of the questionnaire on tick control practices in Anilemo district.....	50
Table 11: Outcome of the questionnaire concerning tick response to acaricides application.....	54
Table 12: Oviposition response of adult <i>A. variegatum</i> and <i>Rh. (Bo.) decoloratus</i> after immersion in tested acaricide at recommended concentration.	55

LIST OF FIGURES

Figure 1: Morphology of hard (right) and soft (left) tick	6
Figure 2: Cement, spermatheca and male tick in mating position	22
Figure 3: Mouth part of different tick species.....	22
Figure 4: Scutum coverage of female (left) and male (right) ticks	23
Figure 5. Map of Ethiopia showing Anilemo district, the study Area.	29
Figure 6: Percentage of tick infestation on each study animal species in Anilemo district.....	35
Figure 7: Photographs of hard tick species collected from domestic animals in Anilemo district ...	40
Figure 8: Average number of ticks laid egg in Ivermectine, Diaznone and control group.....	56

LIST OF ANNEXES

Annex 1: Field data collection format for tick identification	73
Annex 2: Questionnaire format	74
Annex 3: Procedure for invitro efficacy test at recommended dose.....	78
Annex 4: Invitro efficacy test laboratory result report sheet for test of susceptibility of a tick sample	79
Annex 5: Identification key of relevant tick species identified in the Anilemo District	80
Annex 6: Tick collection process (top), treated and control ticks after incubation process (bottom)	83

LIST OF ABBREVIATIONS

AWARDO	Anilemo Woreda Agriculture and Rural Development Office
AIT	Adult Immersion Test
BCS	Body Condition Score
CRGE	Climate Resilient Green Economy
GDP	Gross Domestic Product
GIS	Geographical Information System
GTPII	Growth and Transformation Plan two
FAO	Food and Agriculture organization
Kgbm	kilo gram body mass
LMP	Livestock Master Plan
m.a.s.l	Meters Above Sea Level
MoLF	Ministry of Livestock and Fishery
TBD	Tick Borne Diseases
PAs	Peasant Associations
RH	Relative Humidity
SSA	Sub-Saharan Africa
SNNPR	South Nation, Nationality and Peoples Regional State

ABSTRACT

Ticks are the major health and productivity constraints of ruminants and equines in Ethiopia. A cross sectional study was conducted from September 2018 to May 2019 in Anilemo district with the core intentions to identify tick species infesting ruminants and equines, estimate the prevalence, to assess species diversity, risk factors associated with infestation and the efficacy of the most commonly used acaricides in the study district. To achieve these objectives ticks were collected from animals for identification to species level under stereo-microscope, a questionnaire survey was conducted on acaricide usage and in vitro efficacy evaluation of acaricides was performed following classical procedures. Accordingly, during the study period, a total of 906 ruminants and equines were examined for presence of ticks, 50 persons were interviewed with structured questionnaires about the usage of acaricide in the area and 110 ticks were used to evaluate the efficacy of the commonly used acaricides by adult immersion test. The study revealed that an overall of 34.9% (316/906) animals were infested by one or more ticks during the study period. An overall of 3367 ixodid ticks (adults and immature stages) collected from infested animals were differentiated in to a total of five species that belonged to genus *Amblyomma* and *Rhipicephalus* and the sub genus *Rhipicephalus (Boophilus)*. The major tick species identified in order of their prevalence and predominance were *Rhipicephalus (Boophilus) decoloratus*, *Amblyomma variegatum*, *Amblyomma cohaerens*, *Rhipicephalus evertsi*, *Rhipicephalus pulchellus* and *Rhipicephalus praetextatus*. In the present study *Rhipicephalus (Boophilus) decoloratus* and *Amblyomma variegatum* were the most prevalent (95.2%) and both species ($p < 0.05$) were significantly associated with different risk factors like season, agroecology, age, colour and sex categories of the study animals. Furthermore, a total of 110 ticks were collected for the in vitro Adult immersion test to assess the efficacy of Diaznone and Ivermectine both of which were implicated as the most frequently used acaricides by results of the questionnaire survey. The standard modified adult immersion test (AIT) on a total of 80 *Rhipicephalus (Boophilus) decoloratus* and 30 *Amblyomma variegatum* engorged female ticks showed that Ivermectine was highly effective than Diaznone. In conclusion, findings of this study suggest that ticks were the most important problem of ruminants and equines of the study area. In addition, the study on efficacy of the two most commonly used acaricides in the study area attest the fact that efficacy of these drugs is conserved against both *Rh (B.) decoloratus* and *Amblyomma*

variegatum tick species of the study. Therefore, the possible threat of tick infestation on ruminants and equines warrants all rounded attention regarding strategic application of acaricides and creation of awareness on acaricide usage to prevent and control tick infestation. Further efficacy evaluations using different tick species and more sensitive efficacy evaluation methods involving larval and nymphal stages as well as in vivo efficacy trial at field level should be conducted to obtain more reliable information on efficacies of acaricides in different parts of Ethiopia.

Keywords; *Acaricide usage, Anilemo, Ethiopia, Invitro test, Ruminants and Equines, Ticks.*

1. INTRODUCTION

Agriculture is the backbone of the economies of many developing countries of the world including Ethiopia. Approximately 85% of the population is engaged in the agricultural sector in Ethiopia which 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 (Tessema, 2018). The country has one of 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, 60.5million poultry, 2.1 million horses, 0.4 million mules and 7.88 million donkeys. At national level, in addition to providing industrial raw materials (milk, meat, hides and skins), the livestock sector plays an important role in providing export commodities, such as live animals, hides and skins and honey, contributing 8.3 percent of total GDP and about 20.2 percent of agricultural GDP. The livestock sector in Ethiopia has the potential to deliver the anticipated agricultural-led growth as envisioned in the Growth and transformation plan II (GTP II), Livestock Master Plan (LMP) and the Climate Resilient Green Economy (CRGE) Strategy (MoLF, 2015). The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians.

Livestock mainly ruminants and equines 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 (Bureau and Swinnen, 2018). Despite high livestock population and existing favorable environmental conditions, the current livestock output of the country is far below the expected potential. The widespread and high prevalence of endemic diseases of animals are one of the most important constraints to the livestock productions and productivity in Ethiopia (Shapiro *et al.*, 2017). Ectoparasites are responsible for decreased production and reproduction of these animals as well as leather quality deterioration, down grading and rejection of skins (Ashenafi *et al.*, 2013). Ticks are one of the ectoparasites that directly affects the socio-economic development of resource limited farming community by affecting health and productivity of animals in Ethiopia (Kumsa *et al.*, 2016).

Between 1940 and 2004, the majority of emerging human infectious diseases worldwide were zoonotic. Of these, nearly one-quarter were arthropod vector-borne diseases, with ticks being the

most common vector (Munderloh and Kurtti, 2011). Because of their habit of ingesting blood, ticks are vectors of several diseases that affect humans and other animals (Pinter *et al.*, 2004 ;Brites-Neto *et al.*, 2015). Ticks are widely distributed around the world, especially in warm, humid climates (Zhang *et al.*, 2017). Ticks are one of the most important ecto-parasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses in livestock. For any livestock species, the impact of ticks on animal health can be two fold. These are effects of tick burden as ectoparasites and the effect of diseases transmitted by ticks serving as vectors (Rajput *et al.*, 2006).The major losses, however, caused by ticks are due to their ability to transmit pathogens which are of great economic importance world-wide (Narladkar, 2018) including viruses, bacteria, and protists to vertebrate hosts, including humans, domestic and wild animals. These pathogens cause many viral diseases (e.g., Nairobi sheep diseases, Crimean-Congo hemorrhagic fever, West Nile fever, Omsk hemorrhagic fever, and Colorado tick fever), bacterial diseases (Lyme disease, Q fever, Borreliosis and relapsing fever), fungal diseases (dermatophilosis), protozoal diseases (theileriosis and babesiosis), and rickettsial diseases (anaplasmosis, ehrlichiosis, Brazilian spotted fever, and Rocky Mountain spotted fever) (Abubakar *et al.*, 2018).

In Ethiopia, studies on tick fauna have begun early in the 19th century. Since then, different researchers from abroad and country reported the pattern of ticks and Tick-borne diseases (TBDs) in the country. Ticks are common in all agro-ecological zones in Ethiopia (Abebaw, 2004; Kumsa *et al.*, 2016).The economically most important ixodid ticks of livestock in Ethiopia belong to the sub genus *Boophilus* and the genera *Rhipicephalus*, *Haemaphysalis*, *Amblyomma* and *Hyalomma* (Kumsa *et al.*, 2016) and have been reported from various regions of the country. More than 50 species of ticks infesting both domestic and wild animals have been recorded and 30 of these are known to be most common and are important parasites of livestock (Ferede *et al.*, 2010).

The application of chemicals is still the most effective method of control of ticks in Ethiopia. However, uncontrolled and high frequency of applications of commercial acaricides may have accelerated the emergence of tick resistance to several active ingredients available. Since the introduction of acaricide in Africa in around 1890, tick treatment relying on different application methods have been the main method of tick control in Africa, leading to numerous problems including environmental pollution, development of resistant tick strains and escalating costs (Brito *et al.*, 2011). Likewise, in Ethiopia, over the past decades ticks are mainly controlled by using

variety of acaricides; including organochlorines, organophosphates, macrocyclic lactones, carbamates, amidines or synthetic pyrethroids (Ayana *et al.*, 2013). However, ticks usually develop resistance to acaricides when exposed to favorable factors such as most widespread usage, under concentration, frequent use of organochlorines and organophosphates compounds (Admasu *et al.*, 2015). Therefore, regular studies on dynamics of tick population and species composition of ticks with the current efficacy status of acaricides against the most predominant and economically important tick species of an area is necessary to carry out efficient tick control and/or tick burden reduction (Ticks, 2004).

In southern Ethiopia where livestock and crop production is highly integrated with densely populated land which is highly favorable environmental condition for ectoparasites especially ticks, studies on parasitic arthropods affecting domestic animals is highly important. In addition, information on the status and magnitude of efficacy of the commonly used acaricides has paramount importance in deciding the appropriate tick control strategy in ruminants and equines of the study area. However, In spite of these facts, due attention has not been given to the study and control of ectoparasites affecting ruminants and equines in Anilemo district of Hadiya zone in southern Ethiopia.

Therefore, the present study was designed to attain the following objectives:

- To estimate prevalence and species diversity of ticks infesting ruminants and equines in Anilemo district.
- To investigate the risk factors associated with ticks affecting ruminants and equines in Anilemo district.
- To assess the efficacy and usage of the most commonly used acaricides in the area.

2. LITERATURE ERVIEW

2.1. Ecology of Ticks

The ecology of ticks, the outcome of their interactions with their natural environment is fundamental to the spatial and temporal variation in the risk of infection by tick-borne pathogens. Due to the biology of ticks as blood-feeding parasites, their physical environment includes the host itself. This biotic environment reacts to the tick's presence in both the short and the long term in ways that the abiotic environment cannot do, imposing physiological, population and evolutionary pressures on ticks (Heylen *et al.*, 2019). Ticks, however, are only intermittent parasites, spending the greater part of their life cycle free within their habitat where they are at the mercy of abiotic factors such as habitat structure and climate. They take only one (Ixodid ticks) or a few (argasid ticks) very large blood meals per life stage, as larvae, nymphs and adults, then develop to the next stage, which takes weeks, months or even years, depending on the ambient temperature. This interstadial period is usually passed off-host, although the relatively few two- and one-host ticks (e.g. *Hyalomma excavatum* and *Rhipicephalus (Boophilus) microplus*, respectively) remain on the host for one or both of the interstadial periods (Bowman and Nuttall, 2008).

Tick distribution and their population vary according to their adaptability to ecology, eco-climate, microhabitats, ambient temperature, rainfall and relative humidity which is critical factors affecting life cycle of ticks. The relative humidity on the other hand remains an important factor for survival of ticks by regulating the water balance and prevents dehydrations (Tadesse *et al.*, 2012).

2.2. Taxonomy of Ticks

Ticks are within a member called the phylum (Arthropoda), class (Arachnida), sub class (Acari) and Order (Parasitiformes) (Torr *et al.*, 2004). Within the Parasitiformes, ticks belong to the suborder Ixodida, which contains a single super family, the Ixodoidea, which is divided into two major families, Argasidae (soft ticks) and Ixodidae (hard ticks), and the rare family Nuttalliellidae, with a single African species (Bowman and Nuttall, 2008; Estrada-Peña *et al.*, 2010).

Nuttalliellidae represents only one single species (*Nuttalliella namaqua*; found in South and South-West Africa as a parasite of small mammals). The terms hard and soft refer to the presence of a dorsal scutum or “plate” in the Ixodidae, which is absent in the Argasidae. Their mouthparts are anteriorly attached and visible from dorsal view. If eyes are present, they are located dorsally on the sides of the scutum (Goddard, 2009). The family Ixodidae, or hard ticks, contains some 683 species. As adults, Ixodid exhibit prominent sexual dimorphism: the scutum covers the entire dorsum in males, but in females (and immatures) the scutum is reduced to a small podonotal shield behind the capitulum, thereby permitting great distention of the idiosomal integument during feeding (Jongejan and Uilenberg, 2004). Ixodidae ticks are relatively large and comprise thirteen genera. Seven of these genera contain species of veterinary and medical importance: *Amblyomma*, sub genus *Rh.*(*Boophilus*), *Rhipicephalus*, *Haemaphysalis*, *Hyalomma*, *Dermacentor* and *Ixodes* (Lora, 2001).

The family Argasidae, or soft ticks, consists of about 185 species worldwide and have one important genus that infests cattle, *Ornithodoros* (Latif and Walker, 2004). Adult argasids lack a dorsal sclerotized plate or Scutum, their integument is leathery and wrinkled, their mouthparts are not visible from above, and they show no obvious sexual dimorphism. Argasidae are wandering ticks, which only remain on their host while feeding (Barker and Murrell, 2004).

2.3. Morphology of Ticks

Tick morphology consists of two primary regions, the mouthparts (capitulum) and the body (idiosoma). The mouthparts on hard ticks protrude in front of the body and are visible from above, but the body of soft ticks extends forward above the mouthparts so they are only visible from beneath as indicated in (Figure 1). The body of ticks includes the eyes, legs, and respiratory, digestive and reproductive structures (Nava *et al.*, 2009).

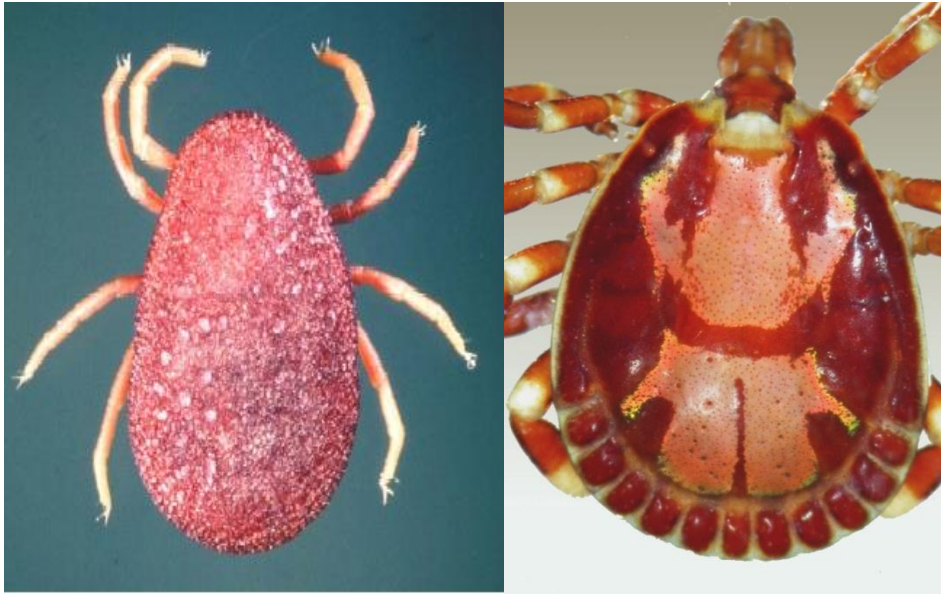


Figure 1: Morphology of hard (right) and soft (left) tick

Source : (Nava *et al.*, 2009)

Ticks differ from other mites; they are larger and have recurved teeth or ridges on the central mouthparts (called the holdfast organ). Ticks do not have wings and they cannot jump and they cannot run, hop, fly or even move quickly. They also have a sensory pit on each of the first pair of legs known as Haller's organ which is packed with chemoreceptor setae used in host location. This pit detects stimuli such as heat and carbon dioxide. Ticks also detect light and dark as well as shapes, shadows and vibrations all stimuli that help them find their vertebrate hosts. Ixodidae ticks are relatively large, ranging between 2 and 20mm in length. Ixodidae ticks are characterized by the presence of a rigid chitinous Scutum that covers the entire dorsal surface of the adult male whereas it extends only for a small area in the female (Estrada-Peña *et al.*, 2010).

The mouthparts (capitulum) have three specialized structures called palps, chelicerae and a hypostome that are attached to a base called the basis capituli. The body (idiosoma) of ticks is typically not hardened to a great extent. 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. Unfolding and stretching of

the soft cuticle along these grooves allows immature and adult female hard ticks to take enormous blood meals and swell to weigh 50 to 100 times their original weight (Lehane, 2005).

2.4. Epidemiology of Ticks

2.4.1. Habitat

Habitat of tick 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 (Nadolny and Gaff, 2018). 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 in and knowledge of the typical physical habitat of a species is an aid in identification. The most important component of the physical habitat of a tick is the climate that is defined by temperature and humidity. When the same tick is on the host it is no longer in danger of drying out or starving, but is in danger of being removed by the host's grooming or having its feeding reduced by host immunity. Most ticks have adaptations in their behavior and physiology of feeding to reduce these host reactions. Usually these adaptations work best for a certain type of host. However the distribution of the potential hosts of a species of tick may often be much larger than the distribution of the tick. Ticks feeding on their hosts may also be eaten by domestic chickens and oxpecker birds (Nadolny and Gaff, 2018).

