

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**IN-VITRO ACARICIDE EFFICACY EVALUATION AND SURVEY OF IXODIDAE
TICKS ON CATTLE IN BORENA PASTORAL AREA, ETHIOPIA**

BY
EYOB ESHETU BEKELE

JUNE, 2012
DEBREZEIT, ETHIOPIA

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**IN-VITRO ACARICIDE EFFICACY EVALUATION AND SURVEY OF IXODIDAE
TICKS ON CATTLE IN BORENA PASTORAL AREA, ETHIOPIA**

A thesis submitted to the school of Graduate Studies of Addis Ababa University
in partial fulfillment of the requirements for the Degree of Master of Science in
Tropical Veterinary Parasitology

BY
EYOB ESHETU BEKELE

JUNE, 2012
DEBREZEIT, ETHIOPIA

**IN-VITRO ACARICIDE EFFICACY EVALUATION AND SURVEY OF IXODIDAE
TICKS ON CATTLE IN BORENA PASTORAL AREA, ETHIOPIA**

BY

EYOB ESHETU BEKELE

Board of External Examiners

Signature

1. Dr. Dereje Gudeta

2. Prof. Getachew Tilahun

3. Dr. Genene Terefe

Academic Advisor:

Dr. Dinka Ayana _____

TABLE OF CONTENTS	PAGES
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	v
LIST OF ANNEXES	vii
LIST OF ABBREVIATIONS	viii
ABSTRACT	ix
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 Classification of ticks	3
2.2 Morphology of ticks	3
2.3 Basic biology of ixodid ticks	4
2.4 Life history of ixodid ticks	5
2.5 The epidemiology and factors affecting tick survival	7
2.5.1 The environment	8
2.5.2 Host preference and availability of suitable hosts	9
2.5.3 Cattle resistance	9
2.5.4 The properties of tick population	11
2.6 The distribution of ticks in Ethiopia	11
2.7 Tick control methods and their limitation	14
2.7.1 Chemical of tick control	16
2.7.2 Acaricide resistance in ticks	17
2.7.3 Factors influencing rate of acaricide resistance	18
2.8 Chemical tick control and acaricide resistance in Ethiopia	19
2.9 Acaricide resistant recognition and testing methods	20
3. MATERIALS AND METHODS	23

3.1 Description of study area.....	23
3.2 Study animals	26
3.3 Study design	26
3.4 Sample size and sampling methods	27
3.5 Study methodology	28
3.5.1 Adult Tick Collections and Identifications	28
3.5.2 In-Vitro acaricide efficacy evaluation/ Adult Immersion Test: AIT	28
3.5.3 Questionnaire survey	30
3.6 Meteorological information data	30
3.7 Statistical data analysis	31
4. RESULTS.....	32
4.1 Questionnaire survey result	32
4.2 Adult tick survey.....	33
4.2.1 Seasonal variation	34
4.2.2 Age variation.....	37
4.2.3 Sex factor.....	38
4.3 In-vitro acaricide efficacy test.....	39
5. DISCUSSIONS	43
6. CONCLUSIONS AND RECOMMENDATIONS	53
7. REFERENCES	55
8. ANNEXES.....	65
9. CURRICULUM VITAE.....	72
10. SIGNED DECARATION SHEET	74

ACKNOWLEDGEMENTS

First of all I would like to express my heartfelt thanks to God for His mercifulness, saving my personal life and keeping my life in all my ways.

I owe a lot of heartily thanks to my academic advisor Dr. Dinka Ayana for his continuous, unwavering guidance and advice throughout the study periods and during writing this paper.

My heartily gratitude goes to my sweet, Mihret Tegene, her patience, love and encouragement enabled me to complete this study. The support given by Getahun T., my mum Etagegnehu G. and my Dad Eshetu B. is also appreciable.

I sincerely appreciate the logistical support from CARE-Ethiopia in general and to the team members of PLI project and CARE- Borana that made my research study and stay in Borena pastoral area a reality; and the Oromia Pastoral Area Development Commission for allowing me to take leave from my job in order to pursue this programme and study.

I would like to extend my thanks to National Animal Health Diagnostic and Investigation Center (NAHDIC) at Sebeta, Ethiopia for their material supports; and provision of training and Certificate for the Participation and Completion on Sample collection, storage and media preparation, Helminthes, Protozoan and Ticks identification techniques.

Even as it is not possible to mention all those who contributed in one way to another, I would like to mention the staff members of Yabello Regional Veterinary Laboratory, in particular to Huka L., for their technical support during sample collection and laboratory processing as well as the pastoralist communities of Borena Pastoral area for sharing their views and perceptions pertinent.

Last but not least I am highly grateful to all my instructors for their unreserved effort in the teaching and learning process during all period of this MSc programme.

LIST OF TABLES

Table 1: Important Ixodids ticks according to the number of hosts needed for their life cycle	7
Table 2: Meteorological data of Yabello, Borena pastoral area for June, 2011 to May, 2012	31
Table 3: Relative tick burden in Yabello and Dire district of Borena pastoral area	33
Table 4: Relative abundance, mean count and M:F of tick species on cattle in Yabello and Dire districts, Borena pastoral area of Ethiopia.....	34
Table 5: Relative abundance and M:F of adult tick species on cattle during short rain and major dry seasons in Borena pastoral area	35
Table 6: T-test analysis of mean tick burden on cattle between short rain and dry season in Borena pastoral area.....	35
Table 7: T-test analysis of mean tick burden on cattle between adult and young cattle in Borena pastoral area	38
Table 8: Prevalence of tick infestation by cattle sex, Borena pastoral area	38
Table 9: T-test analysis of mean tick burden by sex of cattle at Borena pastoral area	39
Table 10: Mean oviposition response of adult <i>A.gemma</i> and <i>Rh.pulchellus</i> after immersion in Amitraz 12.5% and Diazinon 60%EC at recommended concentration and 7 day incubation	41
Table 11: T-test analysis of mean percent <i>A.gemma</i> and <i>Rh.pulchellus</i> oviposition Control between Amitraz 12.5% and Diazinon 60%EC	41
Table 121: Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60%EC at field recommended concentration against adult female <i>A.gemma</i> and <i>Rh.pulchellus</i>	42
Table 13: Analysis of variance of the effects of Amitraz 12.5% and Diazinon 60%EC against oviposition response of <i>A.gemma</i> and <i>Rh.pulchellus</i> in Borena pastoral area, Ethiopia	42

LIST OF FIGURES

Figure 1: Maps of Ethiopia and the study area	25
Figure 2: Monthly population dynamics of five tick species on cattle in Borena pastoral area	36
Figure 3: Monthly mean tick burden variation in relation to temperature, RH and rainfall information recorded at Yabello district of Borena pastoral area	36
Figure 4: Mean tick burden of individual species on adult and young cattle in Borena pastoral area	37
Figure 5: Female <i>Rh.pulchellus</i> and <i>A.gemma</i> laid eggs after immersion in water and incubated at 27°C for 7 days	40

LIST OF ANNEXES

Annex 1: Cattle body regions used for tick collection (Modified from Kaiser, 2004)	65
Annex 2: Laboratory Methodology for Modified Adult Immersion Test (AIT)	66
Annex 3: Some materials and activities during In vitro efficacy trials	68
Annex 4: Questionnaire set to interview pastoralists on herd structure, tick infestation Constraints, acaricide delivery and usage and tick control practice	69
Annex 5: Ovipositing response of engorged adult female <i>Rh.pulchellus</i> and <i>A.gemma</i> after immersion in Amitraz 12.5% and Diazinon 60%EC and 7 days' incubation.....	71

LIST OF ABBREVIATIONS

%C	Percent control
AIT	Adult Immersion Test
BZPADO	Borena Zone Pastoral Area Development Office
CI	Confidence Interval
DD	Discriminating Doses
df	Degree of freedom
ELT	Egg Laying Test
FAO,	Food and Agricultural Organization
LIT	Larval Immersion Test
LPT	Larval packet Test
ML	Macrocyclic Lactones
mm	millimeter
OP	Organophosphates
P	Predictive value
PAs	Pastoralist Association
RH	Relative Humidity
S.D	Standard Deviation
SE	Standard Error
SP	Synthetic pyrethroids
TBDs	Tick Borne Diseases

ABSTRACT

This study was conducted from September 2011 to May 2012 in Borena pastoral area with the objectives of determining the distribution and relative abundance of Ixodidae ticks on cattle in relation to short rain and major dry seasons, cattle age and sex. Moreover, the efficacy of Amitraz 12.5% and Diazinon 60%EC against *Rh.pulchellus* and *A.gemma* was determined using modified Adult Immersion Test; on top of the assessment of pastoralist communities' perceptions on acaricide usage, delivery and methods of tick control practice. A total of 8,912 adult ticks belonging to genera of *Rhipicephalus*, *Amblyomma*, *Boophilus* and *Hyalomma* were collected from 768 indigenous cattle. *Rhipicephalus pulchellus* and *A.gemma* constituted more than 70% of the total collection. Other identified species in order of abundance were *B.decoloratus*, *A.variegatum*, *H.m.rufipes*, *H.truncatum*, *A.lepidium* and *A.cohearens*. All species were collected throughout the study period, but showed higher overall mean tick burden (29.39 ± 14.392) during short rain season than major dry (17.14 ± 7.294). These variations were statistically significant ($t = 14.915$, 95% CI=10.634-13.866, $df = 766$ $P < 0.05$). The abundance and mean burden of each tick species was high in adult cattle than young and females than males; which also showed statistical significant variation ($P < 0.05$) between cattle sex and age groups. Acaricide treatment is the only method for tick control in the area, in which Amitraz was the most widely used, followed by Diazinon. Manual spraying and hand dressing of each acaricide relaying on frequent, over or under concentration, inadequate application and a long time use of one acaricide type was the common practice of application. The result of in-vitro trials revealed that, Amitraz 12.5% has significantly higher ($P < 0.05$) overall mean percent oviposition control (%C= 95.47%) of *A. gemma* and *Rh.pulchellus* than Diazinon 60% EC (%C= 80.9%). But, both acaricides are relatively less efficient against *Rh. pulchellus* oviposition, %C = 90.94% for Amitraz and %C = 71.41% for Diazinon. The results of this study suggested that the two seasons, cattle age and sex were found to be determinant for dynamics of tick population during the study period. Furthermore, Amitraz and Diazinon are the two mostly used acaricides in the area and under in-vitro condition, Amitraz at field recommended concentration provides efficient *Rh.pulchellus* and *A.gemma* oviposition inhibition than Diazinon 60%EC. Therefore, strategic control using such seasonal dynamics of ticks, aimed at primarily reducing *Rh. pulchellus* and *A.gemma* burden on female and adult cattle, with the objectives of allowing maintenance of endemic stability are recommended. Besides, in vivo trial to assess the residual effect of these acaricides was suggested.

Key words: Acaricide, Borena pastoral area, Cattle, Efficacy, In-vitro evaluation, Prevalence, Tick

1. INTRODUCTION

In Ethiopia, pastoralist areas cover 60% of total landmass of the country and majorities are engaged in extensive livestock herding, which forms the backbone of national economy (Solomon, 2004). The pastoralist groups managed 40% of the national cattle herd; and some 90% of the country's live animals for export. Similarly, Borena rangeland area is among the known pastoralist area of Ethiopia and covered 26% of the livestock population of the country (Hassen, 2003). Though, there is huge livestock resource in pastoral area of Ethiopia (Yismashewa, 2005) and pastoralists of Borena, in exacting (Teshale, 2004), due to various constraints, they are not economically benefited out of it. Among others contagious diseases, ectoparasites (ticks and mange mites) and tick borne diseases are the impediments (CARE, 2009; Farmer, 2010).

In most of Africa including Ethiopia, tick and tick borne diseases (TBDs), together with tsetse and trypanosomosis, are economically very important diseases (Belew and Mekonnen, 2011). Since, studies on tick fauna in Ethiopia begun in early the 19th century (Pegram *et al.*, 1981); extensive surveys have been carried out on the distribution of ticks on livestock in different regions and more than 60 species of ticks infesting both domestic and wild animal have been recorded. Among these, 30 species are very widespread and important parasites of livestock (Solomon *et al.*, 2004) and causes significant economic losses to livestock industry. The economic losses incurred from downgrading of hides and skins are enormous, export yields foreign earnings of the country, second only to coffee (Sileshi *et al.*, 2001).

Rhipicephalus pulchellus and *A. gemma* are the predominant species in arid and drier land of the country (Walker *et al.*, 2000) together with arid and drier land of Borena pastoral area (Solomon and Kaaya, 1998; Regassa, 2001; Solomon and Kaaya, 2001). However, the distribution limits of ticks are not fixed and constant, but are determined by a complex interaction of factors (climate, host density, host susceptibility etc) (Solomon *et al.*, 2003). Therefore, relevant update data on such responsible factors are essential for effective tick and TBDs control strategies (Alanr, 2011).

In, most situations, however, efficient and reliable control of ticks and TBDs are still based primarily on intensive use of acaricides, often without the local understanding of those

responsible factors for tick distribution dynamics (Luciana *et al.*, 2011). Since, acaricide introduction in South Africa around 1890, tick treatment relying on different application methods have been the main method of tick control in Africa, and leading to numerous problems; environmental pollution, development of resistant tick strains and escalating costs (Alanr, 2011; Luciana *et al.*, 2011). Therefore, FAO recommends the modified Adult Immersion Test (AIT) for the detection of acaricide resistance in the field (FAO, 2004).

In Ethiopia, over the past decades ticks are mainly controlled by using variety of acaricides; including Organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids (Sileshi, 2001; Yilma *et al.*, 2001). However, with the most widespread, under or over concentration and frequent use of organochlorines and organophosphates compounds; ticks are likely to develop resistance in many countries (Furlong *et al.*, 2007); and in Ethiopia (Adamu, 1996; Yilma *et al.*, 2001; Sileshi *et al.*, 2004). Likewise, in Borena pastoral areas, where mostly use Amitraz 12.5% and Diazinon 60%EC, in various circumstances' animal health personnel's and livestock herders complained on failure of this two acaricides to kill ticks and toxicity associated with Diazinon usage (BZPADO, annual report, 2009/2010).

In view of the aforementioned facts on the dynamics distribution limits of ticks; and with the most inappropriate usage of acaricides, ticks are likely to develop resistance. Continuous studies on the responsible factors for the dynamics of tick population (Alanr, 2011) with the efficacy status of acaricides against the most abundant and important tick in particular area are necessary to carry out efficient tick control and/or tick burden reduction (Solomon, 2001).

Therefore the objectives of this study were:-

- ❖ To determine the distribution and mean burden of adult tick dynamics in different age and sex of cattle and in short rain and major dry seasons of Borena pastoral area.
- ❖ To evaluate the efficacy of Amitraz 12.5% and Diazinon 60% EC against field population of engorged adult female *Rh.pulchilus* and *A.gemma* ticks in in-vitro condition using AIT.
- ❖ To assess the perceptions of pastoralists' communities on the management, ways of acaricide usage and delivery; and methods of tick control practiced in the area.

2. LITERATURE REVIEW

2.1 Classification of ticks

Ticks are within a member called phylum (Arthropoda), class (Arachnida), sub class (Acari) and Order (Parasitiformes) (Torr *et al.*, 2003). Within the Parasitiformes, ticks belong to suborder Ixodida, which contains a single super family, Ixodoidea. This is divided into two major families, Argasidae and Ixodidae; and the rare family Nuttalliellidae, with a single African species (Rodriguez-Vivas *et al.*, 2004).

The family Ixodidae, or hard ticks, contains some 683 species. As adults, ixodids 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 (Lora, 2001). Ixodidae ticks are relatively large and comprise thirteen genera. Seven of these genera (*Boophilus*, *Amblyomma*, *Rhipicephalus*, *Haemaphysalis*, *Hyalomma*, *Dermacentor* and *Ixodes*) contain species of veterinary and medical importance (Rodriguez-Vivas *et al.*, 2004).

The family Argasidae, soft ticks, consists of 185 species worldwide and has 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 (Lora, 2001; Barker and Murrell, 2004).

2.2 Morphology of ticks

The scientific nomenclature (ixos gr.=fly glue, Ixodes=glued) indicates ticks are sticking and stationary parasites. They belong to the super order of Acarina, which have certain characteristics that distinguish them from other arachnids such as spiders. Many ticks resemble spiders in that both groups lack visible abdominal segmentation. However, in spiders the mouthparts are inserted anteriorly on a structure called the cephalothorax, which comprises fused head and thorax. Legs are borne on cephalothorax, which is connected to the abdomen by a narrow pedicel. In contrast, the mouthparts of ticks form a discrete anterior

gnathosoma or capitulum (the "head"), and that part of the body on which the legs are inserted (the podosoma) is broadly joined to the portion of the body behind the legs (the opisthosoma) to form idiosoma (the "body") of the tick (Walker *et al.*, 2003). Ixodid 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, the nymph and larvae to permit the abdomen to swell after feeding (Barker and Murrell, 2004). A more detailed key for morphological features important for identification was given by Walker *et al.* (2003).

2.3 Basic biology of ixodid ticks

The ixodids ticks spend most of their life away from their hosts (Horak *et al.*, 2002). Hard ticks, and a few species of soft ticks, seek their hosts by climbing vegetation, grass, weeds, or brush, and waiting for a suitable animal to pass. In a behavior known as questing, the first pair of legs is extended and used to grasp the host when contact is made. The height at which questing takes place determines the size, and therefore the species, of host selected. Questing heights vary between tick species and among the different life stages of any given species. Adult ticks of the genera *Amblyomma* and *Hyalomma* are active hunters and will run across the ground to seek hosts that are resting nearby (Lora, 2001; Jonsson, 2006).

All feedings of ticks at each stage of the life cycle are parasitic. For feeding, ticks 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 (Horak *et al.*, 2002; Torres, 2008). The feeding of ixodid ticks is slow because the body wall needs to grow before it can expand to take a very large blood meal. Larvae take typically 3 to 5 days to fully engorge with blood; nymphs 4 to 8 days, and females 5 to 20 days. When the ticks have fully engorged with blood they detach from the host skin and drop to the ground. Males of most types of ticks feed but do not expand like the females. They feed enough for their reproductive organs to mature (Minjauw and McLeod, 2003).

Several characteristics of ticks make them outstanding vectors of pathogenic agents. Their wide host range and tendency to feed on several hosts during their life cycle ensures ample opportunity to acquire and transmit pathogens. Their hardiness and longevity enable them to survive long periods of unfavorable environmental conditions (Torr *et al.*, 2003). Ticks those

with high reproductive potential has been also ensuring maintenance of large populations and a high frequency of host-vector contact (Horak *et al.*, 2002). Because of ticks feed slowly and, in the case of ixodids, attached to hosts for relatively long periods, which allow sufficient time for pathogen acquisition and transmission, as well as tick dispersal by migrating or wandering hosts (Jonsson, 2006).

2.4 Life history of ixodid ticks

All ticks have a six-legged larval stage, one or more eight-legged nymphal stages and an eight-legged adult stage. Ixodids ticks have a single nymphal stage, while argasids may have as many as eight. In both families, all stages are feed on blood. Hard ticks generally take one blood meal per stage, followed by molting, and adult females oviposit (Rodríguez-Vivas *et al.*, 2004). Mating takes place on the host, except for *Ixodes* where it may also occur when the ticks are still on vegetation (Walker *et al.*, 2003). After attachment to host the female is inseminated once, before it is ready to fully engorge with blood, and subsequently completes its single large blood meal, but the males feed intermittently and mate with females repeatedly (Latif and Walker *et al.*, 2004). Soft ticks feed intermittently, and adult females may feed and oviposit several times (Minjauw and McLeod, 2003).

During mating, the male ticks crawls under female and after manipulating the genital opening with its mouthparts, transfers the spermatheca, a sac containing spermatozoa, into the opening, presumably with aid of its front legs (Furlong *et al.*, 2007). When the females fully engorged they have enough sperm stored to fertilize all their eggs. Then detaches from the host and averagely deposits up to 18,000 eggs/female in a single batch. The oviposition occurs after a period of digestion and oogenesis in natural shelter, under protective cover of grasses, stone, in plant litter, etc (Sathaporn *et al.*, 2004).

The life cycle of hard ticks varies on the species of tick, level of environmental temperature and humidity, and presence of hosts suitable for reproduction by the adults (Rodríguez-Vivas *et al.*, 2004). After a single oviposition the emptied females die. Under suitable conditions (mostly hot and humid), the egg hatches and six-legged larva emerges (Kirby, 2010). After a period of maturation, it begins to seek the first host, either lying in wait on a grass blade, or moving to hunt actively. Once the host is found, taking of the blood meal lasts for 3-12 days

or more, depending on the species and ambient conditions. Then the larvae moult to nymphs, which is eight-legged. Passing through a period of maturation by feeding, the larvae moults to nymph and sexually differentiated adults developed from these nymphs (Lora, 2001).

Ticks have evolved a great variety of host relationships but are commonly classified as “one-host,” “two-host” and “three-host” species (Walker *et al.*, 2003). Important Ixodids ticks that feed on cattle and according to the number of hosts required to complete their life cycle are listed on Table-1.

One-host ticks complete all feeding and molting on a single animal, usually a large-sized, wandering host; mainly bovines and equines (Minjauw and McLeod, 2003). This strict host specificity is uncommon, being seen in only about a dozen tick species (mostly ixodids), though these may be of great economic importance. In this tick group, eggs are laid on soil. Larvae hatch after several weeks of development and crawl on vegetation to quest for a host (Horak *et al.*, 2002).

In two-host ticks, the molt from larva to nymph occurs on the first host, which is usually a small mammal or bird, and the engorged nymph drops to ground, where it molts to adult stage. The adult must then find a second, usually much larger host. Again, there are only about a dozen two-host ticks; almost all are ixodids and several associated with livestock (Kirby, 2010). All other ixodids are three-host ticks that detach after engorging at each life stage, with molts taking place off the animal. A variety of hosts may be utilized by each life stage and thus, increasing the likelihood that tick will acquire pathogens. But, larvae and nymphs are usually feed on smaller hosts than adults (Silk, 2009).

Table 1: Important Ixodids ticks according to the number of hosts needed for their life cycle

Tick genera	One host tick	Two host tick	Three host tick
Boophilus spps	<i>B. decoloratus</i> , <i>B. annulatus</i> , <i>B. microplus</i>		
Amblyomma spps			All species of <i>Amblyomma</i>
Hyalomma spps	<i>H. scupense</i>	<i>H. m. turanicum</i> , <i>H. d. dentritum</i>	<i>H. truncatum</i> , <i>H. a. anaticum</i> , <i>H. dromedarii</i> , <i>H. m. marginatum</i> , <i>H. excavatum</i>
Rhipicephalus spps		<i>R. e. evertsi</i> <i>R. bursa</i>	<i>Rh. simus</i> , <i>Rh. pravus</i> , <i>Rh. pulchilus</i> , <i>Rh. Appendiculatus</i>
Haemaphysalis spps			<i>Hae. punctata</i>
Ixodes spp.			<i>I. pilosus</i> , <i>I. ricinus</i>
Dermacentor spps	<i>D. albipictus</i>		Most species

Source: Walker *et al.* (2003) and Kirby (2010)

2.5 The epidemiology and factors affecting tick survival

The most important situation to be considered in the epidemiology of ticks is categorized into free-living developmental phase, host finding phase and parasitic phase. Ticks habitat is composed of a variety of living and non-living things in the space in which they lives. That is ticks are adapted to two extremely contrasting components of their habitat: the physical environment and their hosts. Tick populations in any particular environment, with a particular type of animals and management system, fluctuate about a long-term average size (Zahid *et al.*, 2005; Silk, 2009). The size of the fluctuations depends partly upon the extent of variation in environment, but also upon the properties of the tick population. The population dynamics of any organism are the product of the interaction of the demographic process, birth and death, which are determined, by both abiotic (climatic) and biotic (tick density) interactions (Latif and Walker, 2004).

2.5.1 The environment

Abiotic and biotic variables suggest that it is the stage from female to larvae, which is most sensitive to adverse abiotic conditions, specifically low moisture availability, and dryness. One of the most important ecological factors influencing occurrence of ticks in biotope is climate, which is a dynamic factor (Latif and Walker, 2004). The much greater seasonal variation in climatic conditions, particularly temperature, introduces variable development rates and diapauses into the life cycle. Most ticks cannot tolerate direct sunlight, dryness, or excessive rainfall; tick activity decreases during the cold months and increases dramatically during hot time. The proportion of tick development, which takes place each day, depends on the temperature of its surrounding. Temperatures for most tick a range between 18°C to 30°C or suitable below or above survival and development are affected high land species or dessert species have a different range (FAO, 2004). Even if the same factor affects the survival of all tick species to varying degrees, each species has its particular threshold temperature below that diapauses occurs in all in-stars. Obviously, progress of the rate of development is directly related to temperature (Minjauw and McLeod, 2003).

Another important climatic factor influencing the occurrence and survival of ticks in a biotope and is a static factor is relative humidity (Walker *et al.*, 2003). Normally, ticks require as high humidity as they can get about 80%, is the most favorable. Egg and larval development, and egg production in engorged females are inhibited, while immature or unfed adults become quiescent (Radostits *et al.*, 2006).

The activity of most larvae is restricted during cold period, but increases dramatically during hot and humid time. Relative humidity at microclimatic level is essential for the development and survival of eggs and larvae. Each species is adapted to a particular relative humidity in a biotope and it varies with the instars and its size (Desie, 2005). Immatures have very specific requirements, whereas the adults can protect themselves better against evaporation because of their larger size and thicker tegument. As compared to eggs, engorged larvae and nymphs are less susceptible to desiccation, and their success rates in moulting are usually high in the absence of extreme temperatures or dryness. The number of females attached to the host is the most significant indicator of the seasonal activity and population dynamics of ticks (Minjauw and McLeod, 2003; Jonsson, 2006).

2.5.2 Host preference and availability of suitable hosts

The survival of tick population is more dependent on the presence of maintenance hosts suitable for perpetuation. Hence, whenever ticks couldn't get host they will die off starvation. Ticks are limited in variety than the hosts on which larvae and nymphs of three-host ticks can survive; and more limited than those on which adult may attempt to feed but not necessarily survive (Walker *et al.*, 2003). The total proportion of a given tick population, which find a host, under continuous grazing, depends on the rate at which ticks are swept up by hosts and on their average survival time (Desie, 2005).

In Ixodids ticks, the degree of host specificity varies from genera to genera or within certain subgroups of various genera. Hosts are usually in a group of similar species. For instance, all the *Rhipicephalus species* are adapted to feed on cattle, but some may survive by feeding on sheep or antelope (Walker *et al.*, 2003). The majority of ticks, at least 700 of 850 ixodids species, are characterized by more or less strict host specificity and some ticks have adapted to a wide range of hosts. While, others become host specific, *Boophilus species* (Lora, 2001).

Currently, cattle constitute the only available hosts for ticks in an environment transformed by modern agricultural and livestock production system (Kirby, 2010). But, these ticks are not specific to cattle; they were originally associated with either local fauna, rodents and ungulates, which later replaced by cattle, partially or completely (Morel, 1989). It is known from previous studies of Hoogstraal (1956), that *A. cohaerens* was east African buffalo tick. But, now it is frequently reported that it attack cattle in areas where buffalo disappeared due to various reasons.

2.5.3 Cattle resistance

Resistance of cattle to tick infestation was reported to consist of innate and acquired components. The defense mechanisms: tick avoidance, grooming, skin characteristics and more specific immunological responses, are involved in reducing number of ticks parasitizing cattle. Avoidance was attributed to the sighting of ticks (Lora, 2001).