2.4.2. Host finding

Some ticks live in open environments and crawl onto vegetation to 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 genera such as *Rhipicephalus*, *Haemaphysalis* and *Ixodes* the larvae, nymphs and adults will quest on vegetation. The tick grabs onto the host using their front legs and crawl over the skin to find a suitable place to attach and feed. Adult tick of genera *Amblyomma* and *Hyalomma* are active hunters, they run across the ground after nearby hosts (Walker, 2003).

Other ticks, such as nearly all argasid ticks, and many *Ixodes* species, spend their life cycle in or near the nests or shelters of their hosts (= endophilic behaviour). Some ticks are adapted to living in the housing of domestic animals. Attractants released by hosts that stimulate questing by ticks include carbon dioxide and ammonia. For Example; questing larvae of *Rhipicephalus appendiculatus*; the larvae cluster to conserve moisture and they are high on a grass stem for best contact with hosts and become active as soon as they sense a host approaching. Questing female of *Ixodes ricinus* is activated by sensing a host and holds its front legs out for sensing the host and grasping onto the host with paired claws on each leg. The downward facing posture is typical. Amblyomma adults hunt for their hosts over short distances by running to them over the ground. Questing *Hyalomma anatolicum* female is on a mass of cobwebs on the housing of its host and will actively crawl from its resting site to its host, the front legs are held out to sense and grasp the host (Basu and Charles, 2017).

Most ticks have characteristic species of hosts to which they are adapted. These hosts may be a single species but more commonly are a group of similar species. For example all the *Boophilus* species are highly adapted to feed on cattle, but some *Boophilus* may survive by feeding on sheep. The survival of a population of ticks depends on the presence of hosts suitable for reproduction by the adults. These hosts are known as maintenance hosts. These hosts are more limited in variety than the hosts on which larvae and nymphs of three-host ticks can survive. They are also more limited than those on which adults may attempt to feed but not necessarily survive. To use information of tick hosts for identification it is important to realize that a species of tick has a characteristic range of host species but may be found much less commonly on many other kinds of host species. For example, carnivorous mammals may be infested temporarily with ticks which have transferred from their herbivorous prey (Mccoyet *al.*, 2013).

Tick attachment 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. They seek out places on the hosts where they are protected and have favorable conditions for their development and indicated that different ticks have different predilection sites on the host's body. The favorable predilection sites for *Rh. (B).decoloratus* were the lateral and ventral side of the animal; *Am. variegatum*, teat and scrotum; *Am.coherens* udder and *Hy.truncatum*, scrotum and brisket and *Hy.*

rufipes udder and scrotum, *Rh. evertsi* under tail and anus and *Rh. preaxtatus* anus and under tail (Solomon and Authority, 2003; Huruma *et al.*, 2015).

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. Tick location on the host is lined to the possibility of penetration by hypostome. Genera with short hypostome for example *Rhipicephalus*, *Dermacentor* and *Haemaphysalis* species usually attach to hairless area such as under tail and anovulval area (Huruma *et al.*, 2015).

2.4.3. Seasonal occurrence

Most ticks show seasonality in their life cycles; in most species the adult ticks will become active and feed at the start of the rains. This has been noticed by livestock keepers and scientists for years. Because of the great economic importance of certain ticks as vectors of diseases of domestic livestock and in particular cattle, the study of the seasonal occurrence of ticks is of major importance in the control of ticks and tick-borne diseases (Huruma *et al.*, 2015). An increasing number of ticks have been studied during the last decennia and the seasonal responses and adaptations of their life cycles are much more complicated than expected previously. Seasonal activity is mainly determined by the activity or inactivity of the different life cycle stages. The activity of many species of tick is adapted to seasonal variations in climate. In the tropics this is usually to overcome the adverse effects of a long dry season. The survival of many species is improved if they have a seasonal cycle which reduces these risks. For example *Rh. appendiculatus* in Southern Africa has mechanisms, known as *diapause*, that delay the questing of adults so that their feeding and reproduction starts at the beginning of the single rainy season. This is followed by peak numbers of larvae toward the end of the rainy season when humidity is highest. Knowledge of the time of year when adults of a species are likely to be found on their hosts is thus an aid to identification (Huruma *et al.*, 2015).

2.4.4. Geographical distribution

For some species of ticks there are many published records of the geographical sites in which they have been found. These records can be converted into maps which give a general indication of where a species is likely to be found because of where it has been found before. For example the

type of habitat in which the species is found is likely to be much more widely distributed than the current geographical range of the tick. Thus a tick found in a similar habitat but a faraway geographical area from its usual distribution could have become imported recently (Estrada-Peña *et al.*, 2004).

2.5. Life Cycle of Ticks

In the hard ticks mating takes place on the host, except with Ixodes where it may also occur when the ticks are still on the vegetation. Male ticks remain on the host and will attempt to mate with many females whilst they are feeding. They transfer a sac of sperm (spermatheca) to the female. 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. Female argasid ticks lay repeated small batches of eggs. Eggs of all ticks are laid in the physical environment, never on the host (Hendrix and Robinson, 2016).

Members of the family Ixodidae undergo either one-host, two-host or three-host life cycles. Most ticks of public health importance undergo the three-host life cycle. The three hosts are not always the same species, but may be the same species, or even the same individual, depending on host availability for the tick. Argasid ticks have two or more nymphal stages, each requiring a blood meal from a host. Unlike the Ixodidae ticks, which stay attached to their hosts for up to several days while feeding, argasid ticks are adapted to feeding rapidly (about an hour) and then promptly leaving the host (Latif and Walker, 2004).

All feedings of ticks at each stage of the life cycle are parasitic. For feeding, they use a combination of cutting mouthparts for penetrating the skin and often an adhesive (cement) secreted from the saliva for attachment. The ticks feed on the blood and lymph released into this lesion. All ticks orient to potential hosts in response to products of respiration. The feeding of Ixodidae ticks is slow because the body wall needs to grow before it can expand to take a very large blood meal. Males of Ixodidae ticks feed but do not expand like the females. They feed enough for their reproductive organs to mature (Dantas-Torres, 2008; Huruma *et al.*, 2015; Hendrix and Robinson, 2016).

2.5.1. Life cycle of one host ticks

Eggs are laid on soil. Larvae hatch after several weeks of development and crawl onto vegetation to quest for a host. When they have completed feeding they remain attached to the host and moulting occurs there. The nymphs then feed on the same host and also remain attached. After another moult the adults hatch and then feed on the same host. The adults will change position on the same host for mating. Thus all three feedings of any individual tick occur on the same individual host. The life cycle of one-host ticks is usually rapid, for sub genus *Rhipicephalus* (*Boophilus*) it takes three weeks for the feedings on one host and two months for egg laying and larval development. The adult is considered the diagnostic stage, as identification to the species level is best achieved with adults. Few Ixodidae of public health importance follow this pattern; an example is *Rhipicephalus* (*Boophilus*) *annulatus*, which can serve as a vector for Babesiosis (Estrada-Peña *et al.*, 2010). Vertical transmission of *Babesia* via transovarially transmission has been demonstrated for some species of ticks (Kumsa *et al.*, 2014).

2.5.2. Life cycle two host ticks

The two-host life cycle is similar to one-host life cycle but only the larvae and nymphs feed on the same individual host, and the adults will feed on another host. *Hyalomma detritum detritum* and *Rhipicephalus evertsi* have two-host life cycles. The adult is considered the diagnostic stage, as identification to the species level is best achieved with adults. An example of an Ixodidae tick of public health concern with this life cycle is *Hyalomma marginatum*, a vector of Crimean-Congo viral hemorrhagic fever. Two-host Ixodidae ticks have a life cycle that usually spans over two years. Gravid females drop off the second host after feeding to lay eggs. Adults feed on the second host during the summer and mate. In the fall, females drop off the second host to continue the cycle. Humans may serve as first or second hosts for ticks with this life cycle. Also, the second host does not necessarily have to be a separate species, or even a separate individual, as the first host (Latif and Walker, 2004).

2.5.3. Life cycle of three host ticks

This is the commonest type of lifecycle. Larvae develop in the eggs until ready to hatch, usually in several weeks. Larvae feed once on a host, then detach from the host and hide in sites such as soil or

vegetation. They moult to nymphs. Nymphs feed once and moult in the same way as larvae. From the nymphal moult either a female or male hatches. The female feeds once and lays one huge batch of eggs. The depleted female then dies. The male may take several small feeds, mate and then die. Ticks that have recently hatched from eggs or from moulting have soft bodies and are inactive for one to two weeks until the external body wall hardens. The life cycle of three host ticks is slow, from six months to several years. The adult is considered the diagnostic stage, as identification to the species level is best achieved with adults. Most ticks of public health importance follow this pattern, including members of the genera *Ixodes* (Lyme diseases or borreliosis, babesiosis), *Amblyomma* (tularemia, ehrlichiosis and Rocky Mountain spotted fever), *Dermacentor* (Rocky Mountain spotted fever, Colorado tick fever, tularemia, tick paralysis), and *Rhipicephalus* (Rocky Mountain spotted fever, boutonneuse fever). The three hosts do not necessarily have to be different species, or even different individuals. Also, humans may serve as first, second or third hosts (Estrada-Peña *et al.*, 2004).

2.6. Pathogenic Role of Ticks

Direct effects of ticks on cattle are tick worry, blood loss, damage to hides and skins of animals and introduction of toxins (Marufu, 2008). The ecology and physiology of ticks have made them second most important vectors after mosquitoes. Ticks transmit a large variety of intercellular bacteria in the Rickettsia group like Rickettsia, Ehrlichia and Anaplasma. Similarly, several piroplasms protozoa like *T.annulata*, *T.parva* and *Babesia bigemmina* are also transmitted specifically by ticks (Sharma *et al.*, 2013; Tuli *et al.*, 2015).

Tick worry is a generalized state of unease and irritability of cattle severely infested with ticks, often leading to serious loss of energy and weight. This negative effect on the growth of animals and their production is thought to be due to the effects of a toxin in the saliva of ticks (Marufu, 2008).

Anaemia is an inevitable consequence of heavy infestation by any blood-feeding parasite, and cattle deaths attributable to anaemia as a result of tick infestation are common. Engorging Ixodidae females will increase their weight by 100–200 times but the actual amount of blood ingested is

much greater than this, as blood meal is concentrated and fluid excreted in saliva. Estimates of the amount of blood removed vary according to the species under consideration (Marufu, 2008).

Ticks with longer mouthparts such as *Amblyomma* and *Hyalomma* cause more extensive damage than those with shorter mouthparts such as *Rhipicephalus (Boophilus)* and *Rhipicephalus*. The involvement of host reactions leading to tissue damage may be dependent upon recruitment of inflammatory responses characterized by dermal cell infiltrates which form the lesions. Tick wounds may become infested by screwworms or other agents of myiasis, and are also associated with the spread of bovine dermatophilosis caused by *Dermatophilus congolensis* (Brites-Neto *et al.*, 2015).

Tick saliva contains toxins which have a specific pathogenic effect. The toxins affect not only the attachment site but also the entire organs of the host. Some ticks produce neurotropic toxins which induce tick paralysis that is characterized by an acute ascending flaccid motor paralysis. Females of the species *Hyalomma truncatum* produce a dermatropic (epitheliotropic) toxin which causes sweating sickness in calves and some adult cattle (Mans *et al.*, 2004).

2.7. Ticks and Their Distribution in Ethiopia

The distribution and abundance of tick species infesting domestic ruminants and equines in Ethiopia vary greatly across different agro-ecological zones and seasons of the country. The country's environmental condition and vegetation are highly conducive for ticks and tick-borne disease perpetuation (Rajput *et al.*, 2006). The distribution and seasonal dynamics of the following predominant ixodid tick species in Ethiopia is presented below one after the other and their prevalence in different parts of Ethiopia (Table 1).

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, 2003). 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 (Sileshi *et al.*, 2007). Its distribution pattern is simi

lar to that of *Am. variegatum*. In previous records, *Rh.(Bo.) 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 (Abebaw, 2004). Several authors reported this tick from all agroclimatic zones, but it is more common in the highland and midland altitudes in Ethiopia.

Amblyomma variegatum occurs in areas with a wide variety of climates ranging from highland, savannah through to lowlands (Walker, 2003) but its abundance varies greatly across different agroclimatic 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 (Abebaw, 2004). 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 (Mekonnen *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 (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 (Estrada-Peña *et al.*, 2004). 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 (Jongejan and Uilenberg, 2018). *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 (Sileshi *et al.*, 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 (Abebaw, 2004).

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(Walker, 2003). In Ethiopia, according to (Zelege and Bekele, 2004;Mohammed and Admasu, 2015;Kumsa *et al.*, 2015).*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.

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, 2003). 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 (Tiki and Addis, 2011)

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 (Abebaw, 2004).

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, 2003). In Ethiopia, it is present in different agro-climatic zones except in the wetter South-western part of the country (Abebaw, 2004). Previous investigator in Ethiopia collected *Hy. truncatum* as one of the important tick species in central Oromia (Kumsa and Mekonnen, 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).

Table 1. Prevalence of tick species in different study area in Ethiopia with respective authors

Tick species	Study area	Prevalence (%)	Authors
<i>Amblyomma variegatum</i>	Somalia	4.2	(Rahmeto <i>et al.</i> , 2010)
	Northwest, Ethiopia	13.5	(Alemu <i>et al.</i> , 2014)
	Western Ethiopia	17.2	(Kumsa <i>et al.</i> , 2014)
	Southern, Ethiopia	20.8	(Shiferaw and Abebe, 2006)
<i>Rhipicephalus (Boophilus) decoloratus</i>	Western Ethiopia	38.2	(Kumsa <i>et al.</i> , 2014)
	Northern Ethiopia	23.9	(Yacob <i>et al.</i> , 2008)
	Southern Ethiopia	46.6	(Wogayehu <i>et al.</i> , 2016)
	Western Ethiopia	25.8	(Kumsa <i>et al.</i> , 2014)
<i>Rhipicephalus evertsi</i>	North Shewa	20.6	(Tadesse and Sultan, 2014)
	North West Ethiopia	11.5	(Alemu <i>et al.</i> , 2014)
	Somali Ethiopia	40.1	(Tomassone <i>et al.</i> , 2012)
<i>Rhipicephalus pulchellus</i>	Adami Tulu	0.9	(Ayalew <i>et al.</i> , 2014)
	Adami Tulu	19.6	(Ayalew <i>et al.</i> , 2014)
<i>Rhipicephalus praetextatus</i>	Western Ethiopia	0.4	(Kumsa <i>et al.</i> , 2014)
	Ada'a	17.8	(Ayalew <i>et al.</i> , 2014)
<i>Hyalomma rufipes</i>	Somali Ethiopia	13.3	(Tomassone <i>et al.</i> , 2012)
<i>Hyalomma truncatum</i>	Central Oromia	1.5	(Ayalew <i>et al.</i> , 2014)
<i>Amblyomma cohaerens</i>	Bedele District	41.5	(Tamerat <i>et al.</i> , 2015)
<i>Amblyomma cohaerens</i>	Bench Maji zone	4.2	(Onu and Shiferaw, 2013)
<i>Amblyomma gemma</i>	Somali Ethiopia	9.4	(Tomassone <i>et al.</i> , 2012)
	Borana zone	5	(Regassa, 2001)

2.8. Diagnosis of Tick Infestation

2.8.1. Collection of Ticks from Hosts

Tick specimens are usually obtained from their hosts. It is seldom feasible to examine the whole of a livestock animal for ticks, but in some studies the animal is cast to the ground or held in a crush then one half of the body is searched fully. It is often more efficient to examine a sample of fixed areas of the host. This is very useful for ticks which are known to have sites where they prefer to feed (predilection sites). For example, *Rh. appendiculatus* adults on the ears; *Am. Variiegatum* adults on the dewlap, axillae, udder and groin; *Rh. (Boophilus) decoloratus* or *Rh. (Boophilus) microplus* generally on the shoulders, dewlap, and belly; *Hy. Truncatum* adults mostly around the anus (Daniels *et al.*, 2000).

An effective way to detect adult ticks, especially when they are engorging, is to feel the hair coat of the host with the palm of your hand. Smaller domestic animals in a clinic can be examined in the same way. To find immature ticks or unfed adults the hair can be parted systematically using forceps. Protect yourself from ticks attaching to you when collecting them (Zhang *et al.*, 2017). To remove ticks from host skin whilst retaining their good condition for identification use good quality steel forceps. These should be of medium size with blunt points and serrated inner surfaces. The forceps is used to grip the tick firmly over its scutum and mouthparts as closely to the host skin as possible, and then pull strongly and directly out from the skin. Usually the mouthparts will be removed with the rest of the tick and often with a plug of cement. This can be removed later using two fine forceps under a dissecting microscope (Walker, 2003).

For identification of *Rhipicephalus (Boophilus)* species it is important to examine the mouthparts for arrangement of teeth. However, these may be damaged during removal of the tick from the host. For this group and other genera it is very useful to have males in addition to females for identification. Take care to remove the males which often re-attach for mating pressed to the ventral side of engorging females, near their mouthparts (Reyes *et al.*, 2011).

If the ticks are required live for further studies they should be placed in strong tubes containing a piece of damp (moist) paper. During collection it is useful to seal the tube with a rubber membrane made from rubber gloves or similar material and held with a rubber band or tape. This should have a

small slit cut in it through which the ticks are pushed. For transport to the laboratory use a separate ventilated plug. This can be made of cotton wool or a perforated screw cap. These tubes should be labelled then kept in a sealed plastic bag containing wet cotton wool or paper to maintain high humidity. The ticks should be kept cool over ice but take care not to freeze them fatally (Estrada-Peña *et al.*, 2004).

2.8.2. Collection of Ticks from Vegetation and other Environments

Some species of ticks can be collected whilst they are unfed and questing on vegetation. If they are sufficiently dense in numbers adult *Rhipicephalus* and other ticks can be picked by hand from grass stems. More often it is efficient to use a trap which mimics a host. This consists of a 1m square piece of white cloth such as cotton toweling. It is fitted with a bar at the front and a cord for pulling it slowly across the vegetation for 5m to 10m (for approximately 30 seconds of walking and repeated after removing the ticks). Larvae, nymphs and adults will grip temporarily onto the dragging cloth and can be collected with a forceps. This method works well for larvae and nymphs of questing species but is less efficient for adults and hunting species (Daniels *et al.*, 2000).