Spontaneous or acquired resistance may be following infestations, due to the development of cutaneous hypersensitivity. The mechanism responsible for acquired resistance to ticks has been suggested to be a mast cell-dependent eosinophil hypersensitivity (Minjauw and de Castro, 2000). Resistance can be passively transferred with viable lymph node cells but not with serum from resistant hosts. This passage method of tick resistance suggests a delayed hypersensitivity mechanism for the acquisition of resistance (Lodos *et al.*, 2004).

The blood histamine level have been found to be elevated as a result of cutaneous basophiles or mast cells increase in resistant hosts, which has shown to stimulate detachment of tick larvae. But, the infestation rate increases in normally resistant zebus either due to true inhibition by the allergy or by reduced cutaneous pruritis that halts licking that enables to kill larvae by resistant cattle (Torr *et al.*, 2003; Jonsson, 2006).

It has been recognized that various breeds of cattle differ in their response to tick infestations. *Bos indicus* purebreds and crossbreeds were reported to be more innately resistant than *Bos taurus* breeds (Minjauw and de Castro, 2000). According to the observation of these authors, African cattle (*Bos indicus*) are naturally self-groom or groom each other frequently and thoroughly. Therefore, significantly fewer ticks were found on those animals that were able to groom. Some cattle breeds have the ability to reduce the number of ticks they carry and are considered resistant. While, others cannot control the number of ticks they carry and are referred to as sensitive breeds (Latif and Walker, 2004; Zahid *et al.*, 2005).

Previous study conducted to compare tick resistance in 3 breeds of cattle, two indigenous breeds (Arsi and Boran) and one cross breed (Boran x Friesian) following natural infestations at Abernossa ranch in Ethiopia; showed Arsi breed have highest tick resistance and carried 10.3% of all ticks collected. The least resistant cattle, Boran x Friesian, were carried large number of ticks with highest survival periods. However, the most resistance indigenous cattle carried lower number of ticks with lowest survival periods (Solomon and Kaaya, 1998).

A number of physiological and environmental factors can affect the level of host resistance to ticks or the expression of host resistance. Nutrition, sex, pregnancy, lactation, age, exposure to ticks, breeds and tick densities are play key roles (FAO, 2004). Cattle loss resistance with time, and it seems that the older the animal, the lower the resistance. The study conducted by

Okello-Onen *et al.* (1999) on indigenous cattle in a pastoral dry to semi-arid rangeland zone of Uganda showed that calves had very low numbers of ticks. The author also observed more ticks on lactating cows carried than non-lactating cows, except for *Amblyomma variegatum*, where the opposite was true. Pregnant cows were significantly carried a higher number of ticks more than non-pregnant female, mainly during the late stages of pregnancy. The stress of lactation causes a marked decline in the resistance of exotic breeds; it also affect Zebu, but to a much smaller extent. Stable resistance is acquired after several months of exposure to the species of tick to which resistance is required (Minjauw and de Castro, 2000).

2.5.4 The properties of tick population

Most ticks have adaptations in their behavior and physiology of feeding to reduce the host reactions. Two different mechanisms always operate with intensities that vary in proportion to the sizes of tick populations. First, the success rate of larval ticks on pastures in finding a host is strongly dependent upon the density of larvae in the pastures. At high population densities, cattle are able to detect and avoid dense clumps of larvae on the pastures. The growth of tick populations is therefore slowed progressively as the density of larvae on the pasture increases. Secondly, once the larvae succeed in attaching, their success in feeding on that animal will also dependent upon the number of larvae. About 10% of larvae attaching to *Bos indicus* were survive to maturity when 100 larvae attach, whereas only 1% of larvae survive when 20,000 larvae attach. Similarly for *Bos taurus* up to 30% of the larvae may survive at a density of 100-larvae/ animals but only 10% when 20,000 larvae attach (Latif and Walker, 2004).

2.6 The distribution of ticks in Ethiopia

In Ethiopia, studies on tick fauna have begun early in the 19th century (Pegram *et al.*, 1981). Since then, different researchers from abroad and country determine the pattern of ticks and TBDs; and ticks are common in all agro-ecological zones of the country (Pegram *et al.*, 1981; Morel, 1989; Sileshi, 1998). The main tick genera found in domestic animals of Ethiopia are *Amblyomma*, *Hyalomma*, *Rhipicephalus*, *Haemaphysalis* and *Boophilus* (Sileshi *et al.*, 2007).

The most commonly encountered tick genus on cattle is *Rhipicephalus*. Among this genera *Rhipicephalu lunulatus* and *Rh.bergeoni* species were observed in Central Ethiopia (Sileshi *et*

al., 2001), *Rh. muhasmae* in Borena (Regassa, 2001), in wetter western areas of the country (Pegram *et al.*, 1981; de Castro, 1994) and in Arsi (Morel, 1989). *Rhipicephalus lunulatus* parasitizes chiefly cattle and is active only during rainy season (Pegram *et al.*, 1981; Sileshi *et al.*, 2001). Seyoum (2001) was recorded *Rh. humoralis*, *Rh. cliffordi*, *Rh. compositus* and *Rh. distinctus* in Girana valley of Wollo and Northeast areas. *Rhipicephalus cliffordi*, *Rh. compositus* and *Rh. Senegalensis* recorded from western Ethiopia by Pegram *et al.* (1981) and Eastern Ethiopia by Bekele (2002).

Rhipicephalus evertsi evertsi, “Red-legged tick” (Okello-Onen *et al.*, 1999; Walker *et al.*, 2003), is the most widespread species of *Rhipicephalus*. This species was recorded in Borena pastoral area (Solomon *et al.*, 2004; Regassa, 2001), in Western Shoa by Solomon *et al.* (2003), in Central Ethiopia by Sileshi *et al.* (2003); in Western Ethiopia by de Castro (1994), in Eastern Ethiopia (Bekele, 2002), in Metekel Ranch (Sinshaw, 2000), in Asella (Assefa, 2004), in Awassa area (Berhane, 2004) and in Jimma (Getachew, 2004).

Rhipicephalus pulchellus, “Zebra tick” (Okello-Onen *et al.*, 1999; Walker *et al.*, 2003), was recorded as widely distributed in north eastern (Seyoum, 2001), eastern (Bekele, 2002; Zeleke and Bekele, 2004; Rahmeto *et al.*, 2010) and southern rangeland (Solomon *et al.*, 1998; Regassa, 2001; Solomon *et al.*, 2004) part of the country. However, it is often abundant on cattle in Rift Valley and eastwards inhabiting dry, semi-arid and bush land (Pegram *et al.*, 198; Walker *et al.*, 2003).

Rhipicephalus simus is known as “Glossy tick” (Walker *et al.*, 2003). In Ethiopia, it was found in northern (Sinshaw, 2000), eastern (Bekele, 2002; Zeleke and Bekele, 2004, Rahmeto *et al.*, 2010), southern rangeland (Regassa, 2001; Solomon *et al.*, 2004), central (Pegram *et al.*, 1981; Sileshi *et al.*, 2001) and western parts (Pegram *et al.*, 1981; Solomon *et al.*, 2001).

Of the genus *Amblyomma*, four species are commonly infest cattle and includes *Amblyomma variegatum*, *A. gemma*, *A. lepidum* and *A. cohaerens*; and all are known to exist in Ethiopia (Morel, 1989; Bekele, 2002; Walker *et al.*, 2003; Sileshi *et al.*, 2007). The previous observations in Borena Dide-tuyura ranch by Solomon *et al.* (1998) and by Regassa (2001) in Borena zone showed that *A. variegatum*, *A. gemma* and *A. lepidum* distributed in wider areas.

From the observations of Abebaw *et al.* (2004) and Getachew (2004) in Jimma areas and Belay (2004) around Mizan-Teferi *A.variegatum* and *A.cohaerens* are widely distributed in south western Ethiopia. *Amblyomma variegatum* and *A. cohaerens* were recorded as the two most prevalent species in Awassa areas in decreasing order (Berhane, 2004). While, in eastern Ethiopia, *A.variegatum* and *A.gemma* are the two most widely spread species (Bekele, 2002). *Amblyomma variegatum*, *A.cohaerens*, *A.lepidum* and *A.gemma* were *Amblyomma* ticks abundant in central Ethiopia (Sileshi *et al.*, 2001).

Amblyomma gemma, "Gem-like bont tick" (Susan, 1998), is recorded in eastern and southern Ethiopia (Pegram *et al.*, 1981; de Castro, 1994; Walker *et al.*, 2003). It is clearly associated with dry types of vegetation or semi-arid rangelands (Pegram *et al.*, 1981; Morel, 1989). *Amblyomma lepidum*, "East African bont tick"(Susan, 1998), is most commonly inhabits arid habitats and in open bushed shrub or wooded grassland and its distributions overlap with *A. gemma* and *A. variegatum* (Walker *et al.*, 2003). *Amblyomma cohaerens* is hardly abundant at lower altitude and rainfall (de Castro, 1994; Morel, 1989).

Two species of *Boophilus* genus are known to exist in Ethiopia, which include *Rhipicephalus (Boophilus) decoloratus* and *Rhipicephalus (Boophilus) annulatus*. Various previous studies conducted in different parts of the country indicated the distribution of *B.decoloratus*. Likewise, in Borena area (Solomon *et al.*, 1998; Regassa, 2001), in central Ethiopia (Sileshi *et al.*, 2001); in Alemaya (Bekele, 2002), in Asella (Assefa, 2004), in Awassa (Berhane, 2004), in Jimma (Abebaw *et al.*, 2004; Getachew, 2004), in Wolayta (Desie, 2005), in Asella (Abebaw and Tamiru, 2010), in western Shoa zone (Solomon *et al.*, 2003), in central Ethiopia (Sileshi *et al.*, 2001), in Wollo (Seyoum, 2001) and in eastern zone (Bekele, 2002). *Rhipicephalus (Boophilus) annulatus*, "Texas fever tick" (Walker *et al.*, 2003), is known to present in Gambella region and recorded by Pegram *et al.* (1981) and de Castro (1994).

In Ethiopia, about eight species of *Hyalomma* genera that affect cattle are identified, which includes *Hyalomma marginatum rufipes*, *Hy. dromedarii*, *Hy. tuncatum*, *Hy.m. marginatum*, *Hy. impelatum*, *Hy. anaticum excavatum*, *Hy.anaticum anaticum* and *Hy. albiparmatum* (Pegram *et al.*, 1981; Solomon *et al.*, 1998; Sinshaw, 2000; Regassa, 2001; Sileshi *et al.*, 2001; Seyoum, 2001; Bekele, 2002; Assefa, 2004).

Haemaphysalis aciculifer and *Haem. parvata* species of the genus *Haemaphysalis* were found in Ethiopia and distributed in all parts of the country (Pegram *et al.*, 1981; Seyoum, 2001; Sileshi *et al.*, 2001; Walker *et al.*, 2003).

2.7 Tick control methods and their limitation

The aim of tick control campaign is not to control all ticks simultaneously, but a definite species because of its particular role (FAO, 2004). The successful implementation of rational and sustainable tick control programmes in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of tick as it interacts with the host in specific climatic, management and production environments. In most situations, however, efficient and reliable control of ticks and TBDs are based on the use of acaricide application, often without a local understanding of ecology or epidemiology (FAO, 2004, Alanr, 2011).

It is now generally understood that tick control should not only be based on acaricide use, despite the fact that this remains the most efficient and reliable single method of tick control. Complementary approaches have been developed to act in addition or in synergy and are being researched to enable integrated control strategies against ticks and its haemoparasites (Kirby, 2010; Alanr, 2011). Ideally, efficient control strategies should target the parasitic and free-living phases of the life cycle and the role of ticks in the transmission of diseases should not be neglected. Thus, the influence that any of tick control strategies might have on the enzootic stability of diseases always is included in decision making process (Solomon, 2001).

The most common methods used as integrated tick control are ecological, biological, genetic and mechanical control (use of repellents) and natural predation. The availability of each of these options, their advantages and disadvantages, and the cost benefit of each alternative strategy should be assessed before deciding on a type of control programme (Kirby, 2010). These integrated tick control methods are described as follows:

Ecological tick Control:

Ecological control method is used for habitat and host linked treatment. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that

shelters ticks (Kirby, 2010). Walker *et al.*, (2003) stated that pasture management, including spelling and seasonal changes in cattle grazing areas has been used as a tick control strategy and are believed to be responsible for a decrease ticks burden.

Biological tick Control:

A first attempt at tick biocontrol was made with the introduction of tick parasitic wasps from France to USA and Russia. During the past decades, interest in developing antitick biocontrol agents such as birds, parasitoides, entomopathogenic nematodes, entomopathogenic fungi and bacteria have gained momentum (George *et al.*, 2008). In biological tick control, the activities of the hyperparasites chalcid flies *Hunterellus* are probably important in nature, but they are difficult to evaluate and it is still more difficult to manipulate or reproduce them for practical use. The biological agents, which potentially include predators like rodents, birds, ants, spiders, lizards and beetles as well as parasites (Nematodes and fungus) are attack soil living stages of ticks and depending on the conditions, these predators can consume a large number of ticks (Latif and Walker, 2004; Kirby, 2010). Yet, having such effective importance, the development of biological tick control methods has been neglected (Samish and Alekseev, 2001).

Genetical tick Control:

The Australian experience has shown that integrated control, involving a combination of strategies, is necessary because of the development of tick to acaricide. Integrated control has led to the widespread use of tick-resistant zebu cattle and vaccines against TBDs (Santana, 2000; Silk, 2009). A tick-vaccine named "TICK GUARD" is available on the market mainly in Australia and Latin America for the control of *B. microplus*, which is prepared from antigens of tick gut multiplied with genetically engineered *Escherichia coli*. It acts essentially by damaging tick gut thereby reducing tick fertility. The vaccine has been evaluated during the last 10 years and has demonstrated its safety as part of an integrated tick control programs. Unfortunately this vaccine is developed specifically for *B. microplus*, which is the only major tick species of that country (Xu *et al.*, 2005).

2.7.1 Chemical of tick control

Acaricide treatments are commonly used in a *suppressive approach*, applying multiple treatments at regular intervals during high infestation. Suppressive treatments are the most effective in short term; keeping animals almost tick free, thereby reducing the direct effect of ticks and risk of disease transmission. This approach will, however, select heavily for acaricide resistance in ticks. It will also leave cattle susceptible to TBDs (George *et al.*, 2008).

Alternatively, other application method of acaricide is initiated when tick infestations exceed an acceptable level and is known as a *threshold approach*. Threshold treatments may result in some direct production losses, depending on the level of tick infestation permitted before treatments are initiated. There should be a lower probability of TBDs outbreaks, because acquired immunity to these is maintained due to higher levels of exposure of cattle to ticks infected with haemoparasites (Garcia-Garcia *et al.*, 2000; George *et al.*, 2008).

Where there is sufficient ecological information and annual population trends enable it, a *strategic approach* can be used. A strategic approach involves the treatment of specified generations of ticks, usually the first generation in a tick season, even when infestation is at a low level that does little or no damage to the host. The aim is to remove the source of future generations for that year, thereby reducing or eliminating the need for further chemical treatments later in the season. Strategic treatments are more efficient in reducing direct production losses. Overall, the strategic and suppressive approaches might increase the risk of outbreaks of TBDs (Garcia-Garcia *et al.*, 2000; FAO, 2004).

The recent philosophy of minimizing the use of chemical acaricide does not diminish their key role in integrated tick control systems (Kirby, 2010). An ideal acaricide would be cheap, easily applied, with a strong knock down effect and sufficient residual effect on female ticks to prevent egg laying and to protect cattle from reinfestation by larvae. It should not select for resistance through a prolonged, gradual decay on the animal. And also, it should be non-toxic to livestock and humans and have no detectable residues in meat and milk. Unfortunately, such an ideal acaricide has not yet been produced. Generally, although the use of acaricides has limitations and tick resistance to acaricides is an increasing problem and real economic threat to the livestock worldwide, most livestock holders depend completely on acaricides, but

do not have access to guidelines on how to make a profit from their tick control program or how to detect and resolve problems with resistance to acaricides (George *et al.*, 2008).

The choice of acaricide formulation and method of application depends upon acaricide availability and price, the size of the farm and extent of facilities, the production system and the degree to which ticks are seen to be a problem. There are different methods of acaricide application on cattle. Among them the most commonly used method includes manual spraying, hand dressing, dipping, various modes of sprays and subcutaneous injection.

2.7.2 Acaricide resistance in ticks

Resistance in tick was first recognized in 1963 and several tick species are known to be resistance to mainly organophosphorous acaricides (Wharton, 1967). Organophosphates were introduced around 1950, as a replacement for the chlorinated hydrocarbons to which significant resistance had occurred (Shanahan and Hart, 1966). The resistance of ticks to acaricides is an inheritable trait/phenomenon (FAO, 2004), which is achieved by target site insensitivity to acaricides and increased metabolic detoxification by enzymes (Rosario-Cruz *et al.*, 2009). Resistance is usually first recognized as a failure of treatment to eliminate adult tick burdens. Although failure of treatment is often the result of incorrect preparation or application of acaricide, the persistence of ticks after frequent, correctly applied treatments indicates that acaricide resistance is likely. Acaricide resistance is fostered by any approach that uses an increased number of acaricide treatments or one in which a higher proportion of the total tick population is treated at any one time (Pegram *et al.*, 2000).

Due to the use of chemical acaricides has certain drawbacks (Garcia-Garcia *et al.*, 2000) and high cost of research and inevitably slow development of acaricidal substances by the chemical industry as the development of new acaricides is a long and expensive process, which reinforces the need for alternative approaches to control tick infestations (Graf *et al.*, 2004). The number of effective acaricides available at any time has to be regarded as being limited. This number is continuously reduced through development of resistances to new compounds. In some areas, ticks may also be in contact with insecticides aimed at other pests (e.g. agricultural pests, mosquitoes or tsetse flies), which could have an effect on resistance to related acaricidal substances. Once tick population has become genetically resistant or cross-

resistant to an acaricide, there is usually no going back to that acaricide, even after many years. Angus (2003) indicated that resistance to arsenicals was developed in many species of ticks and it was replaced by chlorinated hydrocarbons.

There is a potential for resistance in every livestock tick species to all acaricides and ticks may be resistance to acaricides in one of these three types: ***Vigor tolerance***: Non-specific and is not controlled by a genetic mechanism. The slight increase in tolerance may be due to thicker cuticle, an increase of fat content of ticks etc. Seasonal variation susceptibility may be attributed to this mechanism. ***Behavior resistance***: This is the ability of ticks to avoid acaricide residues. This may be a natural characteristic or may be an induced behavior by the presence of acaricide (Irritability may play a role in this phenomenon). ***Physical resistance***: this type of resistance include less penetration of acaricide through the cuticle, increase metabolism of acaricide into non toxic products, storage of acaricide in tick fat body, excretion of acaricide and decreased sensitivity of target site (Furlong *et al.*, 2000).

2.7.3 Factors influencing rate of acaricide resistance

Internal Factors: which includes, frequency of mutation: the more mutations there are in a given population the faster resistance will be expressed. Genetic variability: the greater the genetic variability, the more chances for recombinant genes occurring leading to mutation. Degree of dominance: if the mutated gene is a heterozygous, and then the mutation will take a long time to express it. It will exhibit itself a weak resistance. A dominant gene develops faster in a population (FAO, 2004; Rosario-Cruz *et al.*, 2009).

External Factors: Persistence of acaricides: acaricide with a short residual protective period have a better advantage of not developing resistance quickly because they are biodegraded in the environment. However, they have a disadvantages because their concentrations become reduced with time, thus subjecting ticks to weak concentrations and hence development of resistance. Frequency of use: frequent use of acaricides hastens the selection process, thus leaving resistant individuals in population. Under strength acaricide: acaricides used at sub-optimal concentrations have disadvantage of leaving heterozygous individual in tick population. Previous use of similar acaricides: if the new acaricides has a similar mechanism of action, thus facilitates resistance (Garcia-Garcia *et al.*, 2000; Graf *et al.*, 2004).

2.8 Chemical tick control and acaricide resistance in Ethiopia

Acaricidal spray has been the commonest method of tick control in Africa since 1890. Similarly, in Ethiopia acaricide application is still the main tick control method. A multitude of acaricide is used and their application seems to be regulated primarily by their availability. Apart from the use of chemical compounds for tick control, certain cultural practices, such as the hand picking of ticks, burning with a hot iron, and application of plant preparations, are widely used by cattle owners in rural areas. Ticks on indigenous cattle are treated whenever the farmers bring their animals to the veterinary clinics either for tick control or for other complaints (Sileshi *et al.*, 2004). In the country, there is no planned programme of tick control except on dairy farms. The majority of tick infestations on local cattle is mild and can be solved by spraying on a localized part of the animal such as axilla, ventrum, and abdomen or udder- common sites for *Amblyomma* ticks (Sileshi, 2001; Yilma *et al.*, 2001).

In the country there are different methods of acaricide application. Manual spraying, hand dressing, dipping, sprays races and subcutaneous injections are among most commonly used methods (Yilma, *et al.*, 2001). According to Sileshi (2001) and Yilma *et al.* (2001), some of the acaricides available in the country are:

Organophosphorous group: Quintofos (Bacdip and Bayer, tick grease), Dioxathion (Bercotox), Diazinon 60%EC and Chlorfenvinphos (Steladone), used on indigenous zebu cattle, with occasional treatment on *Amblyomma* feeding sites such as udder and scrotum.

Carbamate group: Carbaryl (CBM-8) used mainly by private dairy farms where crossbred cattle are kept and acaricide application is irregular.

Synthetic Pyrethroids group: Flumethrin (Bayticol, pour-on) and deltamethrin, Cypermethrin (spot on), used intensively in state dairy farms where exotic and crossbred cattle kept.

In Ethiopia some resistance strains have emerged for instance. Adamu (1996) has indicated that *B.decoloratus* strain was found to be resistant to Deldrine, Diazinon, Chlorfenvinphos and Coumpos. The finding of organophosphate (Diazinon and Coumpos) resistance is the first report of its kind in the country. It is generally presumed that resistant tick population are

emerging at increasing rate in the area warranting for serious attention and providing a fertile ground for future studies (Xu *et al.*, 2005). Therefore, the application of acaricide must be carefully managed in order to maintain as a reserve of effective acaricides as possible, through measures aimed at delaying the onset of resistance and making the best use of each acaricide before resistance makes it obsolescent.

2.9 Acaricide resistant recognition and testing methods

Acaricide resistance in ticks is a widespread phenomenon recognized by farmers, animal scientists and veterinarians in most areas where treatment of livestock against ticks is carried out. The initial problem that must be faced, when a control measures failure occurs, is to ensure that the apparent loss of chemical efficacy is due to procedures which are involved in chemical tick control (George *et al.*, 2008).

As an interim measure, when a field infestation occurs and resistance is suspected there is a lot to be gained by carrying out a closely controlled hand spray test. Adult tick counts on the infested animals, before and after the treatment, on three occasions, will provide a good guide as to whether resistance presents, or whether other possible causes are contributing to the problem. It is only when the results of such field trials are known, and these having been confirmed in the laboratory tests, that one considers an acaricide change (Castro-Janer *et al.*, 2009). The delay between suspicion of resistance, and the availability of test results, represents a problem in determining the significance of resistance mechanism, and the need and choice of an alternative compound. Unfortunately, this delay often precludes the chance of implementing strategies to contain or eliminate the newly emerged resistant strains while it represents only a small proportion of the tick population (Jonsson *et al.*, 2007).

Although there are a variety of acaricide resistance testing methods exists, a lack of standardized techniques for diagnosis appears to be the main difficulty in creating and maintaining a tick resistance monitoring system (FAO, 2004). For a suitable acaricide resistance testing, the test should be sensitive enough to identify resistance early in its emergence. It should also cover the full range of chemical groups that are in use, including the most recently developed active ingredients. However, all involves extensive facilities, infrastructure, and intensive standards and associated high costs. But, the diagnostic test

should be simple and inexpensive and provide a rapid and reliable result, and be suitable for standardization among laboratories in many countries. The most widely used *in vitro* tests are bioassays applied to larvae and engorged female ticks (FAO, 2004; Jonsson *et al.*, 2007).

A questionnaire that was circulated by the FAO Working Group on Parasite Resistance (WGPR) revealed that the most widely used methods for resistance diagnosis in the laboratory was AIT. This group recommends AIT proposed by Drummond *et al.* (1973) for the evaluation of susceptibility to acaricides in tick populations, since it is fast, simple and a relatively cheap method for detection of acaricide resistance in the field. Despite, the high variation of AIT results obtained for SP, OP, Amitraz and moxidectin; AIT is a valuable tool for rapid and cheap detection of loss of susceptibility to a certain acaricide and should be used with this specific objective in resistance management programs (Jonsson *et al.*, 2007).

In-vitro tests:

However, there are several tests are in use for the diagnosis of acaricide resistance in ticks, none of the tests is perfect in all circumstances. But, for the success of any tick management strategy, it is necessary to use a test that is practical, quick, economical and reliable to detect presence of resistance in target population. In-vitro bioassays are relatively simple to perform, have a low cost, and have few requirements for specialized equipment. The most frequently used techniques to detect resistance in cattle tick are: the AIT (Adult Immersion Test), LPT (Larval Packet Test) and LIT (Larval Immersion Test) (Castro-Janer *et al.*, 2009).

Engorged Female Adult Immersion Test (AIT):

It is a bioassay applied to engorged adult female ticks which are immersed in technical or commercial acaricides and are based on rate of oviposition between females of two groups, treated and control. The eggs are analyzed by weight and viability. Additionally, direct mortality can be taken into consideration by comparing females that oviposit or not. In this case, the results will be obtained in 1–2 weeks in contrast with percentage of hatching (5–6 weeks). Although there are many AIT techniques published, the test that is used mostly for the evaluation of resistance at field is adult immersion technique modified by FAO (2004). But, the main limitation is the number of engorged females required to obtain reliable results

(Jonsson *et al.*, 2007). Enthusiasm for the development of standard protocols for modified AITs has been driven by the ease with which the test can be done, lack of any special equipment, and the fact that the test can be completed within only 7 days and this test mimicked the field condition better than the SLIT (Jonsson *et al.*, 2007).

FAO (2004) adopted Larval Packet Test (LPT):

Larval tests are a better alternative to the AIT, because of the high number of specimens used in the experiment greatly increases the power of the statistical analysis over AIT. For this reason, it is possible to accurately and repeatedly test a range of concentrations of several acaricides, if necessary. The LPT cannot be used for all acarine growth regulators and LPT is a laborious test that takes 5–6 weeks to complete and requires a significant amount of monetary and routinely. Therefore, LPT has not been adopted in all countries. In addition, neither molecular nor biochemical assays have yet been developed to the point where they are useful for diagnosis of resistance in the field (FAO, 2004; Jonsson *et al.*, 2007).

Shaw Larval Immersion Test (LIT):

This larval bioassay (Shaw, 1996) is not so widely used for the diagnosis of resistance and has not been promoted by FAO (2004). The method provides a result in six weeks. Different comparative studies have indicated LIT results can be compared with LPT results as there is good agreement between results of the test methods. The SLIT has the advantage that it uses unfed larvae, which are more easily standardized, and the mortality of the larvae can be recorded easily. The larvae are also treated identically which leads to more statistically credible results. One disadvantage of this method is that it magnifies the FOR (Factor of Resistance) and, in addition, the exposure of tick larvae for 10 minutes to an emulsion of a commercial acaricide is also not a satisfactory imitation of the field situation.

In vivo tests

Stall tests: The most conclusive indicators of efficacy. It considers all one host tick stages using the trade formulations at the correct and effective concentration (FAO, 2004).