Endophilic ticks can be collected directly from the nests or shelters of their hosts using forceps to probe in cracks and under pieces of dry dung, spider webs etc. This is very effective for moulting nymphs and adults of *Hyalomma* ticks in cattle sheds. Ornithodoros ticks can be collected in the same way. Ticks are auto-fluorescent in ultraviolet light. This makes them visible in the dark if illuminated with a portable ultraviolet lamp (Barandika *et al.*, 2014).

2.8.3. Preservation of Tick Samples

Long term collections of ticks are stored wet. Laboratory alcohol (90% ethanol) at 80%, plus water at 15% and glycerol at 5% is best. The glycerol prevents drying out when the ticks are examined in air. Colors of ticks fade in alcohol but this can be reduced if 1% of chloroform is added to the alcohol. This is mainly useful if photographs are required of the ticks. For photography it is helpful to first kill the ticks in a way that prevents the legs from curling up. Use Boardman's solution (17% ethanol, 3% ether, 80% water) for 24 hours, and then transfer the ticks to the usual preservative. The tubes ideal for storing ticks in a collection are those with thick glass walls and metal screw caps

with a rubber washer, of 5ml capacity and known as Bijou tubes. If the ticks are to be used for any form of analysis of their nucleic acids or for searches for nucleic acids of transmitted pathogens then the specification of the ethanol or formaldehyde should be conform to the needs of the tests used (Bonnet and Liu, 2012).

Before preservation or transportation for further identification of tick specimen it should be carefully labeled. Labels for tick collections should be written on card using India ink (= China ink). This is carbon based and will not dissolve when placed in preservative. The label should include name of the species (if known), date, collector, and species of host, site and country of collection. The site should be given as both a permanent place name and as latitude and longitude. The use of village names or similar changeable features makes difficulties for later workers. The universally accepted system is to use the global coordinates of latitude and longitude, to at least the nearest minute. These are read from a map of the area, or an instrument to read the global positioning system from satellites (Barandika *et al.*, 2014).

2.8.4. Transportation of Ticks

To transport live ticks to determine the effectiveness of new acaricide (Adult/Larval immersion test), ticks should be put into small boxes, preferably made of cardboard, with a few small holes allowing air to circulate. If the ticks are to be transported over long distances, they should be placed between layers of slightly moistened paper towels in order to keep the environment humid and protect the ticks from damage. Alternatively, freshly cut, green grass or herbage (of a type that has no direct adverse effects on ticks) can be used to provide moisture. More problems occur with samples that are too wet than too dry, provided that transport times and temperatures are not excessive. Where large numbers of ticks are submitted for Adult Immersion Test (AIT), the cardboard containers should be placed in a cool box with a cooler brick (wrapped in paper towels) and transported to the laboratory by the fastest route (Ballweber, 2006).

Another way of transporting live ticks for further study is by placing them in strong tubes containing a piece of damp paper. During collection it is useful to seal the tube with a rubber membrane made from rubber gloves or similar material and held with a rubber band or tape. This should have a small slit cut in it through which the ticks are pushed. For transport to the laboratory

use a separate ventilated plug. This can be made of cotton wool or a perforated screw cap. These tubes should be labeled then kept in a sealed plastic bag containing wet cotton wool or paper to maintain high humidity. The ticks should be kept cool over ice but take care not to freeze them fatally (Kumar, 2013).

2.8.5. Laboratory Identification of Ticks

Ticks can be identified to genus using the naked eye as indicated in (Figure 3) or a simple hand lens of x10 magnification. To identify most species in this guide a dissecting microscope is required. This is a low power stereoscopic microscope. It must have a range of magnification from x10 to x40, and very preferably up to x80. Intense lighting is essential, from a halogen filled lamp bulb or light emitting diodes (Chae *et al.*, 2017).

For preliminary sorting keep the ticks in a dish under the preserving liquid. For some features such as leg coloration it is often useful to observe the ticks under liquid. For final identification it is important to examine ticks dry and cleaned of deposits of glycerol from the preservative. Use tissue paper to blot them dry. When dry the ticks often then appear dirty. The best way to clean them is using an ultrasonic cleaner for 5 minutes whilst they are immersed in 5% sodium or potassium hydroxide solution in water (Labruna *et al.*, 2016).

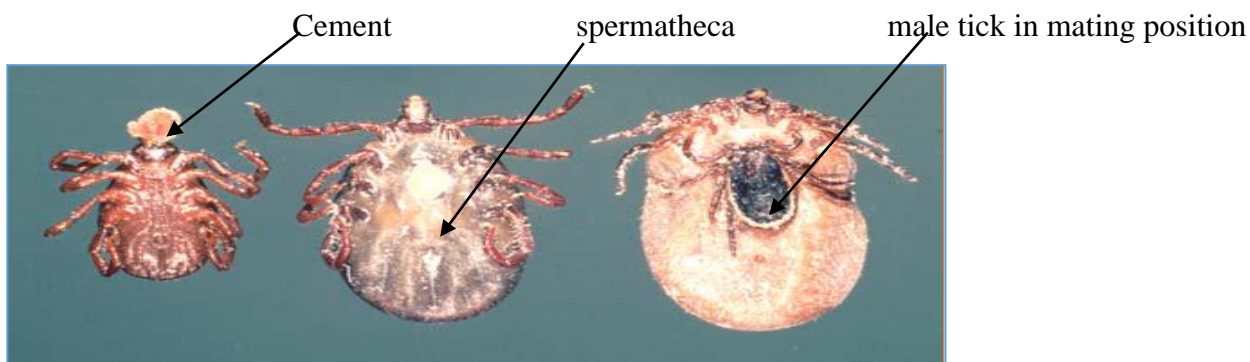


Figure 2: Cement, spermatheca and male tick in mating position

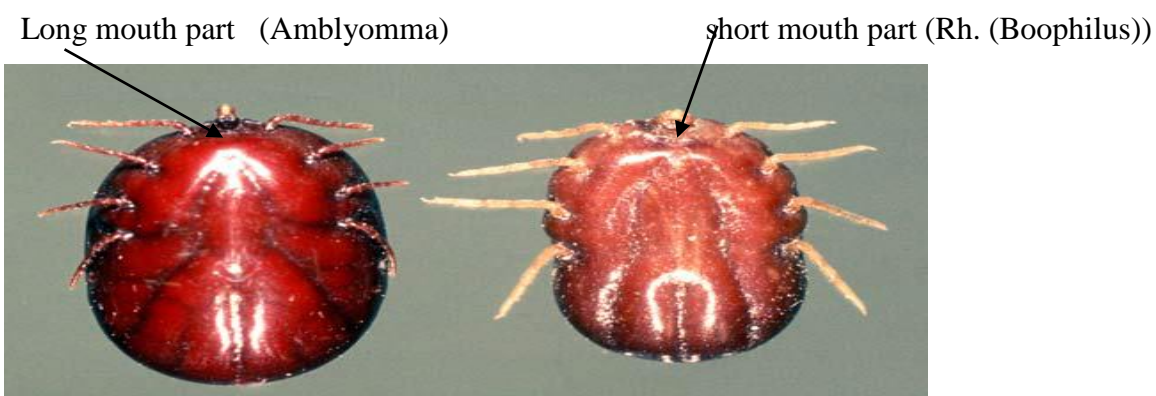


Figure 3: Mouth part of different tick species

Source: (Chae *et al.*, 2017)

It is most important to view the tick from different angles to observe features of superficial texture such as punctations and grooves. The genital aperture of some ticks has important features for identification, especially in *Hyalomma* and *Rhipicephalus*. For detailed examination it is best to cut out the area of integument including the aperture and mount it on a microscope slide with cover slip. Examine the aperture with an ordinary microscope. Use a mounting medium which preserves and clarifies the specimen on the slide. Berlese's medium is suitable: dissolve (Ballweber, 2006).

There are some morphological features which are confusing during identification of tick species. Therefore it is very important to have perfect knowledge about the morphology of different tick species. The relative size of larvae, nymphs and adults when unfed and fed is typified in the

photograph of the life cycle of *Rh. appendiculatus*. It is most important to be familiar with these different sizes (Nijhof *et al.*, 2009).

Engorged female ticks are difficult to examine but most of the necessary features remain visible if the tick is correctly positioned on a viewing stand. The mouthparts may appear unusual because the palps remain splayed apart after the tick has been removed from its host. Additionally a lump of attachment cement may adhere to the mouthparts. Occasionally a male spermatheca remains attached to the genital aperture of a preserved female, appearing as a white sac. The male may remain in the mating position on the female (Figure2) (Walker, 2003).

On the same cattle in some areas there will be *Amblyomma* and *Rhipicephalus* (*Boophilus*) ticks feeding simultaneously. An engorged nymph of *Amblyomma* may appear as large as an engorged female of *Rh.* (*Boophilus*). Examine the mouthparts to differentiate them - long in *Amblyomma*, short in *Rh.* (*Boophilus*) Punctations and female genital apertures need very careful observation under a stereoscopic microscope and Scutum coverage very important to identify male and female (Figure 4) (Blagburn and Dryden, 2009).

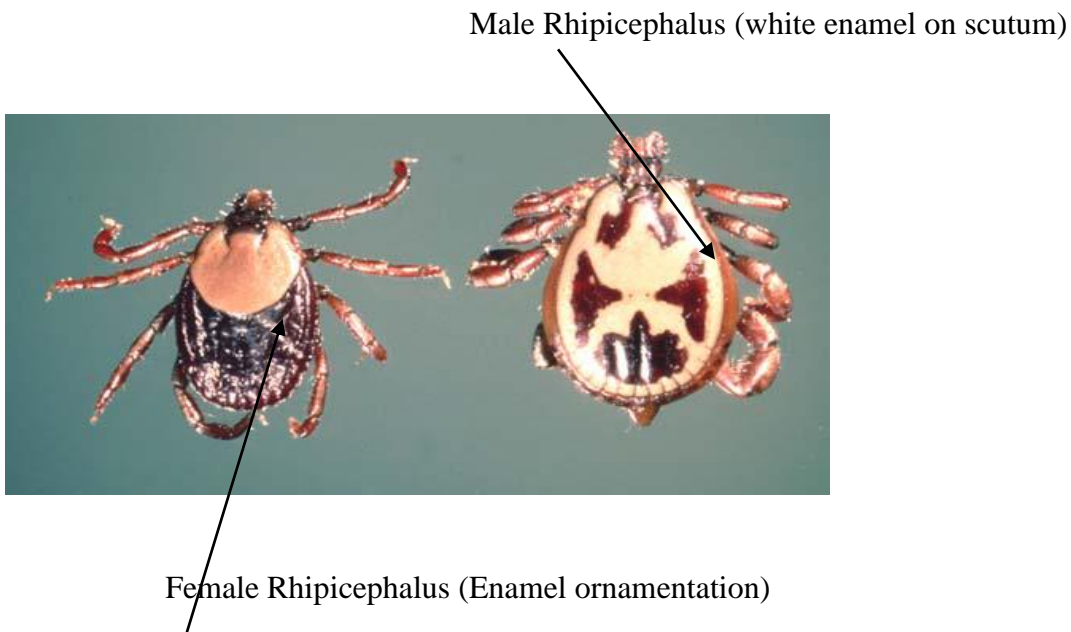


Figure 4: Scutum coverage of female (left) and male (right) ticks
Source: (Ballweber, 2006).

Besides the morphological features; we also make use of their ecological requirements to assist with an accurate diagnosis, the geographic locality at which they were collected, the hosts from which they were collected, the body site on the host from which they were collected, the season of the year during which they were collected, male ticks must be included in any collection sent for identification, label containing all the important collection data and written in pencil should be included with the ticks inside the vial or tube or bottle ball point writing dissolves the moment the alcohol used for tick preservation spills onto it (Madder *et al.*, 2014).

2.9. Control Measures for Ticks

The aim of a tick control campaign is not to control all ticks simultaneously, but a definite species because of its particular role. The strategy should therefore be based on the biological characteristics of the target species. Moreover, there is no perfect control method. The efficacy of these methods depends on rational and methodical use (FAO, 2004).The following are some of the tick control methods;

2.9.1. Chemical tick control

The conventional method of controlling tick infestations in Ethiopia is application of acaricide, either by hand spraying or by hand dressing (Table 2). Therefore, to minimize tick adverse effect appropriate and timely strategic control measures are crucial (Tilahun, 2016). Acaricides are needed to control tick infestations and tick borne diseases. However, the use of acaricides is constrained by their high costs, tick resistance, concerns about residues in food and in the environment. Consequently, the intensity of application of acaricide will depend on whether the aim is to prevent disease transmission or to reduce tick damage and /or worry. Thus a choice can be made as to whether to adopt intensive or strategic/threshold acaricide application or other systems (Abebaw, 2004).

Table 2. Ways of application and concentration of acaricides used for tick control in Ethiopia

Group of compound	Generic name	Trade name	Concentration (%) of active substance and ways of application			Residual effect (days)
			Dip	Spray	Pour on	
Chlorinated hydrocarbons	Toxaphen	Coopertox	0.5	0.75		20-40
	CHC	Lindane	0.75	0.75		30-50
		BCH	0.75	0.75		30-50
Organic phosphorus	Coumaphos	Asuntol	0.1	0.1		7
	Trichlorphon	chlorfos			0.15	2
	Quinthiophos	Bacdip	0.08	0.08		4
	Chlorphenvinphos	Steladone		0.05		2
	Dioxathion	Cattle dip	0.06	0.05		4-7
	Malathion	Malathion	0.05	0.15		2
	Famphur	Famophos			2.6 mg/kgbm	4-7
Carbamates	Carbaryl	Vioxan		0.2		
Pyrethroids	Flumethrin		0.5	0.5	0.75	30
Cyclic amidins	Cymiazole	Besuntol		0.03		7
	Amitraz	Norotraz	0.25	0.25		7
Avermectines	Ivermectin	Ivomec			10ml/50 kgbm	14

Source:(Abebaw, 2004)

2.9.2. Ecological control

Information on the ecology of different instars is used for habitat and host linked treatments. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks. Vegetation is periodically removed by burning, but spontaneous or induced fires have little direct effect on ticks since they occur in the season when adults are not active. Annual dry-season fires are widespread in semiarid regions; the value of these fires is very controversial, as they influence not only the availability of an important source of grazing during long harsh dry seasons, but may also diminish the abundance of ticks and vermin such as rats. The influence of burns on tick abundance varies markedly with the time of year, intensity of burn, and the tick species present (Estrada-Peña *et al.*, 2013).

2.9.3. Genetic control

Use of resistant animal is widely known that *Bos indicus* cattle are more resistant to ticks than are *Bos Taurus* animals. There are great differences between these two breeds of cattle in regard to their susceptibility to parasitism by cattle ticks. Studies are intensifying the crossing of these two groups, aiming to obtain animals that are more resistant to the conditions found in tropical countries and are also good meat producers. Tick resistance among cattle is influenced by a number of factors. The most important are increased levels of histamine at the early stages of the infestation, self-cleaning behavior, increased levels of eosinophils, basophils and mast cells, the presence of specific immunoglobulin patterns, and genes related to the expression of keratins and lipocalins (Maharana *et al.*, 2011; Tabor *et al.*, 2017; Yessinou *et al.*, 2018). However, over 50 tick species are known to exist in Ethiopia. The effects of ticks on indigenous cattle compared to exotic breeds shown to be minimal (Mekonnen *et al.*, 2001). In Ethiopia, Borana and Horo breeds are known tick resistant cattle breeds.

2.9.4. Ethno-Veterinary Control of Tick

However, little work has been done to document and validate these ethno-veterinary remedies in Ethiopia (Abebaw, 2004). But, in north Gondar the use of some plants to control tick in animal using some plants such as *Birbira* (*Milletia ferruginea*), *Zikita* (*Calpurnia auera*), *endod* (*Phytolaccadod ecandra*) and others was reported. And the ethno-veterinary remedies of tick

control practiced in western Ethiopia have been examined by a survey of farmers, followed by in vitro and in vivo testing of treatments that appeared to have potential to control ticks. Some plants with acaricidal properties have some side effects on animals for example application of latexes of *Euphorbiaobo valifoliaca* used alopecia in areas of skin smeared with the latex (Kioko *et al.*, 2015).

2.9.5. Biological control method

Entomopathogens are group of organisms that attack ticks and insects. It can be macro- or microorganisms that affect arthropods. 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. Until this is known, it is not possible to recommend such alternatives to producers for adoption and practical use in the field in Ethiopia.

2.9.6. Immunization (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. 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).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from September 2018 to May 2019 in Anilemo district. Anilemo district is located in Hadiya zone in southern Ethiopia about 18km from the main town of the Zone, Hosanna, and 270 km from main city of the region, Hawassa, and 212 km far from Addis Ababa, capital city of Ethiopia (Figure 5). The district has 27 rural and 1 urban peasant associations (PA's). Anilemo is geographically located between 7° 30'-7° 51' North latitude and 37° 51'-38° 06' East longitude (AWARDO, 2014). According to (AWARDO, 2014) agro-ecologically, the district is categorized into three agro ecological zones (20% highland (named as Bandalicho), 42% midland (named as Laftolenka) and 38% lowland (named as Mentoakebala)) with an altitude of 1000-2500m.a.s.l, with minimum and maximum annual temperature of 15.1-20 degree Celsius, and minimum and maximum annual rainfall of 1001-1200mm. The topography of the area is marked by the presence of a two large mountains, Bandalicho and Adilduna mountains with an altitude of 2500 m.a.s.l and 2480 m.a.s.l, respectively. The area experiences a bimodal rainfall pattern, short rainy season (belg) and long rainy season (kiremt). The short rainy season (belg) lasts between February and May whereas the summer season (the long rainy season) lasts between June and September. Rain that occurs during the long rainy season is very intensive and, hence, the severity of tick infestation is high during this season. Most of the livestock production also takes place during the long rainy season because of the high availability of forage for grazing. The farmers of the study area practice mixed crop-livestock farming system. The area comprises large population of ruminants, equines and poultry. Ruminants are kept to obtain mainly meat, milk and for farming purpose, whereas equines are largely used as the means of transportation in areas where inaccessible to car at relatively lower charges.