3. MATERIALS AND METHODS

3.1 Description of study area

The study was conducted from September 2011 to May 2012 in two districts (Yabello and Dire) of Borena Pastoral area, Southern range lands of Ethiopia. Borena zone, one of the 17 zones of Oromia, is under the administration of Oromia National Regional State. The zone comprises of mainly pastoral area and seldom Agro-pastoral areas. Yabello and Dire districts are among the pastoral areas, and located at distance of 570 and 665 kilometers from Addis Ababa, the capital city of Ethiopia, respectively.

The region has predominantly a semi-arid climate. The annual temperature varying between 21°C and 38°C with little seasonal variations and the rainfall ranges from 350mm to 900mm, with considerable spatial and temporal variability in quantities and distribution (CARE, 2009). The study region is characterized by bimodal type rain with 60% occurring in the long rainy season (*Gena*) extending from mid-March to May and erratic short rain season (*Haggaya*) from mid-September through mid-November. The other two seasons are the cool dry season (*Adolessa*) extending from June to August and the major dry season (*Bonna*) from December to February (BZPADO, 2009/10).

Physiographically, the area is dominated by Savannah vegetation containing mixtures of perennial herbaceous and woody vegetation, mainly of acacia species and thorny shrubs. Several native species of grasses and woody plants provide forage for livestock. Similar to other parts of the country, rapid demographic change is an issue for Borena pastoral area as well. Animal husbandry in the area is characterized by extensive livestock productions system and seasonal mobility. Cattle are the dominant livestock species (CARE, 2009).

Yabello and Dire districts (Figure-1) were the focused area for this study and selected from pastoral areas (districts) of Borena zone. The two districts were selected because of the reasons that on the basis of their rainfall, location and temperature profile in Yabello districts; and however, there is no such profile in Dire districts the climatic condition of this two districts are relatively similar rather than similarities of Yabello district climatic condition

with other pastoral districts. Not only this, because of the availability of reports and rumor in many occasions' field veterinarians and communities usually complained on failure of acaricides to kill ticks and toxicity associated with those locally available acaricides and this two districts having relatively large number of cattle population (BZPADO, 2009/10).

In addition, Dire district is shares boundary with other pastoral districts and international boundary with Kenya, where there is a fear of trans boundary livestock ticks and tick born diseases. On top, Yabello is one of the districts which shares boundary with most of other districts; and the main entry for livestock movement across most districts. In Dire district the study focused on three selected Pastoral Associations (PAs) namely Dubluk, Romso and Megado. Similarly, in Yabello the study focused on Surpha, Dida- hara and El- waya Pastoral Associations. Thus, the sampled districts and PAs are expected to be representatives because the extensive livestock rearing are commons among all areas and the selected PAs of each district shares boundary with adjacent districts.

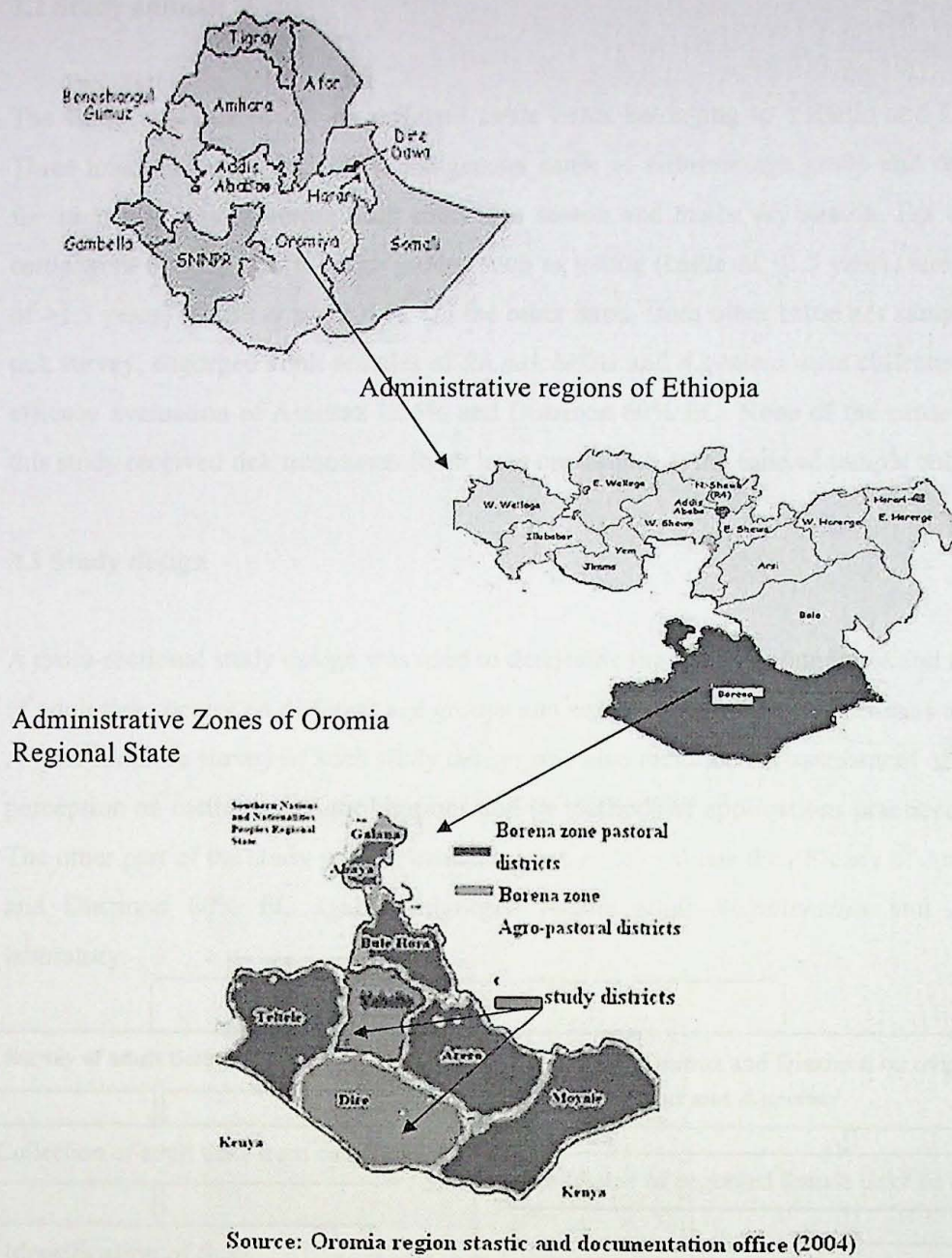


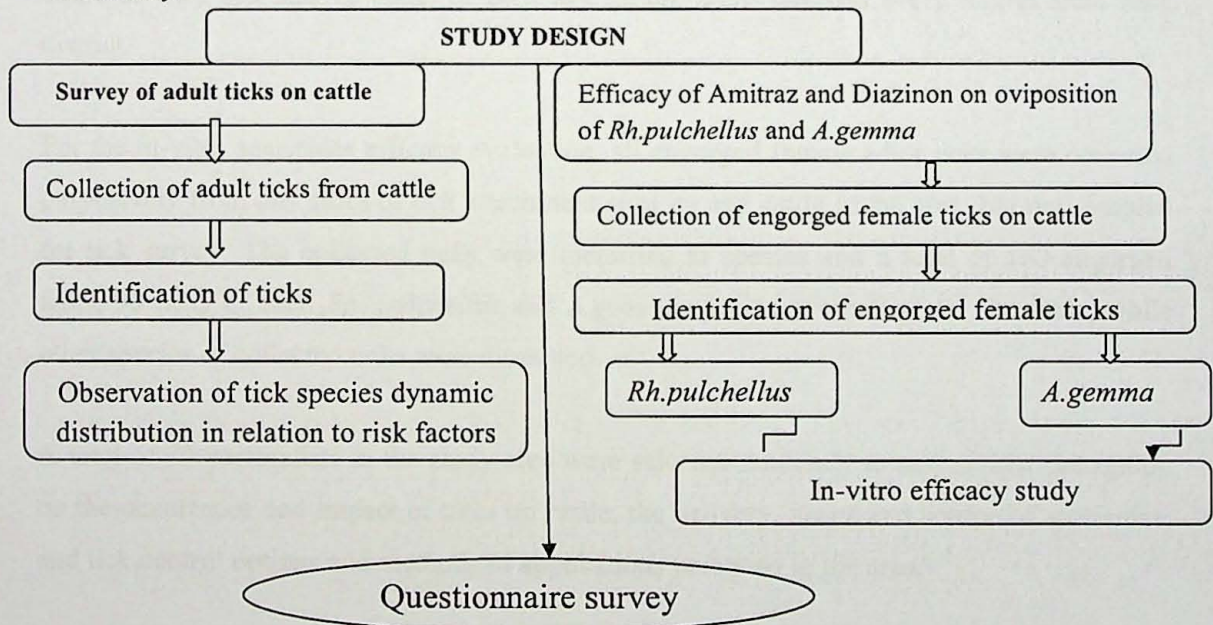
Figure 1: Maps of Ethiopia and the Study area

3.2 Study animals

The study was carried out on different cattle herds belonging to Yabello and Dire districts. Three hundred eighty four (384) indigenous cattle of different age group and sex were used for adult tick survey during each short rain season and major dry season. For convenience, cattle were divided into two age groups such as young (cattle of ≤ 1.5 years) and adult (cattle of > 1.5 years) (Karib *et al.*, 2011). On the other hand, from other cattle not sampled for adult tick survey, engorged adult females of *Rh.pulchellus* and *A.gemma* were collected for in-vitro efficacy evaluation of Amitraz 12.5% and Diazinon 60% EC. None of the cattle sampled for this study received tick treatments for at least one month at the time of sample collection.

3.3 Study design

A cross-sectional study design was used to determine the relative abundance and mean burden of adult tick species on different age groups and sex of cattle and in two seasons of study area. A questionnaire survey of such study design was also included for assessment of pastoralists' perception on cattle tick control options and its methods of applications practiced in the area. The other part of the study was experimental design to evaluate the efficacy of Amitraz 12.5% and Diazinon 60% EC against engorged female adult *Rh.pulchellus* and *A.gemma* in laboratory.



3.4 Sample size and sampling methods

To determine ticks species distribution dynamics and adult tick burden in different sex and age groups of cattle, and two seasons of the area; the study districts (Yabello and Dire) and three PAs in each district were selected purposively. But the study cattle were sampled using Stratified random sampling by considering equal number of cattle in each district as well as equal number of each sex and age groups during each season of the study area.

The sample size was determined using the formula given by Thrusfield (1995) based on 50% expected prevalence because as there was no current study conducted on the overall prevalence of tick infestation on cattle in the area. The desired sample size for the study was calculated using 95% confidence interval at 5% absolute precision.

$$N = 1.96^2 \times P_{\text{exp}} (1 - P_{\text{exp}}) / d^2 = 384 \quad \text{Where } N = \text{required sample size}$$

P_{exp} = expected prevalence

d = desired absolute precision

Therefore, during each season (short rain, early-October to December; and major dry period, January to mid-March) a total of 384 cattle were sampled. That is, a total of 64 cattle (16 cattle of each sex and 16 cattle of each age group) were sampled every month from each district.

For the in-vitro acaricides efficacy evaluation, all engorged female adult ticks were collected purposively from any sides of tick attachment sites on any cattle in the area, but not sampled for tick survey. The collected ticks were identified to species and a total of 180 engorged ticks, 90 ticks of each *Rh. pulchellus* and *A. gemma* species, were used for the trials, while other species of collected ticks were discarded.

A total of 70 pastoralists in the study area were selected randomly to assess their perception on the occurrence and impact of ticks on cattle, the delivery, usage and source of acaricides; and tick control options and methods of applications practiced in the area.

3.5 Study methodology

3.5.1 Adult Tick Collections and Identifications

Ticks were collected in two seasons during the study period: in the short rain season (Mid-September-mid-December-2011) and during the dry period (January-March-2012). Prior to collection, each cattle was casted and restrained (Annex-1B). All adult ticks were collected by hand picking whilst retaining their good condition for identification from eight main tick attachment body sites on alternative sides of the half-body regions of cattle. The eight half body regions for collections were head, dewlap, brisket, belly and back, scrotum/udder, anogenital region, leg and tail (Kaiser, 2004) shown on Annex-1A.

The collected ticks were put in separately labeled universal bottle containing 80% ethanol and 15% water with 5% glycerin. Each label includes locality, identification code, and attachment site and date of collection (Walker *et al.*, 2003). Afterward, ticks were transported to Yabello Regional Veterinary Laboratory. Counting, recording and identification of ticks using hand lens and stereomicroscope took place within one to three weeks of collection. Ticks were identified according to standard taxonomic identification procedures described by Keirans and Robbins (1999); Okello-Onen *et al.* (1999) and Walker *et al.* (2003).

All adult tick species identified in each of the season were counted according to their predilection site on cattle half body: hence, the total number of any tick species and mean tick burden present on cattle was estimated. For analysis the half body tick counts were doubled to obtain whole body adult tick burden. The information on meteorology and other climatic conditions of the area were also gathered.

3.5.2 In-Vitro acaricide efficacy evaluation/ Adult Immersion Test: AIT

To evaluate the efficacy of Amitraz 12.5% and Diazinon 60% EC against oviposition of field collected *Rh.pulchellus* and *A.gemma* ticks a modified AIT was used. This laboratory protocol was first described by Drummond *et al.* (1973) and modified by FAO (2004) and the South Africa Bureau of Standards (SABS) in East London, South Africa.

For the trail, engorged adult female ticks were collected from cattle. Afterward, ticks were placed in identified (including time, date and place of collection and code number) plastic flasks with small holes and free of acaricide residues. In the laboratory, all the collected engorged female adult ticks were identified. Engorged adult female ticks of *Rh.pulchellus* and *A.gemma* species with no signs of injury, was used for the experiment. Both species were carefully washed on a sieve using a clean tap water and dried at room temperature on absorbent paper and divided each species into groups according to their size. Three Groups of ten ticks of each species were randomly formed (Group-I for Amitraz 12.5%, Group-II for Diazinon 60% EC and Group-III for Control/Water) and each group was weighed.