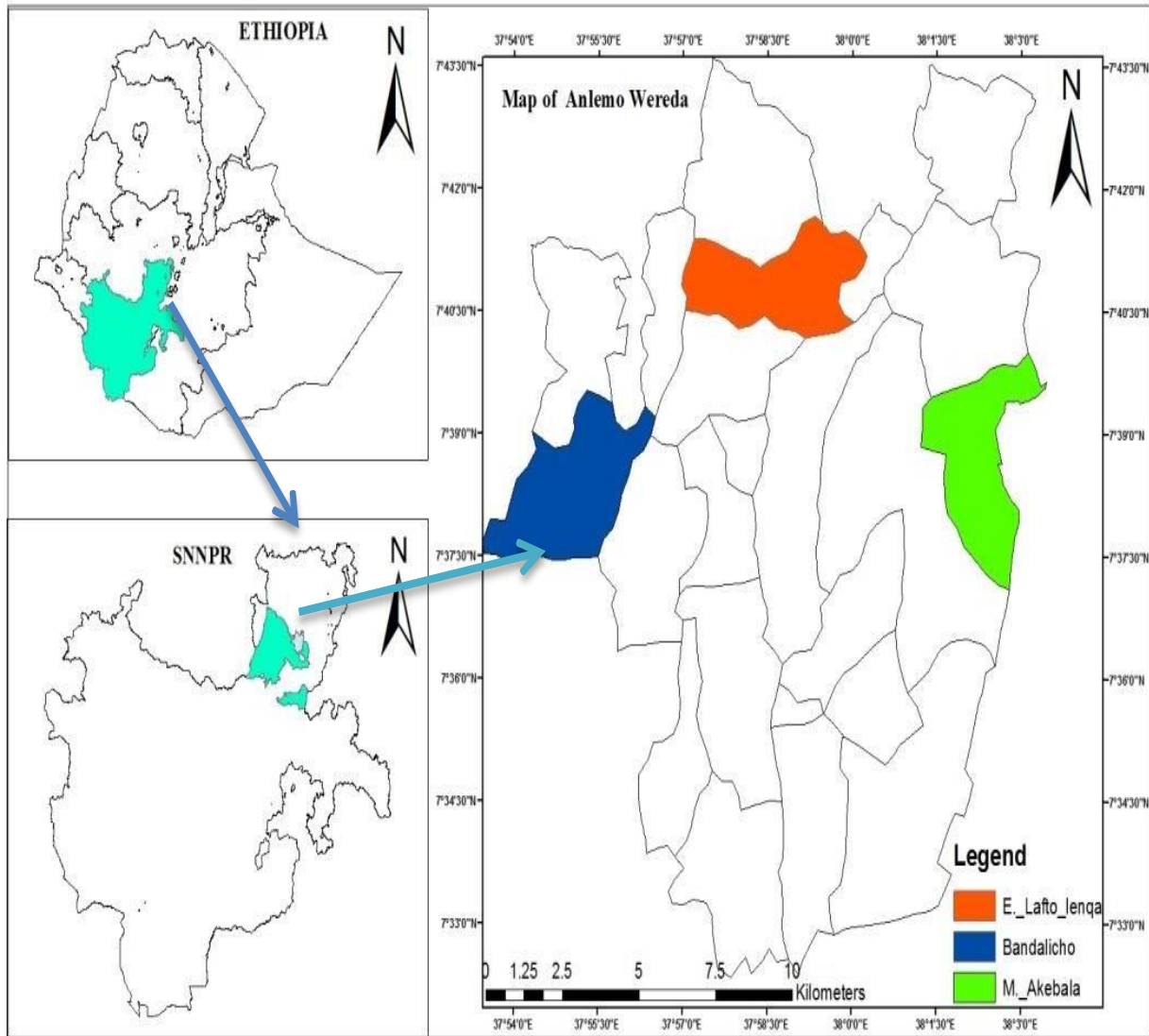


Figure 5. Map of Ethiopia showing Anilemo district, the study Area.

Source: (Ethio GIS, 2004)

3.2. Study Population

The study was conducted on bovine, ovine, caprine, horses and donkeys in selected PAs in Anilemo district in Hadiya zone in Southern Ethiopia. The study included animals of all age, sex, breed, BCS and color. The total population of bovine, caprine, ovine, equine, poultry and bee family in the study area is estimated at 61267, 23875, 21444, 15872, 86932, and 5344, respectively. The total human

population in the district is 101,000. The livelihood of the people in the district depends mainly on mixed, crop and livestock type of farming. The annual crops grown are wheat, teff, potatoes, sorghum, maize, pea, bean, cabbage, carrots and onions. The perennial crops grown in the District are “enset”, chat, sugarcane, avocados, mangoes and timber trees. In the District “enset” is the main perennial crop source of food all year round. Most of the owners keep their animals in semi-intensive production system with part time intensive where animal manure and wasted feed are not regularly cleaned and part time extensive. The available animal feed resource of this area consists of natural grass, concentrates, crop and “enset” residue. The available veterinary services of the district include one veterinary clinic located at the capital town of the district and other four animal health center are found distributed in different areas in the district.

3.3. Study Design

A cross sectional study was conducted from September 2018 through May 2019, with the core intentions of identifying different tick species infesting ruminants and equines and associated risk factors for infestation, and to assess the type and efficacy of the most frequently used acaricides in the study district. Only those animals which were not treated with any acaricides at least for a month prior to sample collection time were included in the present study. Each study animal was randomly selected from the District. Each of the animal selected as sampling unit was checked for any tick infestation and recorded as positive and negative depending on presence and absence of ticks on the body, respectively. Professionals and livestock-keepers/farmers who use acaricides for tick control was approached to respond to structured questionnaire survey, that was used to assess the perception of professionals and livestock keepers towards the various type of acaricides used, frequency of acaricide application, method of application, rotational use of acaricides, use of ethno-veterinary practices to control tick, and their knowledge about ticks, delivery system of acaricide, as well as the different tick control options and methods of applications practiced in the area. The selection of the respondents depends on livestock production and patronage by livestock keepers and animal health workers in the area. Fully engorged female ticks were used to evaluate the efficacy of the two most frequently used acaricides by using Adult Immersion Test (AIT) method during the study period.

3.3.1. Sampling methods

The study sites were purposely clustered by considering the agroecology into three clusters; Laftolenka, Bandalicho and Mentoakebala each contain different study PAs. As there was no previous estimated prevalence of tick infestation of domestic animals in the district, the sample size was determined based on 50% expected prevalence, 5% desired absolute precision at 95% confidence level (Thrusfield and Christley, 2005). As a result, 384 was considered as the minimum sample size required for tick infestation of the study area. However, to increase precision, a total of 906 ruminants and equines from all the three agro-ecologies were examined for the presence of ticks over the whole study period. For efficacy test engorged adult ticks were collected purposely from tick infested cattle.

3.3.2. Tick collection and identification

Ticks were collected by carefully inspecting the skin of each study animal from different predilection/feeding sites such as lateral and ventral side, teat, scrotum, udder, dewlap, tail, anus and ear after being restrained using various methods such as using strong crushes, physical handling and by ropes. During tick collection, age of each study animal was categorized into as young or adult, the sex into male or female, breed as local, cross or exotic, as previously used (Reece and Rowe, 2017). Likewise, the body condition scores (BCS) was taken as good (difficult to feel ribs), medium (ribs cannot be visual distinguished but can be easily felt) and poor (no fat cover over ribs) was recorded in the format including the sample number (Annex 1). All visible ticks (larvae, nymph and adults) were manually collected by using forceps from half regions of the body of the animal in sample number labelled universal bottle and care was taken to avoid decapitulation of ticks during collection. Collected ticks were preserved in 70% ethyl alcohol. The ticks were then transported to the Parasitology Laboratory of College of Veterinary Medicine and Agriculture of Addis Ababa University located at Bishoftu for identification. The collected 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 5) (Walker, 2003 ;Mathison and Pritt, 2014). Glycerol was used to prevent drying out when the ticks were examined. During tick identification in the laboratory the sample were put on Petri dish 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 (Walker, 2003). For each tick, photographs of the dorsal view of larvae, nymph and adult male and female ticks were captured and depicted on figure in the result section.

3.3.3. Questionnaire survey

Semi-structured questionnaire (Appendix 2) was administered to 50 respondents. This sample size was determined using the formula adopted from (Arsham, 2005) as indicated below. Data collected from 16 professionals and 34 farmers about the type of production/mode of husbandry, information on ticks, tick control regimes by ethno-veterinary knowledge and chemical treatment, tick response to acaricides application, and general view of diseases control in the area. Livestock owners in different PAs were interviewed to assess about their knowledge about ticks and TBDs present in the area and the veterinary authorities to know their perception about the main control measures they used to tackle the main ticks in the area.

Sample size =

$$\frac{\frac{z^2 \times p(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p(1-p)}{e^2 N}\right)}$$

Where, N = population size, e = margin of error (percentage in decimal form), Z = z-score. The z-score is the number of standard deviations a given proportion is away from the mean. To find the right z-score to use, refer to the Z- table. And P, the prevalence which is either 50% or from previous study. In this case there was no previous study in the district so the value of p-was 0.5(50%). 95% confidence interval was used to find the z-value. N=101,000, e=0.5, Z=95%=0.95, so the value of z-from the table=1.96, p=50 % (0.5). Sample size (n) =1.96^2*0.5(1-0.5)/0.5^2/1+(1.96^2*0.5(1-0.5)/0.5^2*101,000=385. Thus, 385 indicate the number of respondents from the 28 PAs, but it was distributed at randomly selected 3 PAs so the number of respondents from the three PAs was minimum of 41. To increase precision the number of respondent was maximized to 50.

3.3.4. *In vitro* acaricide efficacy evaluation

In vitro efficacy evaluation of the two commonly used acaricides in the area, Diaznone and ivermectine was conducted against the two most predominantly distributed ticks, *Rh. (Bo.) decoloratus* and *Amblyomma variegatum* collected from animals of the study area. Results of the questionnaire survey suggest that Diaznone and ivermectine are the two most commonly used acaricides in the study district. A total of 110 ticks collected from cattle of the study area were used for this efficacy study after taking treatment history. Ticks were collected at early morning because most engorged ticks drop off the host in the early morning (FAO, 2004). Card board and penstrpt containers with a few small holes to allow air circulation were manually made and used to transport live ticks within 24 hours of collection from Anilemo district to Addis Ababa University College of Veterinary Medicine, Parasitology laboratory. The containers were slightly moistened to keep the environment humid and protect the ticks from damage as has been suggested by FAO (2004). Immediately upon arrival at the laboratory, engorged female ticks were washed by using distilled water to remove eggs that might had laid during transport.

Adult Immersion Test (AIT) method of acaricide efficacy evaluation to determine the oviposition response of ticks to acaricides such as Diaznone 60%EC (manufactured by Egypt Company) and ivermectine1% (manufactured by Uruguay Company) was used in the present study. A total of 80 engorged female *Rh. (Bo.) decoloratus* ticks and 30 *Amblyomma variegatum* ticks of uniform size collected from cattle that were randomly allocated into three groups (Group-1 (n1, n2, n3, n4 = 10), Group-2 (n1, n2, n3, n4 = 10) and Group-3 (n1, n2, n3 = 10) were used for the efficacy evaluation of oviposition inhibition as per the standard guidelines of FAO (2004). Efficacy trial was performed in triplicate. The weights of engorged female tick in all three groups were recorded. All ticks in each group were immersed in each concentration of acaricides preparations recommended at field level whereas the control groups were immersed in the distilled water. The dose of 1ml/1litre for Diaznone and 1g/liter for ivermectine suggested by FAO (2004) was used for adult immersion test. Accordingly, 20ml of diluted acaricides was added into each calibrated plastic container for immersion of a total of 10 engorged female ticks (Annex 3) for efficacy evaluation of acaricides. Then, after 1 minute of immersion all ticks were cleaned and air-dried at room temperature, pasted

onto double-sided adhesive tape on glass test panels with their ventral sides facing upwards keeping their capitula clear of the tape and then were incubated at 25 to 28 degree Celsius and 85-90 % R.H. for 7 days. The effect of each tested acaricides on reproductive capacity of each immersed engorged female tick was determined by comparing with the results of the control groups. All groups were then tested (evaluated) using the egg laying test method (Drummond *et al.*, 1973) modified by (FAO, 2004) by comparing the number of each engorged female ticks laying egg in each tested acaricides with the number of each engorged female ticks laying egg in the control group and finally the percentage of the resistance by each tested acaricide was estimated using the following formula: Percent of resistance = N_t/N_w*100 , Where, N_t = number of treated ticks laid eggs, N_w = number of untreated ticks laid eggs. Similarly, Percent of control for each acaricides calculated as; Number of tick laid egg in treated group minus number of tick laid egg in control and multiplied by hundred.

3.4. Data Management and Analysis

All the collected data were entered and stored into the data base in Microsoft Excel spread sheet (MS -2010) program. Then the stored in MS-excel were imported to STATA and R-software, and the data were coded appropriately and analyzed using STATA version 13.0 statistical software (StataCorp, 2013) and R version 3.4.2 (2017-09-28). The data was summarized by descriptive statistics and displayed by tables and graphs. Poisson regression was used to analyze the number (count) of ticks on animals as a function of different explanatory variables (animal species, BCS, breed, sex, age, agro-ecology, season, color). Furthermore, Negative binomial regression was applied by fitting the model after Poisson regression to overcome over dispersion. Logistic regression was employed to analyze the degree of association of binary outcome (presence or absence of tick) 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. For all statistical analysis a statistical significance level of $p<0.05$ was considered.

4. RESULTS

4.1. Ticks Infesting Ruminants and Equines

4.1.1. Species diversity and relative abundance

Out of the total of 622 cattle, 100 goats, 72 horses, 65 sheep and 47 donkeys examined for tick infestation 282(45.3%) cattle, 9(9%) goats, 9(12.5%) horses, 12(18.5%) sheep and 4(8.5%) donkeys were infested at least with one or more ticks (Figure 6).

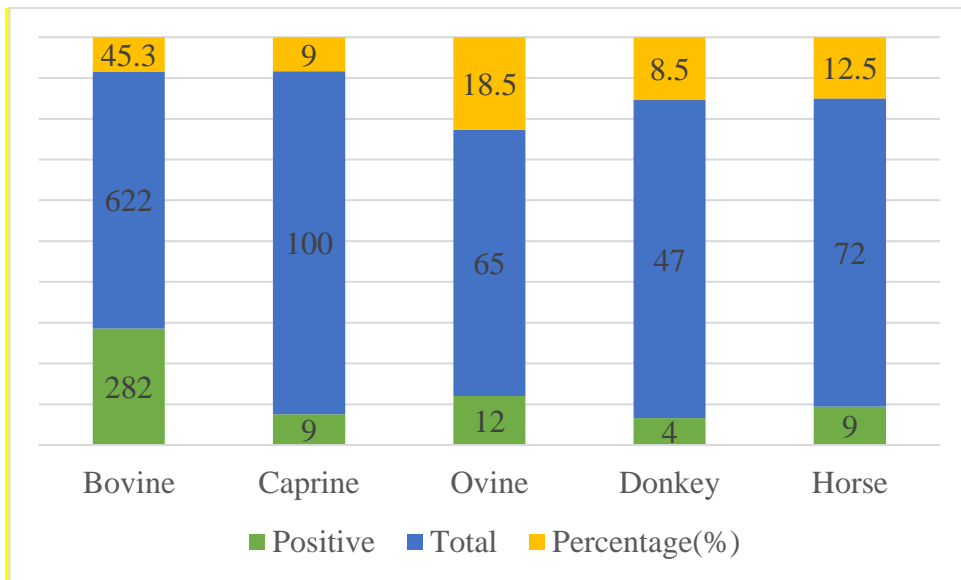


Figure 6: Percentage of tick infestation on each study animal species in Anilemo district

Out of the total of 906 animals examined 316 (34.9 %) of them were infested with one or more ticks. From all infested ruminants and equines an overall of 3367 total ticks comprising 3159 adults (926 males and 2233 females) and 208 immature stages (42 larvae and 166 nymphs) of ixodid ticks were collected during the study period in the districts. Morphological identification of these collected ticks demonstrated that the subgenus *Rhipicephalus (Boophilus)* (70.8%) was the most predominant genus in Anilemo district followed by the genus *Amblyomma* (27.4%) and genus *Rhipicephalus* (1.8% of 3367) (Table 3). Further morphological identification of the collected ticks to the species level revealed the presence of *Rhipicephalus (Boophilus) decoloratus* (70.8%),

Amblyomma variegatum (24.6%), *Rhipicephalus (Boophilus) nymph* (4.9%), *Amblyomma cohaerens* (3%), *Amblyomma nymph* (2.7%), *Rhipicephalus evertsi* (1%), *Amblyomma larvae* (0.8%), *Rhipicephalus pulchellus* (0.7%), *Rhipicephalus (Boophilus) larvae* (0.5%) and *Rhipicephalus praetextatus* (0.1%) (Table 3).

Table 3: Overall count, percentage and relative abundance of tick species identified during the study period in Anilemo district

Species	Sex		Immature tick		Total count (%)
	Male	Female	Nymph	Larvae	
<i>Amblyomma variegatum</i>	594	118	86	26	824/24.6
<i>Rhipicephalus (Boophilus) decoloratus</i>	228	2065	74	16	2383/70.8
<i>Rhipicephalus evertsi</i>	26	8	0	0	34/1.0
<i>Rhipicephalus praetextatus</i>	2	0	0	0	2/0.1
<i>Rhipicephalus pulchellus</i>	12	12	0	0	24/0.7
<i>Amblyomma cohaerens</i>	64	30	6	0	100/3
Total	926/27.5	2233/66.3	166/4.9	42/1.2	3367

Out of the total of 3367 ticks obtained, 91.3% from cattle, 4.1% from goats, 2.7% from sheep, 0.3% from donkeys and the rest 1.7% from horses were collected. Out of the total of 924 *Amblyomma* ticks collected 880 from cattle, 8 from sheep, 6 from goats, 28 from horses and 2 from donkeys were collected. Similarly, out of the total of 2383 subgenus *Rhipicephalus (Boophilus)* ticks collected during the study period 2157 from cattle, 130 goats, 76 from sheep, 12 from horses and 8 from donkeys were collected (Table 4).

Table 4: Total count and percentage of tick species collected from each species of domestic animals during the study period

Animal species	Tick spp						Total count	% on each animal spp
	Am.var	Rh.(Bo.)dec	Rh.ev	Rh.p	Am.coh	Rh.pr		
Bovine(622)	780	2157	22	20	94	0	3073	91.3
Caprine(100)	6	130	2	0	0	0	138	4.1
Donkey(47)	2	8	0	0	0	0	10	0.3
Horse (72)	28	12	10	4	0	2	56	1.7
Ovine (9)	8	76	0	0	6	0	90	2.7
Total	824	2383	34	24	100	2	3367	
% of each tick spp	24.6	70.8	1	0.7	3	0.1		

In (figure 7) different tick species that was identified in the study area that includes adults and immature are mentioned.

Rh. (Bo.) decoloratus male



Rh. (Bo.) decoloratus female

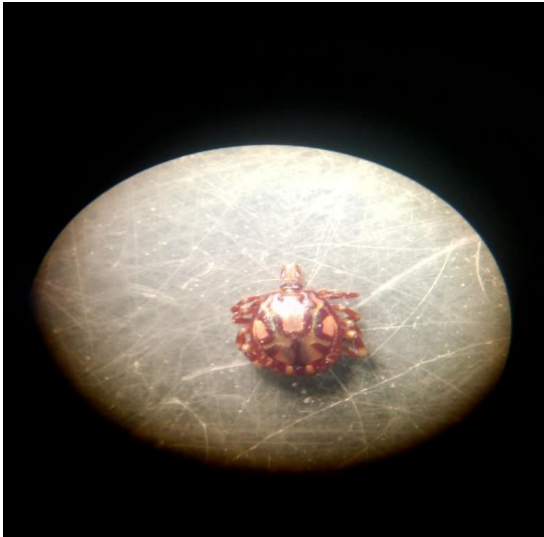




Amblyomma variegatum male



Amblyomma variegatum female



Amblyomma cohaerens male



Amblyomma cohaerens female



Rh. (Bo.) decoloratus larvae

Rh. (Bo.) decoloratus nymph



Amblyomma variegatum larvae

Amblyomma variegatum nymph



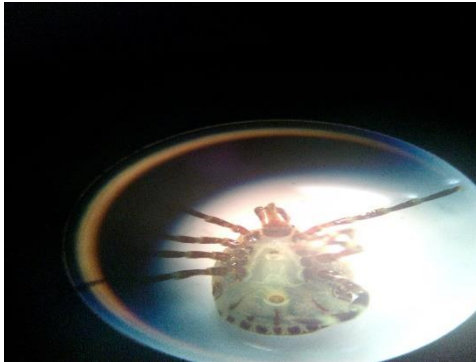
Rhipicephalus evertsi 3 males at top left, one female at top right, *Amblyomma variegatum* one female at bottom left and *Rhipicephalus (Boophilus) decoloratus* one female at bottom right in the first box at the same field, respectively.



Rhipicephalus pulchellus male



Rhipicephalus pulchellus female



Ventral view of *Amblyomma variegatum* male (at left), and Male and female of *Rhipicephalus (Boophilus) decoloratus* at mating position at the middle and *Rhipicephalus praetextatus* male, left.

Figure 7: Photographs of hard tick species collected from domestic animals in Anilemo district (Taken by Mulugeta Melese from September 2018 to May 2019).

4.1.2. Risk factors associated with major tick infestation

The current study showed that the risk of infestation of cattle with *Am. variegatum* was significantly higher by 0.09 than in goats, by 0.06 than in donkeys, by 0.44 than in horses and by 0.12 than in sheep of the study district (P=0.000). The study also indicated the presence of significantly higher infestation of *Am. Variiegatum* by 1.57 and 4.68 times in animals with medium and poor body condition score, respectively than those animals in good body condition animals. Likewise, the infestation of *Am. variegatum* was significantly 1.39 times higher in red colour animals than in black animals whereas the infestation of brown, White red and White study animals was higher 1.11, 1.29 and 1.12 times than the infestation in black colour animals, respectively. *Am. variegatum* infestation in exotic breed animals was 3.13 times higher than cross breed animals (P=0.000) whereas the infestation on local breeds animals was 5.75 times higher than cross breed animals (P=0.02). *Am. variegatum* infestation in male animals was 2.02 times higher than the infestation in female animals. The infestation of animals by *Am. variegatum* in the lowland and midland agroecologies were 28.13 and 1.90 times higher than those animals found in the highland agroecology, respectively. *Am. variegatum* infestation was significantly higher during the rainy season (OR=24.83, P=0.000) than the dry season (Table 5).

Table 5: Association of different risk factors with *Amblyomma variegatum* infestation by using Negative binomial regression analysis

<i>Am. variegatum</i>	Factor	Coef.	OR	95%Conf.interval	P-value	
Animal spp.	Bovine				*	
	Caprine	-2.43	0.09	0.04	0.19	0.000
	Donkey	-2.84	0.06	0.01	0.23	0.000
	Horse	-0.83	0.44	0.29	0.64	0.000
	Ovine	-2.17	0.11	0.06	0.23	0.000
BCS	Good				*	
	Medium	0.45	1.57	1.26	1.95	0.000
	Poor	1.54	4.68	3.89	5.64	0.000
Colour	Black				*	
	Brown	0.10	1.11	0.79	1.55	0.56
	Red	0.33	1.39	1.16	1.67	0.000
	White red	0.25	1.29	0.98	1.69	0.070
	White	0.11	1.12	0.89	1.40	0.317
Breed	Cross				*	
	Exotic	1.14	3.13	1.59	6.11	0.001
	Local	1.75	5.75	3.09	3.94	0.020
Sex	Female				*	
	Male	0.70	2.02	1.73	2.34	0.000
Age	Adult				*	
	Young	0.27	1.31	1.12	1.54	0.001
Agroecology	Highland				*	
	Lowland	3.34	28.13	10.08	78.26	0.000
	Midland	0.64	1.90	1.46	2.48	0.000
Season	Dry				*	
	End rainy	3.21	24.83	9.29	66.02	0.000

*= Reference category, coef= Coefficient, OR=odd ratio

The study also revealed that the infestation of cattle by *Rh. (Bo.) decoloratus* was significantly higher than the infestation in goats, donkeys, horses and sheep with odds of (0.81, 0.09, 0.07, 2.58, respectively). The study also indicated that the infestation by *Rh. (Bo.) decoloratus* was 2.68 (P=0.000) and 5.49 (P=0.000) times higher in animals with medium and poor body condition score, respectively than those animals in good body condition score. The infestation of by brown and white red colour animals by *Rh. (Bo.) decoloratus* was (OR=1.31) and 1.46 time shigher, respectively than infestation in black colour animals (P=0.005). *Rh. (Bo.) decoloratus* tick infestation on local and exotic breed animals was 1.12 and 1.21 times higher respectively, than the infestation in cross breed cattle. Male animals infestation by *Rh. (Bo.) decoloratus* ticks (P=0.000) was 1.18 times higher than the infestation in females animals. The infestation of animals by *Rh. (Bo.) decoloratus* was 3.41 times in lowland and 4.19 times midland agroecology than the infestation of animals' highland agroecology. Similarly, infestation of animals by *Rh. (Bo.) decoloratus* was 1.78 times higher during the end of rainy than the infestation during the dry season of the study period (OR=1.78, P=0.000) (Table 6).

Table 6: Association of different risk factors with *Rh. (Bo.) decoloratus* infestation of the study animals by using Negative binomial regression analysis

<i>Rh. (Bo.)dec</i>	Factor	Coef.	OR	95%Conf.interval	P-value	
Animal spp.	Bovine				*	
	Caprine	-0.21	0.81	0.68	1.03	0.025
	Donkey	-2.35	0.09	0.05	0.19	0.000
	Horse	-2.59	0.07	0.04	0.13	0.000
	Ovine	-0.95	0.37	0.31	0.49	0.000
BCS	Good				*	
	Medium	0.98	2.68	2.36	3.03	0.000
	Poor	1.70	5.49	4.90	6.17	0.000
Colour	Black				*	
	Brown	0.27	1.31	1.08	1.58	0.005
	Red	0.04	1.04	0.87	1.06	0.456
	White red	0.38	1.46	1.27	1.67	0.000
	White	0.06	1.06	0.93	1.19	0.383
Breed	Cross				*	
	Exotic	0.19	1.21	1.08	1.58	0.159
	Local	0.11	1.12	1.13	1.40	0.360
Sex	Female				*	
	Male	0.17	1.18	1.08	1.28	0.000
Age	Adult				*	
	Young	0.46	1.58	1.77	1.40	0.000
Agroecology	Highland				*	
	Lowland	1.23	3.41	2.64	4.39	0.000
	Midland	1.43	4.19	3.42	5.10	0.000
Season	Dry				*	
	End rainy	0.58	1.78	1.52	2.06	0.000

*=Reference category, Coef=coefficient, OR=odd ratio

Results of the study showed that there was no significant difference in the infestation of *Amblyomma cohaerens* among different species of animals ($P>0.05$). Animal with poor BCS were significantly higher level of (OR=1.71, $P=0.000$) infestation with *Amblyomma cohaerens* than those animals with both medium and good BCS. Black ($P=0.003$) colour animals had higher infestation with *Amblyomma cohaerens* than white color animals. Breed of the study animals had never affected the infestation with *Amblyomma cohaerens*. Infestation with *Amblyomma cohaerens* was significantly ($P=0.034$) higher at the end of rainy than during the dry season (Table 7).

Table 7: Association of different risk factors with *Amblyomma cohaerens* infestation by using Negative binomial regression analysis.

<i>Am. cohaere</i>	Factor	Coef.	OR	95%Conf. interval		P-value
Animal spp.	Bovine					*
	Caprine	-16.07	1.05	0.00	∞	0.988
	Donkey	-16.69	5.61	0.00	∞	0.992
	Horse	-16.47	7.01	0.00	∞	0.991
	Ovine	-0.45	0.64	0.28	1.48	0.294
BCS	Good					*
	Medium	0.54	1.71	0.76	3.89	0.196
	Poor	2.38	1.71	5.81	19.89	0.000
Colour	Black					*
	Brown	-16.81	5.02	0.00	∞	0.991
	Red	0.02	1.02	0.64	1.62	0.936
	White red	0.00	1.00	0.52	1.93	0.994
	White	-1.62	0.19	0.07	0.57	0.003
Breed	Cross					*
	Exotic	-0.09	0.91	0.00	∞	1.000
	Local	16.57	2102	0.00	∞	0.994
Sex	Female					*
	Male	-0.22	0.80	0.50	1.28	0.363
Age	Adult					*
	Young	-1.65	0.19	0.08	0.44	0.000
Agroecology	Highland					*
	Low land	15.23	799.45	0.00	∞	0.985
	Mid land	13.94	799.45	0.00	∞	0.986
Season	Dry					*
	End rainy	-1.15	0.34	0.11	0.92	0.034

*=Reference category, Coef=coefficient, OR = Odd ratio

4.1.3. Sex ratio

Higher number of males than females of both *Amblyomma* and *Rhipicephalus* genera was observed in all the study sites during the study period. However, on the contrary to this, higher number of females than males was recorded in the subgenus *Rh. (Bo.) decoloratus* in all the study sites during the study period. The overall male to female ratio was 0.41:1. The male to female ratio of *Am. variegatum*, *Rh. evertsi*, *Am. cohaerens*, *Rh. praetextatus*, *Rh. pulchellus* provided in the following table (Table 8).

Table 8: Overall male/female sex ratio of ticks collected during the study period in Anilemo district

Tick species	Sex ratio			
	M	F	Total count	Over all M/F ratio
<i>Amblyomma variegatum</i>	594	118	712	5.03:1
<i>Rhipicephalus (Boophilus) decoloratus</i>	228	2065	2293	0.11:1
<i>Rhipicephalus evertsi</i>	26	8	34	3.25:1
<i>Rhipicephalus praetextatus</i>	2	0	2	2:00
<i>Rhipicephalus pulchellus</i>	12	12	24	1:01
<i>Amblyomma cohaerens</i>	64	30	94	2.13:1
Total	926	2233	3159	

4.2. Questionnaire Survey

The livestock owners responded that ticks and tick-borne diseases were harmful to their animals; however they did not have any knowledge about the exact role of ticks and tick-borne diseases in causing ill health to their animals. Both sexes were infested, though people paid consideration to females due to teat damage, which affects milk production. Among 34 farmers and 16 professionals (two veterinarian and 14 animal health assistant) all of them knows the tick and its problem in their locality. The most favored habitats by tick according to 94% of the responded farmers and 99% of the professionals is grazing areas whereas the rest lands with grasses. Among all the interviewed

livestock keepers, none of them knows the type of tick and their rank in terms of their negative effects in their locality; however 99% of the professionals know the type of tick as hard ticks and its impact rank estimated as fourth following lumpy skin diseases, endoparasite and black leg in the area. Among the 16 professionals interviewed only 15% of them knows tick borne diseases (TBDs) of man, but 94% of the professionals knows some TBDs about animals. Species of animal mostly infested with ticks are cattle according to 100% of the respondents, whereas breeds of animal comparatively more susceptible to the tick infestation was exotic breed according to all the professional responded. According to 90% and 10% of the professionals responded adult and younger age groups of animal are most affected by ticks, respectively. According to all professionals and farmers responded there was no color preference for tick infestation. According to 99% of the professionals and 35% of the farmers responded tick infest and attach on man or people, in fact none of them have been bitten by ticks (Table 9)

Table 9: Summary of the outcome of the questionnaire on the knowledge and awareness about ticks

Responses of informants/category	Answer/category	frequency		Percentage (%)	
		Animal owners	professional owners	Animal owners	professional owners
Know ticks	yes	34	16	100	100
	no				
Ticks make any health problems	yes	34	16	100	100
	no				
Environment more favored by ticks	grazing areas	32	15	94	99
	lands with	2	1	6	1
	grasses				
Type of ticks you know	hard tick		15		99
	soft tick		1		1
	don't know	34		100	
Common season of tick infestation	end of rainy	6		18	
	beginning of	25	16	74	100
	rainy				
	mid of rainy	3		8	

Rank of ticks in terms of negative impact	third		1		1
	fourth		15		99
Know any tick-borne diseases of animals	yes		15		94
	no		1		6
Know any tick-borne diseases of man	yes		2		15
	no		14		85
Know any tick control method	yes	34	16	100	100
	no				
Livestock mostly infested by ticks	cattle	34	16	100	100
Breed of animal more affected by ticks	exotic	3	16	10	100
	cross	10		30	
	local	21		60	
Age group of animals mostly affected	adult		14		90
	young		2		10
Sex of animal more affected	male	3		7	
	female	31		93	
	equal		16		100
Colour of animals more susceptible	mixed	34	16	100	100
	red				
	white				
Infest and attach on man or people	yes	12	15	35	99
	no	22	1	65	1
Bitten by ticks	yes				
	no	34	16	100	100

Results of the questionnaire survey showed that all the professionals treated tick infested animals by modern/chemical treatment on the other hand 98% of the farmers use both modern and traditional (ethno-veterinary) treatment while 2% of them used only modern treatment. Both animal owners and professionals informed that animals were treated only when infested with ticks. Government and private clinics were described as the common sources of acaricides. Majority of animal owners (88%) informed that government veterinarians prescribed and administered acaricides and the rest

8% by private veterinarian and 4% by animal owners themselves. All professionals informed they followed manufacturers' instruction to determine the concentration of the acaricide used. Majority of the professionals (94%) indicated that organophosphates and macrocyclic lactones were the most commonly used acaricides while 22% of them believe that there was history of tick resistance while 78% of the professionals think that there was no tick resistance history. Poisoning and skin damage post treatment in animals due to acaricides was known by 68% of the professionals while only 4% of the farmers know this situation. All the farmers indicated that they applied acaricides whenever ticks were observed on the body of their animals. Majority (57%) of the professionals informed that they noticed ticks remaining post-treatment with Diaznone while 37% of them informed ticks remained after treatment with ivermectine. The details for other results of the questionnaire on tick control practices in Anilemo district is provided on table 10.