For each acaricide, the test concentration was prepared by performing dilution of commercial acaricide in distilled water at field concentration recommended by the manufacturer. Then, all the procedure of modified AIT protocol described on Annex-2 was followed. During the study period, three replicates of trails for each acaricide treatment and control group were done.

To estimate the efficacy of each acaricide, both groups (treated and control) of each tick species were then evaluated using the egg laying test (ELT). The overall mean efficacy was then estimated from all the three replica of trials. ELT method involves the comparison of the egg mass of ticks treated with acaricide and the egg mass of untreated ticks and finally estimates the percentage control value, using the following formula:

$$\text{Percent control} = \frac{\text{MEC} - \text{MET}}{\text{MEC}} \times 100$$

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

Descriptions of Acaricides Used for the Study:

Acaricides used for in-vitro efficacy evaluation study were those widely used in Borena pastoral area for cattle tick infestation treatment and control, and are commercially available in local market, Government veterinary clinic and private pharmacies and includes:-

Commercial name: Amitraz 12.5

Name of Manufacturer: LABORATORIOS MICROSULES URUGUAY S.A.

Registration number: M.G.A.P.N°A-1430, Uruguay

Active ingredient: Amitraz in the form of emulsifiable concentrate

Batch no, EM090627-1

Purity: 12.5%% (m/v)

Field recommended concentration: 1.6:1000

Manufactured date: April, 17-2010 Expired date: April, 17-2013

Packaging: 1 liter bottle

Commercial name: Diazinon

Name of Manufacturer: Shandong Luxi Anim. Med. Share CO. LTD. (China)

Active ingredient: Diazinon in the form of emulsifiable concentrate

Batch number: 20090708

Purity: 60% (m/v)

Field recommended concentration: 1:1000

Manufacturing date: July, 08-2010 Expired date: July, 08-2013

Packaging: 1 liter bottle

3.5.3 Questionnaire survey

A total of 70 questionnaires, mixed type questions (i.e. technical and opinion pools), with open ended and closed types were included and delivered to 70 pastoralist respondents. This was to assess the perception of pastoralists on the occurrence of ticks, the delivery, usage and source of acaricides for treatment of their cattle and different tick control options and methods of applications practiced in the area. A face to face, personal interview was the way of completing a questionnaire (Annex-4).

3.6 Meteorological information data

One year records, June, 2011 to May, 2012, of meteorological data (Table-2), which includes the study periods were obtained from Yabello Oromia Agricultural Research Institute for

Yabello districts. The influence of these parameters on the distribution and abundance of tick species were observed. The meteorological data from these stations was used for the study sites, because the area is relatively homogeneous and such data were not available on spot of the other sites.

Table 2: Meteorological data of Yabello, Borena pastoral area from June, 2011 to May, 2012

Data type	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Rain fall	20.6	11.8	4.3	11.2	26.2	7.5	10.4	17.3	18.1	26.5	70.4	53.8
RH %	72.3	63.5	62.8	58.6	63.9	41.7	44.4	40.2	43.8	65.2	60.9	75.3
Soil temp	17.6	15.5	13.6	12.9	14.8	18.2	17.4	19.1	20.8	20.1	19.2	18.2
Max Temp	24.7	22.6	21.6	24.4	27.3	29.4	31.8	30.6	29.8	30.3	26.4	25.2
Min Temp	16.7	15.8	16.9	13.8	12.6	17.6	21.4	23.3	19.8	19.4	18.2	17.4

Source: Yabello Oromia Agricultural Research Center, (2011/12)

3.7 Statistical data analysis

All collected data were entered into Microsoft Excel 2003 computer program then displayed by graphs and tables. All statistical analyses were performed using Statistical Package for Social Science (SPSS)-Version 19 for windows. The difference in the relative proportion and prevalence of tick species were analyzed using Chi-square test. Percent control (% C) obtained with Egg Laying Test (ELT) for each acaricide was used to evaluate its effectiveness. Whereas, Independent sample t-test was used to examine either differences mean tick burden between seasons, sexes and age groups or difference mean % C between acaricides. A P-value less than 0.05 at 95% confidence intervals was considered for significance.

4. RESULTS

4.1 Questionnaire survey result

Through the responses of interviewed pastoralists, 80% of the respondents preferred to use Amitraz 12.5% while 17.1% of them were in favor of Diazinon 60%EC. In the area, substitution of acaricide bases did not occur in systematic ways; when it occurred, the reason they given was mainly depends on their observation of died ticks after treatment, low price and ease of availability of acaricides in the local market.

Moreover, almost all interviewed herders, responded that they started to use Amitraz 12.5% before 2-3 years, but they used Diazinon 60% EC since over a decade. At a time of study, all respondents had complained on failure of these two acaricides to kill ticks and fear of using Diazinon 60%EC, because of its toxicity on cattle during the last one/two years in the area.

The source of acaricides in the area was private pharmacies for 61.4% of respondents, but 15.7% and 22.9 % them have got acaricides from government veterinary clinic and local markets, respectively. Nevertheless, none of the interviewed respondents had scheduled and/or regular tick treatment programme. Therefore, 97.1% of respondents treated cattle for their own whenever they observed high number of ticks on cattle, particularly in milking cows (93.7% of respondents). The frequency of treatments against ticks was depends on the extent of infestation. The most often used method of acaricide application in the area was spraying with snap sac hand sprayer, but seldom applied with hand dressing.

All respondents had no chance of awareness previously either on proper usage and handling of acaricides, development of ticks acaricide resistance and its consequences for livestock production. As a result, majority of respondents (87.1%) indicated that concentration and dilution of acaricides was based on the extent of tick infestation, but 12.9% of respondents indicated as they based on cattle population. No one of respondents was observed acaricides withdrawal period and expired date before its application.

4.2 Adult tick survey

In the survey, a total of 8,912 adult ixodid ticks were collected from half body region of 768 cattle in Yabello and Dire districts of Borena pastoral area. Of totals collected, 4,488 ticks were collected from cattle in Yabello while 4,424 tick from cattle in Dire district. Eight tick species belonging to four genera were collected in both districts (Table-3). The tick genera identified were: *Rhipicephalus* (46.79%), *Amblyomma* (35.28%), *Boophilus* (13.05%) and *Hyalomma* (4.98), but with diverse level of infestation in different age and sex groups of cattle as well as during the short rain and major dry seasons of the area. The overall mean tick burden/cattle were 23.35 ± 12.27 in Yabello and 23.18 ± 13.61 in Dire district; and which showed no statistical significance ($t = 0.178$, 95% CI= -1.668 to 2.002, $P > 0.05$).

Table 3: Relative tick burden in Yabello and Dire district of Borena pastoral area

Tick Genera	Yabello	Dire	% out of total tick
<i>Rhipicephalus</i>	46.2% (2073)	47.22% (2089)	4162 (46.79%)
<i>Amblyomma</i>	35.36% (1587)	35.19% (1557)	3144 (35.28%)
<i>Boophilus</i>	13.41% (602)	12.68% (561)	1163 (13.05)
<i>Hyalomma</i>	5.01% (226)	4.91% (217)	443 (4.98%)

Rhipicephalus pulchellus was the most abundant tick species identified and having male to female tick sex ratio (M:F) of 4.26:1; and followed by *A.gemma*, having M:F of 3.93:1. Thus, *Rh.pulchellus* and *A.gemma* were the two most prevalent tick species in cattle representing more than 70% of total collected ticks. While, *A.cohearens* was least abundant and having M:F of 5.7:1 (Table-4). The mean burden variation of each tick species between the two districts has not shown statistical significance ($P > 0.05$).

Table 4: Relative abundance, mean count and M:F of tick species on cattle in Yabello and Dire districts, Borena pastoral area of Ethiopia

Tick species	Yabello		Dire		t-value	Overall	Overall
	P (%)	Mean±SD	P (%)	Mean±SD		Prevalence	M:F
<i>A.gemma</i>	23.66	3±2	23.62	3±1	0.405	23.64%	3.93:1
<i>A.variegatum</i>	8.6	1±1	8.1	1±1	0.95	8.35%	3.83:1
<i>A.lepidium</i>	1.8	0	1.81	0±1	-0.072	1.78%	4.28:1
<i>A.cohearens</i>	1.33	0	1.7	0	-1.133	1.5%	5.7:1
<i>B.decoloratus</i>	13.41	2±2	12.68	1±2	-0.178	13.05%	0.93:1
<i>H.truncatum</i>	2.11	0	2.22	0±1	0.78	2.17%	7.39:1
<i>H.m.rufipes</i>	2.9	0±1	2.69	0±1	0.871	2.81%	4.53:1
<i>Rh.pulchellus</i>	46.2	5±3	47.22	5±3	-0.221	46.7%	4.26:1

P (%) = Prevalence, SD= Standard Deviation, M:F=Male to female tick sex ratio

4.2.1 Seasonal variation

To assess the seasonal distribution of adult tick in cattle of Borena pastoral area, two seasons namely, short rain and major dry periods, were considered. All the identified tick genera and species were collected throughout the period; but highest number of all species was collected in short rain season (Table-5), mainly in October month. The total count of each tick species indicated significant seasonal variation ($P < 0.05$). An overall M:F of 2.92:1 in short rain and M:F of 4.21:1 in major dry season were collected.

During both seasons, *Rh. pulchellus* was found to be the most abundant species and followed by *A.gemma*, with similar peak occurrence in October month. The monthly population dynamics of each tick species on cattle showed on Figure-2. The high mean tick burden of one month coincides with the meteorological information data recorded in the preceding month (s) relatively necessary for development of oviposition female, hatching of eggs, moulting of larvae and nymphs of tick species (Figure-3).

Table 5: Relative abundance and M:F of adult tick species on cattle during short rain and major dry seasons in Borena pastoral area

Tick species	Short rain season		Major dry season	
	% from the season	M:F	% from the season	M:F
<i>Rh.pulchellus</i>	2457 (43.66%)	3.34:1	1705 (51.92%)	6.65:1
<i>A.gemma</i>	1201 (21.34%)	3.72:1	906 (27.6%)	4.24:1
<i>A.variegatum</i>	536 (9.53%)	3.91:1	208 (6.34%)	3.62:1
<i>A.lepidium</i>	118 (2.11%)	5.1:1	41 (1.25%)	2.73:1
<i>A.cohearens</i>	104 (1.85%)	7.67:1	30 (0.92%)	2.75:1
<i>B.decoloratus</i>	159 (16.35%)	0.98:1	91 (7.4%)	0.75:1
<i>H.truncatum</i>	920 (2.83%)	11.1:1	243 (7.63%)	4:1
<i>H.m.rufipes</i>	133 (2.36%)	6.67:1	60 (1.83%)	2.67:1

M:F= male to female tick sex ratio

The overall mean adult tick burden has shown statistical significant seasonal variation ($t=14.915$, 95% CI=10.634-13.866, $df=766$, $P<0.05$) and higher during short rain season (29.39 ± 14.392) than major dry period (17.14 ± 7.294). During both seasons, maximum mean adult tick burden was observed in case of *Rh.pulchellus*, 12.80 ± 7.818 during short rain and 8.88 ± 3.932 in dry season; and the least was in case of *A.cohearens*, 0.54 ± 1.051 during short rain and 0.16 ± 0.644 in dry period (Table-6).

Table 6: T-test analysis of mean tick burden on cattle between short rain and dry season

Tick species	Season	Mean	SD	SE	t-value	df	95%CI	S
<i>Rh.pulchellus</i>	S/rainy	12.80	7.818	0.399	8.77	766	3.040-4.793	0.000
	dry	8.88	3.932	0.201				
<i>A.gemma</i>	S/rainy	6.26	3.271	0.167	7.272	766	1.122-1.951	0.000
	dry	4.72	2.537	0.129				
<i>A.variegatum</i>	S/rainy	2.79	2.339	0.119	12.14	766	1.432-1.984	0.000
	dry	1.08	1.457	0.074				
<i>A.lepidium</i>	S/rainy	0.61	1.173	0.060	5.695	766	0.263-0.539	0.000
	dry	0.21	0.727	0.037				
<i>A.cohearens</i>	S/rainy	0.54	1.051	0.054	6.127	766	0.262-0.509	0.000
	dry	0.16	0.644	0.033				
<i>H.m.rufipes</i>	S/rainy	0.83	1.305	0.067	4.054	766	0.183-0.526	0.000
	dry	0.47	1.107	0.057				
<i>H.truncatum</i>	S/rainy	0.69	1.057	0.054	5.471	766	0.244-0.517	0.000
	dry	0.31	0.859	0.044				
<i>Rh. (Booph). decoloratus</i>	S/rainy	4.79	3.383	0.173	16.818	766	3.114-3.938	0.000
	dry	1.27	2.333	0.119				

S/rainy= Short rain season, SD= Standard deviation, SE= Standard error, S= Significance