Table 10: Outcome of the questionnaire on tick control practices in Anilemo district

Responses of informants/category	Answer/category	frequency		Percentage (%)	
		Animal owners	professional	Animal owners	professional
Treat tick infested animals	chemical	1	16	2	100
	herbal				
	both	33		98	
Treat only the infested animals	yes	34	16	100	100
	no				
Preferred sources of acaricides	government clinics	34		100	
	private clinics				
	illegal sellers				
More preferred acaricides	Diaznone	34		100	
	ivermectine				
	both		16		100
Causes of variation for preferences	effectiveness	20	16		100
	cost	10			

	accessibility	4			
Prescribe and administer acaricides	government vets	30			88
	private vets	3			8
	owner	1			4
Method of application	spraying	4			12
	pouring on	30			88
Application equipment	knap sack sprayer				
	hand sprayer	4			12
	scrubbing cloth	30			88
Method of restraints	crush	4	16		12
	rope	30			88
Water for acaricide mixing	clean natural	33	15		97
	tap	1	1		3
Supervises mixing and application	owner	4			12
	animal health assistant	30			88
Class of acaricide	organophosphate				
	macrocyclic				
	lactone				
	both		15		94
	don't know		1		6
Measures taken to avert tick resistance	Not sure and no record				
	Change acaricide		13		81
	Increase the frequency		3		19
The coping strategies effective	yes		15		99
	somehow		1		1
Time spent with previous before change to current acaricide	Less than 5 months		2		11
	1-2 years		14		89

Mixing two or more acaricides together	Not applicable		16		100
Mixing solve acaricide failure	Not sure		16		100
Distance neighboring farm	with no gap	34	16	100	100
Interaction of animals with that of neighbors	daily	22		65	
	weekly	12		35	
Knowledge of acaricide use by neighbors	yes	33		99	
	no	1		1	
knowledge neighbors sprays	yes	34		100	
	no				
New animals introduced in the farm	yes	34		100	
	no				
Quarantine animal sprayed before mixing	yes	34		100	
	no				
Location where acaricide is stored	Designated store		16		100
	Residential house	34		100	
Animals with damaged skin due to acaricide	yes	32	11	96	68
	no	2	5	4	32
Most affected by negative effects	cattle	34	16	100	100
Adverse effects of acaricide on man	yes	34	16	100	100
	no				
Type of adverse effect on man	Eye and skin	29	3	85	19
	Itching				
	Eye itching	5	13	15	81
Equipment used for acaricide measurement	Non calibrated bottle top	33		99	
	calibrated bottle top	1		1	

	syringe		16		100
Apply acaricide during	weekly				
dry season	1 to 3 times	34	11	100	69
	twice a week				
	fortnight		5		31
Apply acaricide during	weekly				
rainy season	monthly				
	twice a week				
	fortnight	34	16	100	100

Majority (97.1%) of the professional respondents confirmed the presence of ticks on their animals after application of acaricides (Table 11). Most farmers noticed more ticks on animals during spring followed by summer but less tick infestation was observed during winter and autumn seasons. Majority (87%) of the professional respondents reported one fifth of the ticks never dropped after application of acaricides. Majority (97.1%) of the professionals reported ticks remained on their animal after application of Diaznone while the rest 2.9% reported after injection of Ivermectine (Table 11).

Table 11: Outcome of the questionnaire concerning tick response to acaricides application

Responses of informants/category	Answer/category	frequency		percentage	
		owner	professional	owner	professional
Tick seen after application of acaricides	yes	33	15	97.1	97.1
	no	1	14	2.9	2.9
Time it take before drop	a week		16		100
	three days				
	more than a week				
Ticks remaining after application/injection	Diaznone		15		97.1
	Ivermectine		1		2.9
	others				

4.3. Invitro Efficacy of acaricides

The result of efficacy evaluation of acaricides by the method of the adult immersion test following the guidelines used by FAO revealed that ivermectine was more efficacious than Diaznone in preventing laying of eggs by engorged females of both *Rh. (Bo.) decoloratus* and *Amblyomma variegatum* as compared to engorged female ticks in the control. The detail on survived ticks and overall oviposition inhibition ability of each tested acaricides is provided on the table 12.

Table 12: Oviposition response of adult *A. variegatum* and *Rh. (Bo.) decoloratus* after immersion in tested acaricide at recommended concentration.

Trial	Tick species	Acaricides	N	Eng.wgt(gm)	No of ticks laid egg	%C	%R
1	<i>Rh.(Boophilus) decoloratus</i>	Diaznone	10	5	5	50	50
	<i>Rh.(Boophilus) decoloratus</i>	Diaznone	10	6	4	60	40
	<i>Rh.(Boophilus) decoloratus</i>	Diaznone	10	7	6	40	60
	<i>Rh.(Boophilus) decoloratus</i>	Control	10	6	10	0	100
2	<i>Rh.(Boophilus) decoloratus</i>	Ivermectine	10	5.1	1	90	10
	<i>Rh.(Boophilus) decoloratus</i>	Ivermectine	10	5.25	1	90	10
	<i>Rh.(Boophilus) decoloratus</i>	Ivermectine	10	6	2	80	20
	<i>Rh.(Boophilus) decoloratus</i>	Control	10	5	10	0	100
3	<i>Amblyomma variegatum</i>	Diaznone	10	8.23	5	50	50
	<i>Amblyomma variegatum</i>	Ivermectine	10	8	0	100	0
	<i>Amblyomma variegatum</i>	Control	10	8	10	0	100

NB: N= number of ticks immersed, Eng. Wgt (g) =Average Engorgement weight in gram, %R= Percent of resistance.

The average number of tick that laid egg in each treatment group indicated in the box plot in (figure 8) below (with χ^2 -test at P-value=1).

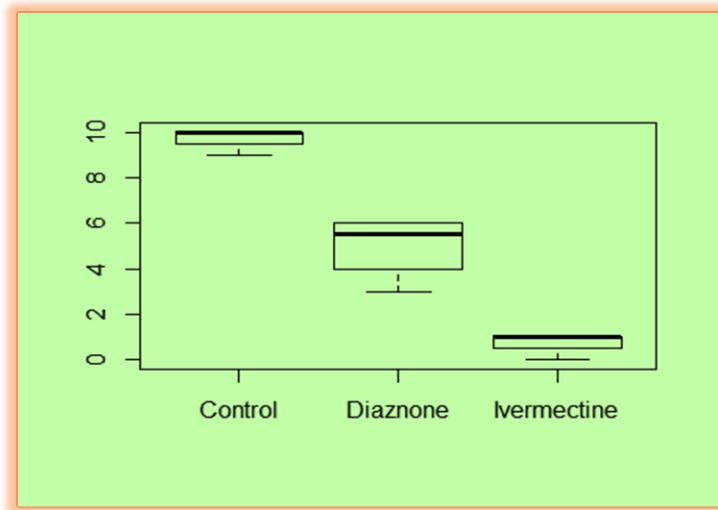


Figure 8: Average number of ticks laid egg in Ivermectine, Diaznone and control group

5. DISCUSSION

5.1. Tick Infestation and Associated Risk Factors

The present study provided additional information regarding the diversity of ticks affecting both ruminants and equines in Anilemo district of Hadiya zone in southern Ethiopia from where previously very scanty information is available. However, several previous studies documented this type of information only from either of these two host species. The results of the current study showed that *Amblyomma variegatum*, *Rh.(Boophilus) decoloratus* and *Am. cohaerens* were the most commonly encountered and the predominant tick species in the study district across all the agro-ecological zones and during both the end of rainy and dry seasons. On the other hand, very few collections of *Rh. evertsi*, *R. praetextatus* and *R. pulchellus* were made only in very few numbers

The observation of *Rh.(Boophilus) decoloratus* as the most abundant tick species (70.8%) in the present study is in agreement with previous several studies from Ethiopia (Ayalew *et al.*, 2014; Aboma *et al.*, 2017) and bordering countries like Kenya as well (Moumouni *et al.*, 2015). In this study *Rh.(Boophilus) decoloratus* was collected from all the animal species found in all the three agro-ecological of the district. *Rh.(Boophilus) decoloratus* is a potential vector responsible for the transmission of *Anaplasma marginale* and *Babesia bigemina* in Ethiopia (Kumsa *et al.*, 2012).

The findings of *Amblyomma variegatum* as the second most abundant tick species (24.5%) in the current study is in line with several previous studies including (Ayalew *et al.*, 2014) who reported 30.3% in wet season, which also infested all animal species in all the agro-ecologies. *Amblyomma variegatum* is a potential vector responsible for the transmission of the causative agent of cowdriosis, dermatophilosis and Nairobi sheep disease in Ethiopia (Bayisa *et al.*, 2012). Furthermore, it can cause serious damage to skin and hides because of its long mouthparts. Notably, these ticks preferentially feed on the udder and teats of cattle (Fanos *et al.*, 2012) and may cause serious problems in the suckling of calves due to blindness of teats of cows.

The observation of significantly higher infestation of *Rh.(Boophilus) decoloratus* and *Amblyomma variegatum* infestation in cattle than all the other animal species in the present study concurs with previous studies from Ethiopia (Kumsa *et al.*, 2015b; Beyecha *et al.*, 2014; Mohammed and

Admasu, 2015) and Pakistan (Rehman *et al.*, 2017). This was most probably linked with the grazing nature of cattle in environments most suitable for the survival and developments of ticks as has been argued before (Sajid *et al.*, 2008), and also the browsing nature of goats protect from tick in which plant leaf is not suitable for tick survival. And also in equines the thicker skin due to the compact fibrous component within the deeper parts of the dermis of the back and hind quarters is less suitable for tick attachment than in cattle and other ruminants (Taylor *et al.*, 2002). Host genetics is also implicated to play an important role (Jonsson *et al.*, 2014). Limited information is available about tick infestation in small ruminants and equine in Ethiopia, as only a few studies have focused on these animals.

The observation of highest mean total tick count during the end of the rainy season than the dry season in the present study is in agreement with the observations of (Solomon and Kaaya, 1998). The findings of lower infestation of *Rh.(Bo) decoloratus* and *Amblyomma variegatum* in highland agroecology than in both the lowland and midland agro-ecologies in the present is in agreement with previous reports from Ethiopia (Ayalew *et al.*, 2014). This was most probably attributed to the fact that the highland area is located at a higher elevation and hence receives lower annual mean temperatures (minimum and maximum) as compared to the lowland and midland areas which is one of the important factors that determines tick abundance, load and distribution of an area.

The observation of higher tick burdens ($P=0.000$) on male animals than on females is inconsistent with previous studies carried out on ruminants (Sajid *et al.*, 2008). This might be due to the fact that in the study area in Anilemo district female animals are mainly used for milk production and breeding purposes throughout the year, and therefore, they receive more attention, like frequent grooming including the manual removal of ticks, which would result in low tick burdens. A significantly ($P=0.000$) higher tick burden in young as compared to adult animals agrees with previous studies (Swai *et al.*, 2005; Lorusso *et al.*, 2013). The lower tick burdens recorded in adult could be due to a combination of factors, including the frequent grooming of adult, especially head, ears and neck regions, as compared to young. Furthermore, adult animals seem to be more capable of protecting themselves from ticks by innate and cell mediated immunity (Okello-Onen *et al.*, 1999). The observation of higher tick infestation ($p < 0.05$) in animals with poor body condition than those animals with medium and good body condition score in the present study most probably reflects the fact that poorly conditioned animals had low resistant to tick infestation and lack enough

body capacity to build resistance whereas animals with good and medium body condition showed reasonable combat to the infestation according to (Manan *et al.*, 2007). On the way, tick infestation might be a cause for poor body condition instead of vice versa.

Even though tick has no host color preference for infestation due to the fact that ticks locate and infest their blood host targets from the chemical cues including volatile organic compound (VOC) such as carbon dioxide, ammonia, heat and others produced by host skin microbes after they metabolize skin gland secretions (Verhulst and Takken, 2015). But some unpublished source and cultural believe implicated that animals with black colour are less infested than animals with other colors. To obtain information if color of study animals may affect tick infestation it was taken as risk factor. Findings of the present study revealed that red animals were significantly higher infestation by *Amblyomma variegatum* than the black animals ($P < 0.05$) whereas white red and brown animals had significantly higher infestation by *Rh.(Boophilus) decoloratus* than ($P = 0.000$) black colour animals. This might be due to large number of animal population with red, white red, brown and white color than black rather than host preference of the tick species.

The observation of higher *Rh.(Boophilus) decoloratus* infestation in exotic breeds (1.21 times higher than (OR=1.21)) local breed is in line with previous reports from Ethiopia (Regassa, 2001). Higher tick infestation in exotic breeds as compared to indigenous cattle has also been reported in other countries like Pakistan (Rehman *et al.*, 2017). Resistance to one-host ticks, e.g. *Rh. microplus* is related to the proportion of zebu genes in the breed (Jonsson *et al.*, 2014). In *Bos indicus* and their crosses, the resistance against different tick species has been found a highly heritable trait.

5.2. Sex Ratio of Ticks

The findings of male dominance in the genera *Amblyomma* and *Rhipicephalus*, however, on the opposite the female dominance in the subgenus *Rhipicephalus (Boophilus) decoloratus* ticks recorded in the present is in line with the previous reports from 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; (Beyechea *et al.*, 2014). This is most probably attributed to the fact that fully engorged female ticks of the genera *Amblyomma* and *Rhipicephalus* “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.

5.3. Invitro Acaricide Efficacy

Results of the present study attest the fact that ivermectine showed higher in vitro efficacy than Diaznone against both *Amblyomma variegatum* and *R.(Bo.) decoloratus* and produced maximum efficacy (100%) in ivermectine and minimum efficacy (40%) in Diaznone only at their recommended dose. This suggests that, ivermectine is the more preferable than Diaznone for treatment of *R. decoloratus* ticks. This finding agrees with the previous finding of Sajid *et al.*, (2009) and Mekonnen (2002). However, it disagrees with the finding of El-Bahy *et al* (2015) who reported 100% efficacy of Diaznone on *Boophilus annulatus* in Southeast Brazil. Likewise, ivermectine showed greater efficacy against *Amblyomma variegatum* than Diaznone at the recommended dose after 7 days post exposure. This finding is consistent with the earlier report of Turkson and Botchey (1999) in Ghana who reported that field strain of *Amblyomma variegatum* is resistant to organophosphates like Diaznone. Overall the present study demonstrated the fact that ivermectine had better efficacy than Diaznone against both *Amblyomma variegatum* and *Rhipicephalus (Boophilus) decoloratus*. The result also revealed that *Rhipicephalus (Boophilus) decoloratus* was resistant to both acaricides than *Amblyomma variegatum* ticks. The differences in the efficacy of the two evaluated acaricides (Ivermectine and Diaznone) were most likely attributed to one of the wide spread frequency, irregular application, inadequate spraying, and improper mixing of acaricides and on the use of acaricides stored for a long time after dilution.

5.4. Questionnaire Survey

Findings of the questionnaire survey on tick control practice of the present study revealed that the entire respondents have good knowledge on the economic importance of ticks. This finding corroborates with previous works (Ayalew *et al.*, 2014; Jonsson *et al.*, 2014).The observation of ticks more on cattle than other species of animals and findings of tick infestation throughout the

year, especially at the beginning of rainy season is in agreement with the previous findings reported from Jimma Zone (Abebaw, 2004) and in southern Ethiopia (Tamerat *et al.*, 2015). The observation that the majority of the respondents in the area do not have sufficient knowledge about tick-borne diseases might show the fact that indigenous breeds are relatively resistant to tick-borne diseases (Regassa, 2001;Jonsson *et al.*, 2014) and thus the knowledge about the diseases transmitted by ticks to animals is very poor among livestock owners of the present study district..

6. CONCLUSION AND RECOMMENDATIONS

This study showed that *Rh. (Bo.) decoloratus* and *Am. variegatum* are the most predominant and dominant tick species in the district and cattle is the animal species responsible in harboring most of the tick collections. Diaznone and ivermectine are implicated as the acaricides most commonly used in the district and in vitro efficacy evaluation using adult immersion test suggest that ivermectine has more efficacy against both the tested tick species. The predominance of the two species of ticks of ruminants and equines and their associated risk factors in the study districts deserves much attention at all levels to minimize the impacts on the health and productivity of domestic animals in the district to improve the living standards of farmers of the study area where there is integration of livestock and crop production takes place in which livestock are mainly used for draught power, milk and meat production and source of manure (fertilizer).

Therefore, based on above conclusion the following recommendations are forwarded

- Improving the awareness of livestock owners and the whole community about the negative impacts of ticks on lowering animal productivity and human health should be the priority.
- Develop and practice good drug use policy and responsible use of acaricides by avoidance of uncontrolled utilization, dependence on limited type of acaricides and appropriate and good application of acaricides as prescribed by manufacturers.
- Strategies involving the early detection of lowered efficacy of acaricides and the use of integrated tick control method.
- Advanced molecular studies on tick identification, tick-borne animal and human diseases, and detail studies in vitro and in vivo acaricides efficacy test in wider parts of Ethiopia is needed.

7. REFERENCES

- Abebaw, G.K., (2004): Seasonal dynamics of ticks (*Amblyomma cohaerens* and *Boophilus decoloratus*) and development of a management plan for tick and tick borne diseases control on cattle in the Jimma zone, southwestern Ethiopia. Niedersächsische Staats-und Universitätsbibliothek Göttingen.
- Aboma, H., Kebede, A. and Abdurahaman, M. (2017): Further studies on Bovine Ixodide Ticks in and around Bedelle, Southwest Ethiopia. *African J. Agric. Res.* **12**, 1922–1929.
- Abubakar, M., Perera, P.K., Iqbal, A. and Manzoor, S. (2018): Introductory Chapter: Ticks and Tick-Borne Pathogens, in: Ticks and Tick-Borne Pathogens. *IntechOpen*, pp. 1–8.
- Admasu, P., Wakayo, B.U., Megersa, M. and Feyera, T. (2015): In vitro and in vivo acaricidal efficacy study of amitraz and diazinon against some tick species infesting *Camelus dromedarius* around Jigjiga, Eastern Ethiopia. *African J. Pharm. Pharmacol.* **9**, 850–855.
- Alemu, G., Chanie, M., Mengesha, D. and Bogale, B. (2014): Prevalence of ixodid ticks on cattle in Northwest Ethiopia. *Acta Parasitol. Glob* **5**, 139–145.
- Arsham, H., (2005): Questionnaire design and surveys sampling. Retrieved January 14, 2008.
- Ashenafi, H., Tolossa, Y.H. and Yebegaeshet, M. (2013): Impact of sheep and goats ectoparasites on the tanning industry in Tigray Region, Ethiopia. *Ethiop. Vet. J.* **17**, 63–76.
- AWARDO, (2014): Annual Progressive Report of Annilemo Woreda Agriculture and rural development office.
- Ayalew, T., Hailu, Y. and Kumsa, B. (2014): Ixodid ticks infesting cattle in three agroecological zones in central Oromia: species composition, seasonal variation, and control practices. *Comp. Clin. Path.* **23**, 1103–1110.
- Ayana, D., Eshetu, E., Waketole, H. and Abunna, F. (2013): In-vitro Acaricidal efficacy evaluation trial of Ixodid ticks at Borana, Ethiopia. *Ethiop. Vet. J.* **17**, 85–99.
- Ballweber, L.R., (2006): Diagnostic methods for parasitic infections in livestock. *Vet. Clin. Food Anim. Pract.* **22**, 695–705.
- Barandika, J.F., Olmeda, S.A., Casado-Nistal, M.A., Hurtado, A., Juste, R.A., Valcárcel, F., Anda, P. and García-Pérez, A.L. (2014): Differences in questing tick species distribution between Atlantic and continental climate regions in Spain. *J. Med. Entomol.* **48**, 13–19.

- Barker, S.C. and Murrell, A.(2004): Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology***129**, S15–S36.
- Basu, A.K. and Charles, R.(2017): Ticks of Trinidad and Tobago-an Overview. Academic Press.
- Bayisa, D., Berhanu, A., Fentahun, T. and Chanie, M.(2012): Occurrence of Bovine Dermatophilosis in Ambo town, West Shoa Administrative Zone, Ethiopia. *Am. J. Sci. Res.* **7**, 172–175.
- Beyechea, K., Kumsa, B. and Beyene, D.(2014): Ectoparasites of goats in three agroecologies in central Oromia, Ethiopia. *Comp. Clin. Path.* **23**, 21–28.
- Blagburn, B.L. and Dryden, M.W.(2009): Biology, treatment, and control of flea and tick infestations. *Vet. Clin. Small Anim. Pract.* **39**, 1173–1200.
- Bonnet, S., Liu, X., (2012): Laboratory artificial infection of hard ticks: a tool for the analysis of tick-borne pathogen transmission. *Acarologia* **52**, 453–464.
- Bowman, A.S. and Nuttall, P.A., (2008): Ticks: biology, disease and control. Cambridge University Press.
- Brites-Neto, J., Duarte, K.M.R. and Martins, T.F., (2015): Tick-borne infections in human and animal population worldwide. *Vet. world***8**, 301.
- Brito, L.G., Barbieri, F.S., Rocha, R.B., Oliveira, M. and Ribeiro, E.S.(2011): Evaluation of the efficacy of acaricides used to control the cattle tick, *Rhipicephalus microplus*, in dairy herds raised in the Brazilian southwestern amazon. *Vet. Med. Int.* 2011.
- Bureau, J.-C. and Swinnen, J.(2018): EU policies and global food security. *Glob. Food Sec.* **16**, 106–115.
- Chae, J.-B., Kang, J.-G., Kim, H.-C., Chong, S.-T., Lee, I.-Y., Shin, N.-S. and Chae, J.-S.(2017): Identification of Tick Species Collected from Wild Boars and Habitats of Wild Boars and Domestic Pigs in the Republic of Korea. *Korean J. Parasitol.* **55**, 185.
- Chali, E., Melaku, M. and Negero, J. (2017): Study on cattle tick species frequency distribution in and around Jimma province of Ethiopia. *J. Parasitol. Vector Biol.* **9**, 34–38.
- Daniels, T.J., Falco, R.C. and Fish, D.(2000): Estimating population size and drag sampling efficiency for the blacklegged tick (Acari: Ixodidae). *J. Med. Entomol.***37**, 357–363.
- Dantas-Torres, F.(2008): The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806)(Acari: Ixodidae): from taxonomy to control. *Vet. Parasitol.* **152**, 173–185.
- Daubney, R. (1930): Natural transmission of heart-water of sheep by *Amblyomma variegatum* (Fabricius 1794). *Parasitology***22**, 260–267.

- De Meneghi, D., Stachurski, F. and Adakal, H.(2016): Experiences in tick control by acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. *Front. public Heal.* **4**, 239.
- Drummond, R.O. et al, Ernst, S.E., Trevino, J.L., Gladney, W.J. and Graham, O.H., (1973): *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. *J. Econ. Entomol.* **66**, 130–133.
- El-Bahy, N.M., Bazh, E.K. and Shaheen, H.M.(2015): Efficacy of deltamethrin, diazinon, and ivermectin on *Boophilus annulatus* ticks (in vitro and in vivo study). *Parasitol. Res.* **114**, 29–36.
- Estrada-Peña, A., Bouattour, A., Camicas, J.L. and Walker, A.R.(2004): Ticks of domestic animals in the Mediterranean region.
- Estrada-Peña, A., Gray, J.S., Kahl, O., Lane, R.S. and Nijhoff, A.M.(2013): Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats. *Front. Cell. Infect. Microbiol.* **3**, 29.
- Estrada-Peña, A., Mangold, A.J., Nava, S., Venzal, J.M., Labruna, M.B. and Guglielmo, A.A.(2010): A review of the systematics of the tick family Argasidae (Ixodida). *Acarologia* **50**, 317–333.
- Fanos, T., Gezali, A., Sisay, G., Bersissa, K. and Tariku, J.(2012): Identification of tick species and their preferred site on cattle's body in and around Mizan Teferi, Southwestern Ethiopia. *J. Vet. Med. Anim. Heal.* **4**, 1–5.
- FAO, F. and A.O. of the U.(2004): Guidelines for resistance management and integrated parasite control in ruminants. Rome.
- Ferede, B., Kumsa, B., Bsrat, A. and Kalayou, S.(2010): Ticks of donkeys in central Oromia regional state, Ethiopia. *Rev. Med. Vet. (Toulouse)*. **161**, 121–126.
- Goddard, J.(2009). *Infectious diseases and arthropods*. Springer Science & Business Media.
- Hendrix, C.M. and Robinson, E.D.(2016): *Diagnostic Parasitology for Veterinary Technicians-E-Book*. Elsevier Health Sciences.
- Heylen, D., Lasters, R., Adriaensen, F., Fonville, M., Sprong, H. and Matthysen, E.(2019): Ticks and tick-borne diseases in the city: Role of landscape connectivity and green space characteristics in a metropolitan area. *Sci. Total Environ.*
- Huruma, G., Abdurhaman, M., Gebre, S. and Deresa, B.(2015): Identification of bovine tick species and their prevalence in and around Sebeta Town, Ethiopia. *J. Parasitol. Vector Biol.* **7**, 1–8.

- Jongejan-Director, F. and Uilenberg-Retired, G.(2018): Vectors: Ticks.
- Jongejan, F. and Uilenberg, G.(2004): The global importance of ticks. *Parasitology***129**, S3–S14.
- Jonsson, N.N., Piper, E.K. and Constantinoiu, C.C.(2014): Host resistance in cattle to infestation with the cattle tick *Rhipicephalus microplus*. *Parasite Immunol.* **36**, 553–559.
- Kassa, S.A. and Yalew, A.(2012): Identification of Ixodid ticks of cattle in and around Hararamaya district, Eastern Ethiopia. *Sci. J. Crop Sci* **1**, 32–38.
- Kioko, J., Baker, J., Shannon, A. and Kiffner, C.(2015): Ethnoecological knowledge of ticks and treatment of tick-borne diseases among Maasai people in Northern Tanzania. *Vet. world***8**, 755.
- Kumsa, B.(2013): Demonstration of diagnostic methods for parasitic infections/infestations of animals. Dep. Vet. Microbiol. 154.
- Kumsa, B., Laroche, M., Almeras, L., Mediannikov, O., Raoult, D. and Parola, P.(2016): Morphological, molecular and MALDI-TOF mass spectrometry identification of ixodid tick species collected in Oromia, Ethiopia. *Parasitol. Res.* **115**, 4199–4210.
- Kumsa, B., Signorini, M., Teshale, S., Tessarin, C., Duguma, R., Ayana, D., Martini, M. and Cassini, R.(2014): Molecular detection of piroplasms in ixodid ticks infesting cattle and sheep in western Oromia, Ethiopia. *Trop. Anim. Health Prod.* **46**, 27–31.
- Kumsa, B., Socolovschi, C., Almeras, L., Raoult, D. and Parola, P.(2015a): Occurrence and genotyping of *Coxiella burnetii* in ixodid ticks in Oromia, Ethiopia. *Am. J. Trop. Med. Hyg.* **93**, 1074–1081.
- Kumsa, B., Socolovschi, C., Parola, P., Rolain, J.M. and Raoult, D. (2012): Molecular detection of *Acinetobacter* species in lice and kids of domestic animals in Oromia Regional State, Ethiopia. *PLoS ONE* **7**, e52377.
- Kumsa, B., Socolovschi, C., Raoult, D. and Parola, P.(2015b): New *Borrelia* species detected in ixodid ticks in Oromia, Ethiopia. *Ticks Tick. Borne. Dis.* **6**, 401–407.
- Kumsa, B.E. and Mekonnen, S.(2011): Ixodid ticks, fleas and lice infesting dogs and cats in Hawassa, southern Ethiopia. *Onderstepoort J. Vet. Res.* **78**, 1–8.
- Labruna, M.B., Nava, S., Marcili, A., Barbieri, A.R.M., Nunes, P.H., Horta, M.C. and Venzal, J.M.(2016): A new argasid tick species (Acari: Argasidae) associated with the rock cavy, *Kerodon rupestris* Wied-Neuwied (Rodentia: Caviidae), in a semiarid region of Brazil. *Parasit. Vectors***9**, 511.
- Latif, A.A., (1984): Resistance to *Hyalomma anatolicum anatolicum* Koch (1844) and *Rhipicephalus*

- evertsi Neumann (1897)(Ixodoidea: Ixodidae) by cattle in the Sudan. *Int. J. Trop. Insect Sci.* **5**, 509–511.
- Latif, A.A. and Walker, A.R.(2004): An introduction to the biology and control of ticks in Africa. *ICTTD-2 Proj.* 1–29.
- Lehane, M.J.(2005): The biology of blood-sucking in insects. Cambridge University Press.
- Lora, R.B.(2001): Veterinary Parasitology: The Practical Veterinaria, Arthropods. Butterworth-Heinemann, a Member of the Reed Elsevier Group. Library of Congress Cataloging, United State of America.
- Lorusso, V., Picozzi, K., de Bronsvort, B.M.C., Majekodunmi, A., Dongkum, C., Balak, G., Igweh, A. and Welburn, S.C.(2013): Ixodid ticks of traditionally managed cattle in central Nigeria: where *Rhipicephalus* (*Boophilus*) *microplus* does not dare (yet?). *Parasit. Vectors* **6**, 171.
- Madder, M., Horak, I. and Stoltz, H.(2014): Tick identification. *Fac. Vet. Sci. Univ. Pretoria* 58.
- Maharana, B.R., Baithalu, R.K., Allaie, I.M. and Samal, L.(2011): Mechanism of Immunity to Tick infestation in Livestock. *Vet. World* **4**, 131.
- Manan, A., Khan, Z. and Ahmad, B.(2007): Abdullah, Prevalence and Identification of Ixodid. Ministry of Economic Development and Cooperation Survey of Livestock and Fisheries Development.
- Mans, B.J., Gothe, R. and Neitz, A.W.H.(2004): Biochemical perspectives on paralysis and other forms of toxicoses caused by ticks. *Parasitology* **129**, S95–S111.
- Marufu, M.C.(2008): Prevalence of ticks and tick-borne diseases in cattle on communal rangelands in the highland areas of the Eastern Cape province, South Africa.
- Mathison, B.A. and Pritt, B.S.(2014): Laboratory identification of arthropod ectoparasites. *Clin. Microbiol. Rev.* **27**, 48–67.
- Mccoy, K.D., Léger, E. and Dietrich, M.(2013): Host specialization in ticks and transmission of tick-borne diseases: a review. *Front. Cell. Infect. Microbiol.* **3**, 57.
- Mekonnen, S.(2002): Acaricide resistance profiles of single and multi-host ticks in commercial and communal farming areas in the Eastern Cape and North-West Provinces of South Africa.
- Mekonnen, S.(2001): In vivo evaluation of amitraz against ticks under field conditions in Ethiopia: research communication. *J. S. Afr. Vet. Assoc.* **72**, 44–45.
- Mekonnen, S., Hussein, I. and Bedane, B., (2001): 'The distribution of ixodid ticks (Acari: Ixodidae) in central Ethiopia.

- Merino, O., Alberdi, P., Pérez de la Lastra, J.M. and de la Fuente, J.(2013): Tick vaccines and the control of tick-borne pathogens. *Front. Cell. Infect. Microbiol.***3**, 30.
- Mohammed, K. and Admasu, P.(2015): Prevalence of Ixodid ticks in small ruminants in selected districts of Fafen zone, Eastern Ethiopia. *Eur. J. Appl. Sci.* **7**, 50–55.
- MoLF.(2015): An Overview of the Ethiopian Livestock Overview of the Ethiopian Livestock sector sector' 's role in livelihood and food s role in livelihood and food safety. Addis Ababa.
- Moumouni, P.F.A., Aboge, G.O., Terkawi, M.A., Masatani, T., Cao, S., Kamyngkird, K., Jirapattharasate, C., Zhou, M., Wang, G. and Liu, M.(2015): Molecular detection and characterization of Babesia bovis, Babesia bigemina, Theileria species and Anaplasma marginale isolated from cattle in Kenya. *Parasit. Vectors***8**, 496.
- Munderloh, U.G. and Kurtti, T.J.(2011): Emerging and re-emerging tick-borne diseases: new challenges at the interface of human and animal health. Natl. Acad. Sci. Washington, DC, Crit. needs gaps Underst. Prev. Amelior. Resolut. Lyme other tick-borne Dis. short-term long-term outcomes A142–A166.
- Murrell, A. and Barker, S.C.(2003): Synonymy of Boophilus Curtice, 1891 with Rhipicephalus Koch, 1844 (Acari: Ixodidae). *Syst. Parasitol.* **56**, 169–172.
- Nadolny, R.M. and Gaff, H.D.(2018): Modelling the effects of habitat and hosts on tick invasions. *Lett. Biomath.* **5**, 2–29.
- Narladkar, B.W.(2018): Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. *Vet. world***11**, 151.
- Nava, S., Mangold, A.J. and Guglielmone, A.A.(2009): Field and laboratory studies in a Neotropical population of the spinose ear tick, Otobius megnini. *Med. Vet. Entomol.* **23**, 1–5.
- Nijhof, A.M., Balk, J.A., Postigo, M. and Jongejan, F.(2009): Selection of reference genes for quantitative RT-PCR studies in Rhipicephalus (Boophilus) microplus and Rhipicephalus appendiculatus ticks and determination of the expression profile of Bm86. *BMC Mol. Biol.* **10**, 112.
- Okello-Onen, J., Tukahirwa, E.M., Perry, B.D., Rowlands, G.J., Nagda, S.M., Musisi, G., Bode, E., Heinonen, R., Mwayi, W. and Opuda-Asibo, J.(1999): Population dynamics of ticks on indigenous cattle in a pastoral dry to semi-arid rangeland zone of Uganda. *Exp. Appl. Acarol.***23**, 79–88.

- Onu, S.H. and Shiferaw, T.Z.(2013): Prevalence of ectoparasite infestations of cattle in Bench Maji zone, southwest Ethiopia. *Vet. World***6**, 291–294.
- Pegram, R.G., Hoogstraal, H. and Wassef, H.Y.(1981): Ticks (Acari: Ixodoidea) of Ethiopia. I. Distribution, ecology and host relationships of species infesting livestock. *Bull. Entomol. Res.***71**, 339–359.
- Pinter, A., Dias, R.A., Gennari, S.M. and Labruna, M.B.(2004): Study of the seasonal dynamics, life cycle, and host specificity of *Amblyomma aureolatum* (Acari: Ixodidae). *J. Med. Entomol.* **41**, 324–332.
- Rahmeto, A., Thedrous, F., Mesele, A. and Jemere, B.(2010): Survey of ticks (Acari: Ixodidae) infesting cattle in two districts of Somali regional state, Ethiopia. *Vet. World***3**, 539–543.
- Rajput, Z.I., Hu, S., Chen, W., Arijo, A.G. and Xiao, C.(2006): Importance of ticks and their chemical and immunological control in livestock. *J. Zhejiang Univ. Sci.* **B7**, 912–921.
- Reece, W.O. and Rowe, E.W.(2017): Functional anatomy and physiology of domestic animals. John Wiley & Sons.
- Regassa, A.(2001): Tick infestation of Borana cattle in the Borana Province of Ethiopia. Onderstepoort *J. Vet. Res.* **68**, 41–45.
- Rehman, A., Nijhof, A.M., Sauter-Louis, C., Schauer, B., Staubach, C. and Conraths, F.J.(2017): Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan. *Parasit. Vectors***10**, 190.
- Reyes, J.L., González, M.I., Ledesma-Soto, Y., Satoskar, A.R. and Terrazas, L.I.(2011): TLR2 mediates immunity to experimental cysticercosis. *Int. J. Biol. Sci.***7**, 1323.
- Sajid, M.S., Iqbal, Z., Khan, M.N. and Muhammad, G.(2009): In vitro and in vivo efficacies of ivermectin and cypermethrin against the cattle tick *Hyalomma anatolicum anatolicum* (Acari: Ixodidae). *Parasitol. Res.* **105**, 1133–1138.
- Sajid, M.S., Iqbal, Z., Khan, M.N. and Muhammad, G.(2008): Point prevalence of hard ticks (Ixodids) infesting domestic ruminants of lower Punjab, Pakistan. *Int J Agric Biol***10**, 349–351.
- Shapiro, B.I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Gezahegn, A. and Henok, M.(2017): Ethiopia livestock sector analysis: A 15 year livestock sector strategy.
- Sharma, A., Singla, L. Das, Tuli, A., Kaur, P., Batth, B.K., Javed, M. and Juyal, P.D.(2013): Molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* in dairy animals from