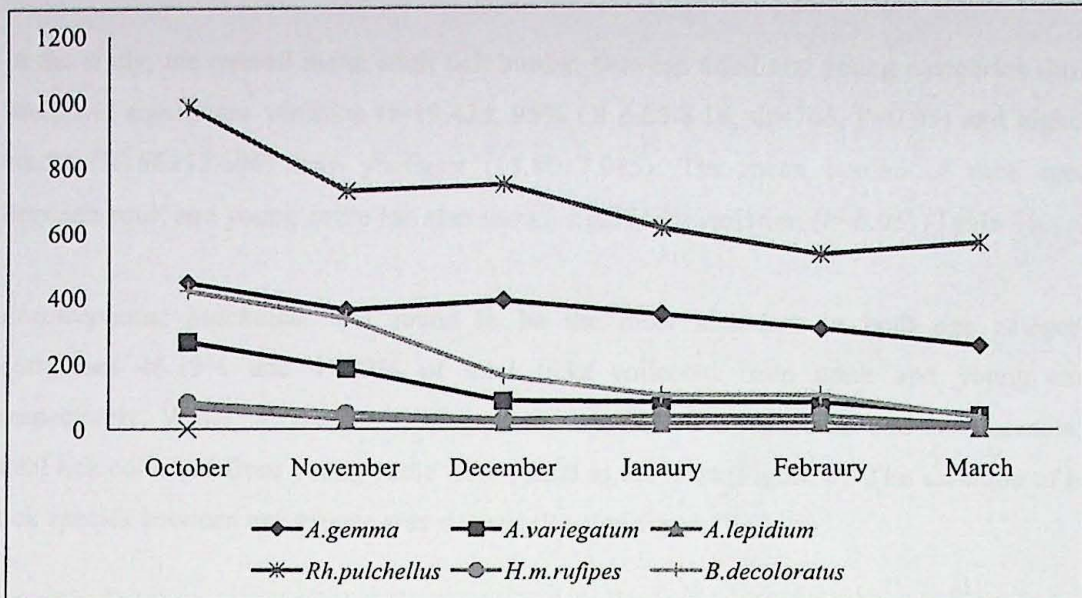


Figure 2: Monthly population dynamics of six tick species on cattle in Borena pastoral area

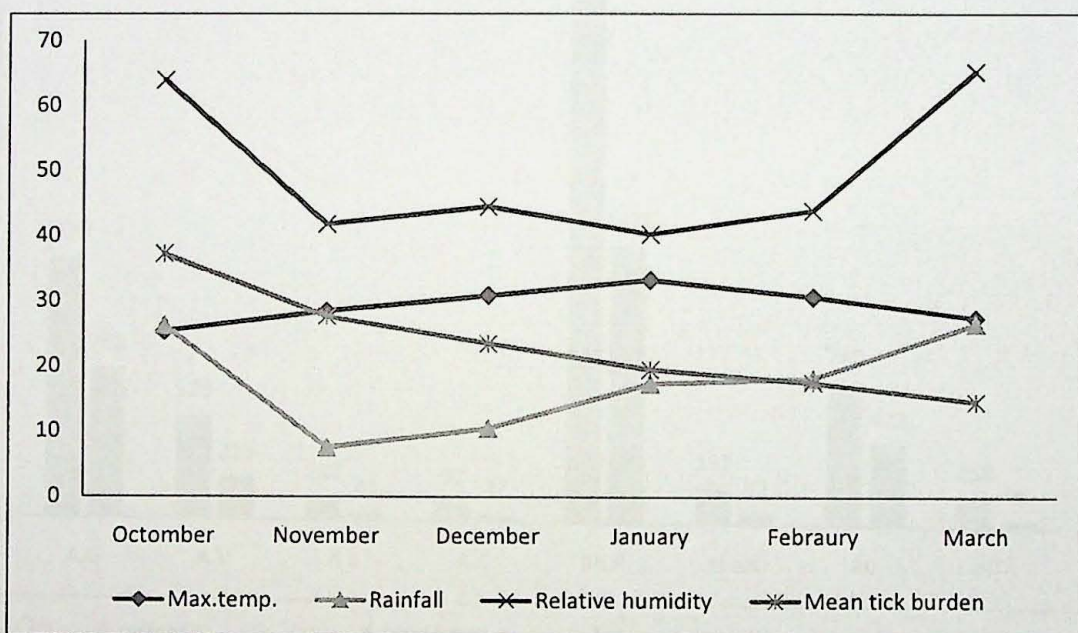
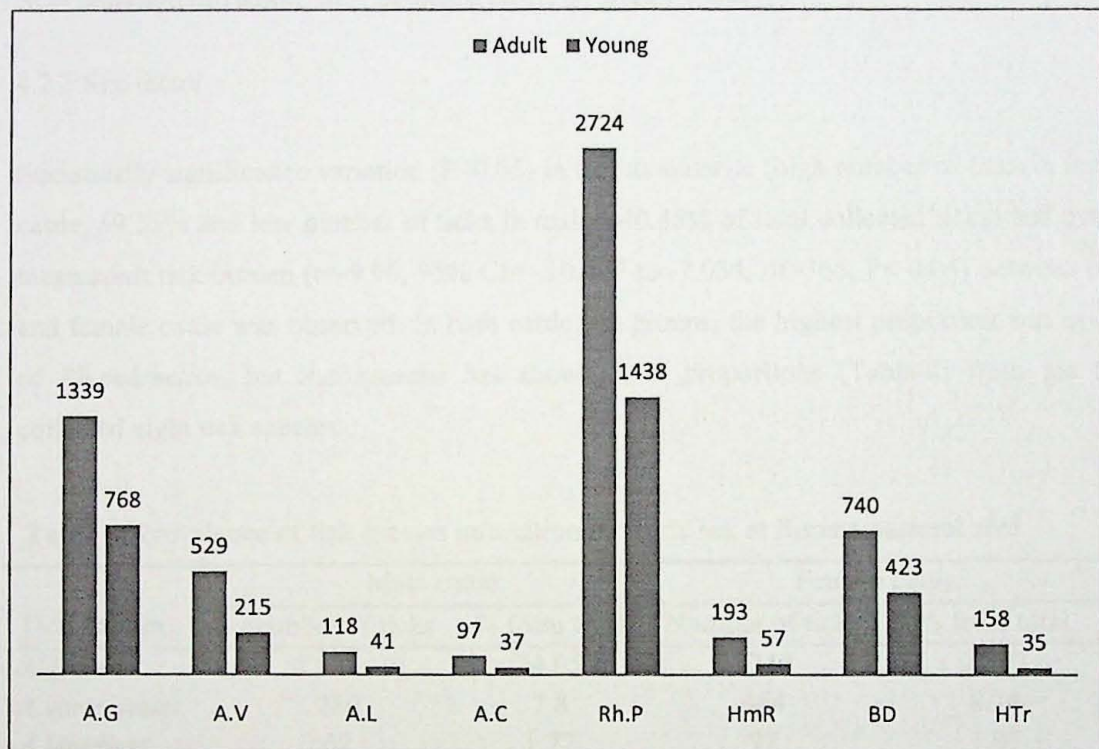


Figure 3: Monthly mean tick burden variation in relation to temperature, relative humidity and rainfall information recorded at Yabello district of Borena pastoral area

4.2.2 Age variation

In the study, the overall mean adult tick burden between adult and young categories showed statistical significant variation ($t=19.423$, 95% CI 6.65-8.16, $df=766$, $P<0.05$) and higher in adults (30.66 ± 12.696) than youngest (15.80 ± 7.945). The mean burden of each species between adult and young cattle has also shown significant variation ($P<0.05$) (Table-7).

Rhipicephalus pulchellus was found to be the most abundant in both age categories; comprises 46.19% and 47.77% of total ticks collected from adult and young cattle, respectively. While, *A.cohearens*, of total ticks collected from adults and *H.truncatum*, of total tick collected from young cattle were found as the least (Figure-4). The variation of each tick species between age groups was statistically significant ($P<0.05$).



A.G= *A.gemma*, A.V= *A.variegatum*, A.L= *A.lepidium*, A.C= *A.cohearence*, Rh.P=*Rh.pulchellus*, HmR= *H.m.rufipes*, BD= *B.decoloratus*, HTr= *H.truncatum*

Figure 4: Mean tick burden of individual tick species on adult and young cattle, Borena pastoral area

Table 7: T-test analysis of mean tick burden in adult and young cattle at Borena pastoral area

Tick species	Age	Mean	SD	SE	t-value	df	95% CI	S
<i>A.gemma</i>	Adult	6.94	3.027	0.154	15.244	766	2.541-3.292	0.000
	Young	4.02	2.207	0.113				
<i>A.variegatum</i>	Adult	2.74	2.306	0.117	11.370	766	1.336-1.894	0.000
	Young	1.13	1.554	0.080				
<i>A.lepidium</i>	Adult	0.61	1.223	0.062	5.631	766	0.258-0.535	0.000
	Young	0.21	0.637	0.033				
<i>A.cohearens</i>	Adult	0.50	1.053	0.054	4.868	766	0.184-0.433	0.000
	Young	0.19	0.659	0.034				
<i>Rh.pulchellus</i>	Adult	14.11	7.031	0.358	16.318	766	5.793-7.377	0.000
	Young	7.53	3.592	0.184				
<i>H.m.rufipes</i>	Adult	1.00	1.450	0.074	8.295	766	0.536-0.868	0.000
	Young	0.30	0.797	0.041				
<i>Booph. decoloratus</i>	Adult	3.83	3.614	0.184	6.796	766	1.152-2.087	0.000
	Young	2.21	2.953	0.151				
<i>H.truncatum</i>	Adult	0.82	1.168	0.059	9.482	766	0.504-0.767	0.000
	Young	0.18	0.596	0.030				

SD= Standard deviation, SE= Standard error, S= Significance

4.2.3 Sex factor

Statistically significance variation ($P < 0.05$) in tick abundance (high number of ticks in female cattle, 59.55% and low number of ticks in males, 40.45% of total collected ticks) and overall mean adult tick burden ($t = -9.96$, 95% CI= -10.487 to -7.034, $df = 766$, $P < 0.05$) between male and female cattle was observed. In both cattle sex groups, the highest proportion was in case of *Rh.pulchellus*, but *A.cohearens* has shown least proportions (Table-8) from the total collected eight tick species.

Table 8: Prevalence of tick species infestation by cattle sex at Borena pastoral area

Tick species	Male cattle		Female cattle	
	Number of ticks	% from total	Number of ticks	% from total
<i>A.gemma</i>	867	24.05	1240	23.37
<i>A.variegatum</i>	280	7.8	464	8.74
<i>A.lepidium</i>	62	1.72	97	1.83
<i>A.cohearens</i>	52	1.44	82	1.55
<i>Rh.pulchellus</i>	1654	45.88	2508	47.26
<i>H.m.rufipes</i>	84	2.33	166	3.13
<i>B.decoloratus</i>	534	14.82	629	11.85
<i>H.truncatum</i>	72	2	121	2.28

The overall mean tick was higher, 27.67 ± 14.518 in female cattle and lower, 18.89 ± 9.291 in males. The mean burden of each tick species between male and female cattle during the study also showed statistical variation at 5% level of significance (Table-9).

Table 9: T-test analysis of mean adult tick burden by sex of cattle at Borena pastoral area

Tick species	Sex	Mean	SD	SE	t-value	df	95% CI	S
<i>A.gemma</i>	Male	4.52	2.464	0.126				
	Female	6.46	3.218	0.164	-9.392	766	-2.349 to -1.537	0.00
<i>A.variegatum</i>	Male	1.46	1.746	0.089				
	Female	2.42	2.356	0.120	-6.405	766	-1.252 to -0.665	0.00
<i>A.lepidium</i>	Male	0.32	0.805	0.041				
	Female	0.51	1.149	0.059	-2.546	766	-0.323 to -0.042	0.00
<i>A.cohearens</i>	Male	0.27	0.744	0.038				
	Female	0.43	1.014	0.052	-2.435	766	-0.282 to -0.030	0.00
<i>Rh.pulchellus</i>	Male	8.61	4.260	0.217				
	Female	13.06	7.497	0.383	-10.11	766	-5.312 to -3.584	0.00
<i>H.m.rufipes</i>	Male	0.44	0.999	0.051				
	Female	0.86	1.380	0.070	-4.913	766	-0.598 to -0.256	0.00
<i>B.decoloratus</i>	Male	2.78	3.437	0.175				
	Female	3.28	3.344	0.171	-2.022	766	-0.975 to -0.014	0.00
<i>H.truncatum</i>	Male	0.38	0.821	0.042				
	Female	0.63	1.105	0.056	-3.634	766	-0.393 to -0.117	0.00

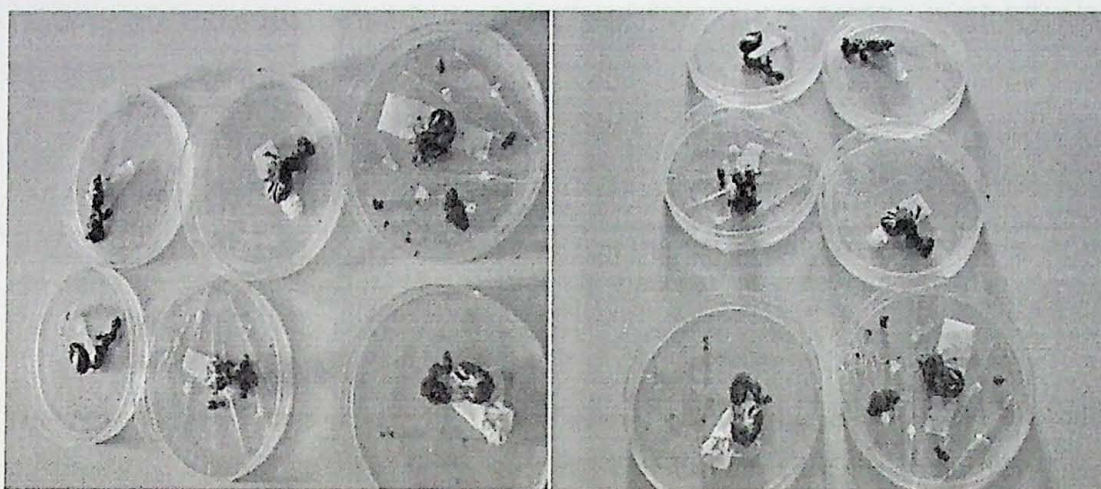
SD= Standard deviation, SE= Standard error, S= Significance

4.3 In-vitro acaricide efficacy test

The mean percent oviposition control (%C) of Amitraz 12.5% and Diazinon 60%EC at field recommended concentration against the oviposition of each *A.gemma* and *Rh.pulchellus* were calculated by comparison of the mean egg mass laid by each tick species after treated in each acaricide and the mean egg mass of each acaricide untreated tick species. Subsequently, the mean %C results of each acaricides, after three replicates, against oviposition of each tick species was determined and presented on Table-10.