- Punjab, India, by duplex PCR: a step forward to the detection and management of concurrent latent infections. *Biomed Res. Int.* 2013.
- Shiferaw, D. and Abebe, G.(2006). Cattle tick dynamics in different agro-ecological zones of Wolayta, Southern Ethiopia. *Ethiop. Vet. J.* **10**, 85–99.
- Sileshi, M., Pegram, R.G., Solomon, G., Abebe, M., Yilma, J. and Sileshi, Z.(2007): A synthesis review of ixodid (Acari: Ixodidae) and argasid (Acari: Argasidae) ticks in Ethiopia and their possible roles in disease transmission. *Ethiop. Vet. J.* **11**, 1–24.
- Solomon, A. and Authority, E.L.M.(2003): Livestock marketing in Ethiopia: a review of structure, performance, and development initiatives. ILRI (aka ILCA and ILRAD).
- Solomon, G. and Kaaya, G.P.(1998): Development, reproductive capacity and survival of *Amblyomma variegatum* and *Boophilus decoloratus* in relation to host resistance and climatic factors under field conditions. *Vet. Parasitol.* **75**, 241–253.
- Solomon, G., Nigist, M. and Kassa, B.(2003): Seasonal variation of ticks on calves at Sebeta in Western Shoa zone Ethiopia. *Ethiop. Vet. J* **7**, 17–27.
- Swai, E.S., Mbise, A.N., Kessy, V., Kaaya, E., Sanka, P. and Loomu, P.M.(2005): Farm constraints, cattle disease perception and tick management practices in pastoral Maasai community-Ngorongoro, Tanzania. *Livest. Res. Rural Dev.* **17**.
- Ethio.G.I.S.(2004): A Geo-referenced Database and Digital Terrain Model of Ethiopian Spatial Information Prepared in a Geographical Information System (GIS).
- Tabor, A.E., Ali, A., Rehman, G., Rocha Garcia, G., Zangirolamo, A.F., Malardo, T. and Jonsson, N.N.(2017): Cattle Tick *Rhipicephalus microplus*-host interface: a review of resistant and susceptible host responses. *Front. Cell. Infect. Microbiol.* **7**, 506.
- Tadesse, B. and Sultan, A.(2014): Prevalence and distribution of tick infestation on cattle at Fitchelale, North Shewa, Ethiopia. *Livest Res Rural Dev* **26**.
- Tadesse, F., Abadfaji, G., Girma, S. and Jibat, T.(2012): Identification of tick species and their preferred site on cattles body in and around Mizan Teferi, Southwestern Ethiopia. *J. Vet. Med. Anim. Heal.* **4**, 1–5.
- Tamerat, N., Erba, F., Muktar, Y. and Kemal, J.(2015): Identification and prevalence of ixodid tick in bovine at Bedele district, Oromiyia Regional State, Western Ethiopia. *J. Parasitol. Vector Biol.* **7**, 156–162.
- Taylor, P.M., Pascoe, P.J. and Mama, K.R.(2002): Diagnosing and treating pain in the horse: where

- are we today? *Vet. Clin. Equine Pract.* **18**, 1–19.
- Tessema, Y.A.(2018): Drivers of Climate Sensitive Farm Level Adjustments: Climate Change, Economic and Technical Factors, and Resource Availability.
- Thrusfield, M. and Christley, R.(2005): Veterinary epidemiology. Wiley Online Library.
- Ticks, F.A.O.(2004): Acaricide resistance, diagnosis, management and prevention. Guidel. Resist. Manag. Integr. parasite Control ruminants Agric. Dep. Roma 1.
- Tiki, B. and Addis, M.(2011): Distribution of ixodid ticks on cattle in and around holeta town, Ethiopia. *Glob. Vet.* **7**, 527–531.
- Tilahun, A.N.B.(2016): Epidemiology and Control of Bovine Theileriosis in Ethiopia. *Epidemiology* 23.
- Tomassone, L., Grego, E., Callà, G., Rodighiero, P., Pressi, G., Gebre, S., Zeleke, B. and De Meneghi, D., (2012): Ticks and tick-borne pathogens in livestock from nomadic herds in the Somali Region, Ethiopia. *Exp. Appl. Acarol.* **56**, 391–401.
- TORR, S., EISLER, M., COLEMAN, P., MORTON, J. and MACHILA, N.(2004): Integrated control of ticks and tsetse. Report for the DFID Advisory and Support Services Commission. Natural Resources Institute (NRI), Chatham, UK.
- Tuli, A., Singla, L. Das, Sharma, A., Bal, M.S., Folia, G. and Kaur, P.(2015): Molecular epidemiology, risk factors and hematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). *Acta Parasitol.* **60**, 378–390.
- Turkson, P.K. and Botchey, M.(1999): Acaricide resistance in the cattle tick, *Amblyomma variegatum*, in the coastal savanna zone of Ghana. *Ghana J. Agric. Sci.* 32, 199–204.
- Verhulst, N.O. and Takken, W.(2015): Skin microbiota and attractiveness to mosquitoes. *Encycl. Metagenomics Environ. Metagenomics* 591–595.
- Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Pena, A., Horak, I. G., Latif, A. A., Pegram, R. G. and Preston, P.M.(2014): Ticks of domestic animals in Africa: a guide to identification of species. Edinburgh, UK.
- Walker, A.R.(2003): Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports Edinburgh.
- Walker, J.B., Keirans, J.E. and Horak, I.G.(2005): The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Cambridge University Press.
- Wall, R. and Shearer, D.(2001): Veterinary Ectoparasites: Biology, Pathology and Control. Blackwells

Science Ltd.

- Willadsen, P.(2006): Tick control: thoughts on a research agenda. *Vet. Parasitol.* **138**, 161–168.
- Wogayehu, Y., Wossene, A., Getachew, S. and Kabtyimer, T.(2016): Epidemiological Study of Ticks and their Distribution in Decha Woreda of Kafa zone, SNNPRS. *Int. J. Res.* **7**.
- Wolde, A. and Mohamed, A.(2014): Prevalence of ixodid ticks on Bovine in Soddozuria districts, Wolaita Zone, Ethiopia. *Acta Parasitol. Glob.* **5**, 188–197.
- Yacob, H.T., Ataklty, H. and Kumsa, B.(2008): Major ectoparasites of cattle in and around Mekelle, northern Ethiopia. *Entomol. Res.* **38**, 126–130.
- Yessinou, R.E., Adoligbe, C., Akpo, Y., Adinci, J., Youssao Abdou Karim, I. and Farougou, S.(2018): Sensitivity of Different Cattle Breeds to the Infestation of Cattle Ticks *Amblyomma variegatum*, *Rhipicephalus microplus*, and *Hyalomma* spp. on the Natural Pastures of Opkara Farm, Benin. *J. Parasitol. Res.* 2018.
- Zelege, M. and Bekele, T.(2004): Species of ticks on camels and their seasonal population dynamics in eastern Ethiopia. *Trop. Anim. Health Prod.* **36**, 225–231.
- Zhang, J., Liu, Q., Wang, D., Li, W., Beugnet, F. and Zhou, J.(2017): Epidemiological survey of ticks and tick-borne pathogens in pet dogs in south-eastern China. *Parasite***24**.

Annex 2: Questionnaire format

1. Personal Information

- Name _____ Tel (mobile) _____ Peasant Association (PA) _____ sex; Male _____ Female _____ (Tick where appropriate)
- Age (years) (circle the correct number) a) Below 25 years b) 26-30 c) 31-40 d) 41-50 e) 51-60 f) above 60 years
- Education Level a) Illiterate b) local school c) elementary d) High School e) College Diploma f) Degree
- Experiences of keeping animals in years a) <5 years b) 5-10 years c) >10 years) 10-15years d) 15-20 years e) more than 20 years

2. Type of production/mode of husbandry

- Livestock management system? a) Communal b) tethering c) paddocks d) ranching e) zeroes grazing
- Are livestock kept plus other agricultural system like Crop growing? a) Yes b) No. If yes which one is more profitable entity? _____
- Kind of livestock kept; a) Cattle b) sheep c) goats d) equine e) poultry f) all. Among those which species of animals are more important for the district and for what purpose? _____
- Where do you get the replacement stock? a) Develop your own b) market c) breeders
- Is there Wildlife-livestock interaction? a) Yes b) No

3. Information on ticks

- Do you know ticks? a) Yes b) No
- Are ticks make any problems in your locality? a) Yes b) No
- Which type of areas or habitat is more favored by ticks? a) Vegetation b) grazing areas c) areas covered with crops d) lands with crops e) lands with grasses
- Do you think ticks have any health or productivity problems in animals or man? a) Yes b) no
- Type of ticks you know in your locality; a) Hard ticks b) Soft ticks c) both
- Tick infestation is more common in which season of the year? a) At the end of rainy season b) At the beginning of rainy season c) In mid of rainy season d) At the dry season
- What is the rank of ticks in terms of negative effects on animals? a)1st b)2nd c)3rd d)4th e)5th f)<5th
- Do you know any tick-borne diseases of man? a) Yes b) No
- Do you know any tick-borne diseases of animals? a) Yes b) No
- Do you know any tick controlling system? a) Traditional b) modern c) both

- Species of livestock, mostly infested with ticks a) cattle b) sheep c) goats d) equines e) others_____
- Breed of animals comparatively more susceptible to the tick infestation in your locality a) Indigenous breed b) Cross breed c) exotic breed d) all are equally infested
- Which age groups of animals are most affected? a) Young b) adult c) same d) all
- Which sex, category of animals is most affected? a) Female b) male c) equal d) don't know
- Color of animals more susceptible to tick infestation inn your locality? a) Black animals b) white animals c) gray animals d) red animals e) mixed f) others_____
- Do you think tick infest & attach on man or people? a) Yes b) No
- Have you been bitten by ticks? a) Yes b) no
- If yes, how many times? a) One b) two c) three d) four e) more than five times

4. Tick control regimes

- How do you treat tick infested animals? a) Herbal treatment b) Chemical treatment c) Others d) None
- Do you treat only the animals that are infested with ticks? a) Yes. b) NO
- Do you think that Tick and tick borne diseases are the major problem of animals in this area? If your answer is yes, what is the better alternative to reduce the impact of this disease?

- Common sources of acaricide used? a) Government Clinics b) Private Clinics c) Open market d) Illegal sellers e) Others_____
- Which one is more preferred sources of acaricide used?
a) Government Clinics b) Private Clinics c) Open market d) Illegalsellers e) Others_____
- Do you have differences in preference for various acaricides? a) Yes b) NO
- If yes, for which acaricides you give more preference? _____
- What are the causes of variation in preferences? _____
- Who is prescribing and administering acaricides? a) Government Vets b) Private Vets c) Open market d) Illegal sellers e) The animal's owner
- What concentration used? _____
- Method of application; a) Dipping b) spraying c) pouring on d) hand picking f) none
- Equipment used; a) knap sack sprayer b) bucket/foot pump c) dip d) hand sprayer e) spray race f) scrubbing cloth g) none
- Method of restraints: a) crush b) Boma c) ropes d) dip
- Source of water for acaricide mixing; a) tap water b) well c) dirty natural d) clean natural e) swamp e) no water needed
- Number of cattle sprayed with 20 liters of mixed acaricide wash? FAO recommended (2 cattle) a) At most 7 b) 8–20 c) 21–40 d) >40 e) Not applicable (dip)

- Who supervises mixing and application? a) Herdsmen b) Owner c) Farm manager d) Children
- Class of acaricide used: a) Amitraz b) Synthetic pyrethroid c) Organophosphate d) Co-formulation e) Do not use acaricide f) Not sure and no record
- History of tick resistance: a) Yes b) No
- Measures taken to avert tick resistance: a) Change acaricide b) Double concentration and change of acaricide c) Double concentration d) Increase the frequency of spraying) f) Triple acaricide concentration g) Use Ivermectineh) injectable and change acaricide i) not applicable
- Are the coping strategies, effective? a) Yes b) Somehow c) No
- Time spent with previous acaricide before changing to current: a) Less than 5 months b) 6–9 months c) 1–2 years d) Above 2 years e) Not sure
- Source of advice on mixing two or more acaricides together: a) Fellow farmer b) Trial and error c) Not applicable
- Does mixing two or more acaricide together solve acaricide failure? a) Yes b) No c) Not sure
- Distance with neighboring farm (kilometers)? _____ and Interaction of animals with that of neighbors? a) Daily b) Weekly c) Monthly d) Never e) Not sure
- Knowledge of acaricide use by neighbors? a) Yes b) No
- Knowledge on the days of the week when a neighbor sprays? a) Yes b) No
- New animals introduced at the farm: a) Yes b) No
- Origin of new animals (cattle) introduced in the farm? a) Cattle market b) Neighboring districtsc) Neighboring farm d) None
- Did you quarantine animal sprayed before mixing with others? a) Yes b) No c) Not applicable
- Location where acaricide is stored? a) At dip tank b) Drug box c) In the bush at the kraal d) Designated store e) Residential house f) No response
- Have you seen or heard of animals with damaged skin due to acaricide (Poisoning)? a) Yes b) No c) Not sure
- Which animal species are most affected by the negative effects of skin damage due to acaricide or any other poisoning? a) Equinesb) cattle c) sheep d) goats e) dogs and cats f) any other _____
- Has any person applying acaricide on this farm suffered from adverse (bad) effects of acaricides? a) Yes b) No; if yes, which effects? a) Death b) Blindness c) Coughing d) Dizziness e) Eye and skin Itching f) Eye is itching g) Skin itching h) Skin itching and Coughing i) Any other symptoms j) None
- Equipment used for acaricide measurement? a) Calibrated bottle top b) acaricide bottle for dip c) non calibrated bottle top d) syringe
- How often do you spray / apply acaricide during dry____ and rainy____ season? a) Weekly b) twice a week c) thrice a week d) fortnight e) monthly

- Source of advice on tick control a) drug shop attendant b) drug shop attendant and fellow farmer c) drug shop attendant and veterinarian d) fellow farmer e) fellow farmer and veterinarian f) veterinarian g) none

5. Tick response to acaricides application

- Are tick seen after application of acaricides? _____
- If yes, what number _____ do they drop later or not? _____
- If they drop how long does it take before they drop? _____
- If they do not drop what do you do? _____
- Which acaricides in particular do you experience ticks remaining after application? (name any) a) _____ b) _____ c) _____ d) _____
- Is there tick resistance to acaricides? (answer with yes or no) _____
- What do you think should be done to avoid acaricides resistance?

6. General view of diseases, controls

- Mention in order of importance the names of the most important animal diseases in the area/PA/district?
i) _____ ii) _____ iii) _____ iv) _____
- Is there sufficient Veterinary care in the area? (Answer with yes or no) _____
- Is veterinary intervention around the area affordable/expensive? _____
- How regularly do you vaccinate your animal? _____

Thank you for the information you have provided. All this shall be taken into account in trying to assist to control the diseases in the district that will be of great importance in recognizing the full potential in keeping animals i.e. maximizing profit.

Annex 3: Procedure for invitro efficacy test at recommended dose

Source: (FAO, 2004)

1. Acaricides was diluted to the recommended discriminating doses.
2. 20 ml of the diluted acaricide was added to 100 ml plastic containers with screw-cap lids. 20 ml of water was added to another container as a control. The containers were labeled.
3. For each container 10 healthy, clean, engorged female ticks taken from cattle within 48 h of the test was added.
4. Ticks were immersed in acaricide solution for 1 minute at about 25°C and the containers was shook gently.
5. After 1 minute, the acaricide solution was poured off into a safe storage container and the ticks were dried gently on paper toweling.
6. The ticks were transferred from one container, with ventral side up, onto double-sided sticky tape in a Petri dish.
7. The dishes were incubated in a larger polystyrene container at about 25 to 30°C for 7 days. The container was kept moist with damp paper or toweling. The container did not shake till as the egg batches from each tick have to be observed.
8. After 7 days the number of ticks that have laid eggs was counted.

Annex 4: Invitro efficacy test laboratory result report sheet for test of susceptibility of a tick sample

Trial	Tick species	Acaricides	N	Eng.wgt(gm)	S	No.LE	Egg.M(gm)	%C
Trial1								
Trial 2								
Trial 3								

Annex 5: Identification key of relevant tick species identified in the Anilemo District

Rhipicephalus (Boophilus) decoloratus (Murrell and Barker, 2003)

Female	Male
Porose areas shape is a narrow oval	Cornua are distinct
Hypostomal teeth are in 3 + 3 columns	Coxae 1 spurs length is short
Palp articles 1 internal margin has a protuberance with pectinate setae	Ventral plate spurs are distinct
Coxae 1 spurs are distinct	Caudal appendage is narrow in fed males
Coxae 2 and 3 spurs are present	Ventral plate spurs are visible dorsally

Amblyomma variegatum (Daubney, 1930) modified by (Walker, 2003)

Female	Male
Mesial area of enamel ornamentation on scutum is elongate (it may be indistinct in its anterior part).	Eyes are distinctly convex
Lateral areas of enamel ornamentation on scutum are small	Primary punctation sizes on scutum are small to medium
Scutum sides are straight	Primary punctation distribution on scutum is regular
Scutum posterior angle is broad	Enamel colour pink to orange
Leg colouration is with pale rings	Genital aperture posterior lips have a broad U shape

Rhipicephalus evertsi evertsi (Latif, 1984)modified by (Walker, 2003)

Female	Male
Interstitial punctation size is small to medium. Interstitial punctation distribution is dense. Setiferous punctations are indistinct.	Interstitial punctation size is small to medium. Interstitial punctation distribution is dense. Setiferous punctations are indistinct.
Basis capituli lateral angles are blunt.	Coxae 1 anterior spurs are visible
Porose areas separation is narrow	Cervical fields depression is not apparent. Cervical fields texture has wrinkled areas
Palp pedicels are short	Eyes are very convex
Cervical fields shape is not apparent. Cervical fields texture has wrinkled areas	
Scapular grooves profile is shallow	Conscutum colour is dark
Eyes are very convex	Accessory adanal plates are small (they may be absent)
Spiracle plate areas have dense setae	Adanal plates shape is broad and curved
Scutum posterior margin is slightly sinuous. Scutum colour is dark	Caudal appendage is absent in fed males
Legs are coloured a distinctive pale orange, uniformly over each segmen	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, uniformly over each segment.)

Rhipicephalus praetextatus (Walker *et al.*, 2005)

Female	Male
Basis capituli lateral angles are blunt	Eyes are slightly convex
Porose areas separation is broad	Conscutum colour is dark
Palp pedicels are long	Accessory adanal plates are large
Cervical fields shape is large and curved	Adanal plates shape is broad and curved
Spiracle plate areas have sparse setae	Caudal appendage is broad in fed males
Scutum posterior margin is slightly sinuous	Spiracle plate areas have sparse setae
Scutum colour is dark	Posterior grooves are absent

Rhipicephalus pulchellus (Walker *et al.*, 2005).

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.

Annex 6: Tick collection process (top), treated and control ticks after incubation process (bottom)