Amitraz 12.5% showed higher mean percent oviposition control of both tick species than Diazinon 60%EC at field recommended concentration under in-vitro condition. In the trial none of *A.gemma* species treated with Amitraz was laid eggs, while few of *Rh.pulchellus* laid small batch of eggs weighted at mean of 0.033gm. In contrast, both tick species immersed in

Diazinon 60%EC were laid eggs with mean weight of 0.037gm and 0.12gm by *A.gemma* and *Rh.pulchellus*, respectively. However, there was no statistical significance variation ($P>0.05$) between the two acaricides against the oviposition of each tick species (Table-11). In this case, both tick species in the control group laid relatively large batch of eggs with mean weight of 0.33gm by *A.gemma* and 0.373gm by *Rh.pulchellus*. The engorgement weight, survival and oviposition response of each *A.gemma* and *Rh.pulchellus* after being immersed in each acaricide and incubated for 7 days (in all the three replica trial) was given in Annex-5.



A. Female *Rh.pulchellus* laid eggs after immersed in water and incubated at 27°C



B. Female *A.gemma* laid eggs after immersed in water and incubated at 27°C

Figure 5: Engorged female *Rh.pulchellus* and *A.gemma* laid eggs after immersed in water and incubated at 27°C for 7 days

Table 10: Mean oviposition of adult *A.gemma* and *Rh.pulchellus* after immersion in Amitraz 12.5% and Diazinon 60%EC at recommended concentration and 7 day incubation

Treated ticks	Treatment	N	M ₁	S	M ₂	%C
<i>A.gemma</i>	Amitraz 12.5%	10	2.63	0	0.000	100.00
	Water control	10	2.45	8.67	0.333	0.000
<i>Rh.pulchellus</i>	Amitraz 12.5%	10	2.83	0.67	0.033	90.94
	Water control	10	2.75	9.67	0.373	0.00
<i>A.gemma</i>	Diazinon 60% EC	10	2.56	1.0	0.037	88.85
	Water control	10	2.45	8.67	0.333	0.000
	Diazinon 60% EC	10	2.79	1.33	0.12	71.41
<i>Rh.pulchellus</i>	Water control	10	2.75	9.67	0.373	0.00

N=Number of immersed female ticks; M₁=Engorgement weight (gm); S=Number of tick survived after 7 days incubation; M₂=Average egg mass per treatment group (gm)

Table 11: T-test analysis of mean percent *A.gemma* and *Rh.pulchellus* oviposition control between Amitraz 12.5% and Diazinon 60%EC at recommended concentration.

Ticks	Acaricide	%C	N	Mean	SD	t-value	df	95% CI	S
<i>A.gemma</i>	Amitraz	100	3	100.0	0.0				
	Diazinon	88.85	3	88.85	7.9	2.438	4	-1.55-23.85	NS
	Amitraz	90.94	3	90.94	11.3				
<i>Rh.pulchellus</i>	Diazinon	71.41	3	71.33	9.9	2.26	4	-4.48-43.71	NS

%C=Percent control; N= Number of trails; SD=Standard Deviation; NS= Not significant

The overall mean %C of Amitraz 12.5% and Diazinon 60%EC were calculated by comparison of the mean egg mass laid by both tick species treated in each acaricide with that of the mean egg mass of acaricide untreated groups. Afterward, the overall mean %C of each acaricide and their respective standard deviations as well as their minimum and maximum mean efficacy during the three replica of trial is presented in Table-12.

Table 12: Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60%EC at field recommended concentration against adult female *A.gemma* and *Rh.pulchellus*

Acaricides	Min. Efficacy (%)	Max. Efficacy (%)	Mean efficacy (%±SD)
Amitraz 12.5%	78.38	100	95.47±8.663
Diazinon 60%EC	61.11	97.06	80.09±12.537

A mean %C for Amitraz 12.5% at recommended dose was found higher than for Diazinon 60%EC. The variance analysis showed that, regard less of the tick species acaricides had variable efficacy, according to the F-test value (ANOVA) at 5% significance (Table-13).

Table 13: Analysis of variance of the effects of Amitraz 12.5% and Diazinon 60%EC against oviposition response of *A.gemma* and *Rh.pulchellus* in Borena pastoral area, Ethiopia

	Sum of squares	df	Mean Square	F	Significance
Between groups	31530.515	2	15765.258		
Within groups	1161.072	15	77.405	203.673	0.000
Total	32691.587	17			

df= Degree of freedom; F= F-value

5. DISCUSSIONS

The present questionnaire survey result revealed that the entire pastoralist respondents' livelihood depends on extensive livestock production, mainly cattle rearing. Cattle rearing accounts for 61.68% of livestock and it is primarily for milk, gift of dowry and social prestige; however, they suffer from a number of constraints. About 77.1% of interviewed herders ranked tick infestation (locally called, "Silmi" or "Dirandisa") as a second constraint, next to draught, because of ticks infest all livestock, for the most part cow teat or udder damage and/or lesion, which in turn affects milk production, growth and birth rate. According to the respondents, tick infestation occurs throughout the year, but infestation was more than ever during the long rain followed by during short rain seasons. This finding was supported by the work done in Jimma Zone (Abebaw, 2004) and in Borena pastoral area (CARE, 2009; Teshale, 2004).

The analysis of the present questionnaire survey result also indicated that tick control in the area entirely depends on drugs treatment, in which 80% and 17.1% of interviewed herders responded for their use of Amitraz 12.5% and Diazinon 60%EC, respectively. Whiles, 7.1% of interviewed herders were preferred to use subcutaneous injection of Ivermectin. According to 84.24% and 12.86% of respondents, the most commonly used acaricide application methods were manual spraying and hand dressing, respectively.

In the present study area, the questionnaire survey result indicated that the livestock herders were most responsible for the dilution and application of acaricides; hence, 92.9% of respondents were treated tick on cattle for their own when they observe high tick infestation on cattle. This observation was in agreement with the investigation made by Natala *et al.* (2005) in Ghana and he found that most of the farmers were often adding an unknown amount of acaricide and water. The present study also revealed that there was no regular tick treatment programme in the study area. Other work done in Ethiopia revealed that ticks on indigenous cattle are treated whenever the farmers bring their animals to the veterinary clinics either for tick control or for other complaints. There is no planned programme of tick control except on dairy farms (Sileshi *et al.*, 2001).

Information on the status and magnitude of acaricide resistance is of paramount importance in deciding the appropriate tick and tick-borne disease control strategy in different localities in Ethiopia (Solomon *et al.*, 2004). However, the situation is compounded by the absence of an effective legislation for acaricide importation, marketing and monitoring. No one could know the origin of acaricides available on local markets and monitored it. Similarly, the present questionnaire survey indicated that private pharmacies, government veterinary clinics and local markets were the source of acaricides in the study area as responded by 61.4%, 15.7% and 22.9% of interviewed herders, respectively.

None of the respondents had gain awareness on the proper usage and handling of acaricides and on the development of acaricides resistance by tick and its consequences for the livestock production. Not only these they all were not observed withdrawal periods of acaricides. Thus, milking of cows and application of acaricides was often done at the same time and it will may create a serious human health risk.

In this study, a survey was accomplished to determine the dynamics of adult tick distribution and infestation level on cattle in relation to seasonal changes (short rain and major dry) and the host susceptibility (sex and age). The survey was limited to adult ticks. This was due to the fact that adult ticks are more visible, relatively easier to collect and believed to be the most important ectoparasite stage in causing reduced productivity in cattle (Regassa, 2001).

During the study period a total of 8,912 ticks belonging to genera of *Rhipicephalus* (46.79%), *Amblyomma* (35.28%), *Boophilus* (13.05%) and *Hyalomma* (4.98%) were found on cattle in both Yabello and Dire districts of Borena pastoral areas, Ethiopia. Previously a closely similar result with greater frequency of *Rhipicephalus* was reported in these areas by Solomon *et al.* (1998) and Regassa (2001) as well as in eastern part of Ethiopia by Zeleke and Bekele (2004).

The result of this adult tick survey indicated that all the identified species were distributed in both study districts in a closely similar abundance manner. The identified tick species were *Rh.pulchellus*, *A.gemma*, *A.lepidium*, *A.variegatum*, *A.cohearens*, *B.decoloratus*, *H.truncatum* and *H.m.rufipes*. This finding was in line with Rahmeto *et al.* (2010) investigation, who recorded the distribution of similar eight tick species in two pastoral districts of Somalia regional state. Such closely similar occurrences of tick species in two districts was most likely

associated with the fact that similar livestock rearing practice, environmental condition, vegetation coverage and presence of only indigenous cattle breed of the two districts (CARE, 2009). Hence, these factors are highly determined the distribution, survival and development of ticks. Abebaw (2004) and Bekele (2002) seeing that climatic factors and cattle rearing practices associated with different breeds have a direct influence on the biology and ecology of ticks. Desie (2005) and Rahmeto *et al.* (2010) also robustly agree with the fact that unrestricted livestock movement from area to area in search of water and grazing, which is a common phenomenon in the area, increases and promotes tick-host contact between and/or among districts. Thus, contributes for tick distribution similarities.

All the identified tick genera and species were collected throughout the study period; but the total count of each tick species indicated significant seasonal variation ($P < 0.05$). Higher numbers of ticks were collected in short rain season, mainly in October. This observation was in consistence with the previous finding in Borena province by Solomon and Kaaya (2001), in which higher rate of tick infestation on cattle was observed in October, April, May and June. Moreover, an investigation with similar proportion and seasonal variations for majority of this tick species were indicated by previous studies by Zeleke and Bekele (2004) in Eastern Ethiopia; Sileshi *et al.* (2007) and Abunna *et al.* (2009) in West Harargie of Oromia region and Rahmeto *et al.* (2010) in pastoral area of Somalia. This study also agrees with other study conducted in Southern Sudan by Salih *et al.* (2008).

During this study period, the abundance of adult tick population in one month depends on the meteorological information recorded on climatic factors (RH and atmospheric and soil temperature) of the preceding month (s). This might be due to the fact that the number of adult tick annual generation is limited by conditions necessary for development of ovipositing females, hatching of eggs, moulting of larvae and nymphs. Hence, all these necessary conditions are of paramount importance to the future continuation of the tick life cycle (Solomon and Kaaya, 1998; Rodríguez-Vivas *et al.*, 2004). For that reason, it is most likely to be that adult female ticks exposed during wet season (March, April and May of the study area) having optimal RH and rainfall could lay large number of eggs with a high level of hatching (Rodríguez-Vivas *et al.*, 2004; Furlong *et al.*, 2007). Thus, might result the development of more larvae and ultimately a high population on the pasture during the late peak rain season. The larvae that hatched at such wet season, had long survival, high moulting

rate and it can remain at an exposed vantage point on the vegetation (Horak *et al.*, 2002) during the cool dry period; June to August of the present study area. This might attribute to the collection of high adult tick population in October month of the short rain seasons.

However, female ticks those exposed during the major dry and cool months either failed to lay expected normal batch of eggs or produced very few eggs, which did not hatch at all (Minjauw and McLeod, 2003). The climatic condition of these months (December, July and August) most likely attributed to adversely affect egg laying by most ticks, aligned for the collection of small number of adult tick for their respective successive month (s) (Walker *et al.*, 2000; FAO, 2004). Furlong *et al.* (2007); Abebaw *et al.* (2004) and Solomon and Kaaya (2001) as well observed this variation.

The variation of tick abundance and mean burden during the short rain and major dry season in the present study might also most likely depends on vegetation coverage, immunological response of hosts and livestock keeping practice during short rain season of the area. As vegetation coverage following small rain and seasonal dependant crop production might have great importance to create shades or suitable habitat for ticks (Bekele, 2002). Moreover, a significantly higher mean tick burden was viewed in the area where practice of keeping large herds of cattle together during rainy season, that is when there was no area to area movement in seeking of watering and grazing land, which often tend attributed to ticks to find suitable hosts (Rahmeto *et al.*, 2010). Similar analysis was also revealed in Southern Sudan by Salih *et al.* (2008). The effect of stock rates on tick burden, further, explained by FAO (1984) as cited in Desie (2005). Minjauw and de Castro (2000) have been explained low tick load association with the dry season.

It is known that the number of female ticks attached to the host is the most significant indicator of the seasonal activity and population dynamics of ticks (Minjauw and McLeod, 2003; Jonsson, 2006). During this study too, low M:F of 2.92:1 during short rain and high M:F of 4.21:1 during dry season was observed. Similar to this finding the highest collection of ticks combined with a relatively small male/female ratio obtained during rainy season of October by Solomon *et al.* (2003). Salih *et al.* (2008) also indicated to rainy season is favorable for the survival and breeding of female ticks in Sudan.

In all cases, except for *B. decoloratus*, males outnumbered females; this is most probably because fully engorged female ticks drop off to the ground to lay eggs while males tend to remain on the host up to several months later to continue feeding and mating with other females on the host before dropping off (Solomon *et al.*, 1998; Minjauw and McLeod, 2003; Abebaw *et al.*, 2004). Host grooming easily remove semi-engorged or engorged females as compared to males (Solomon *et al.* 1998). The females of *B. decoloratus* outnumbered males in this study probably due to the small size of the male which could not be seen. This might be one of the contributory factors for missing males. Similar report was indicated in the country by Solomon *et al.* (2003); Desie (2005) and Abebaw and Tamiru (2010).

In this finding, however, each identified tick species was collected from both young and adult cattle; age of cattle was found to be a risk factor for the variation of ticks abundance and mean burden. The proportion of the overall tick count was relatively higher in adult cattle (66.18% of total collection) than in youngest (33.82% of total collection). This finding was in agreement with the previous work of Okello-Onen *et al.* (1999) in Uganda and with that of Yakhchali and Hasanzadehzarza (2004) in Oshnavich .

This study also revealed the collection of all the identified tick species from both male and female cattle. However, there was abundance and mean tick burden variation in male and female cattle and found high number of tick count in female cattle (59.55% of total collection) than in male cattle (40.45% of total collection). This variation has shown a statistical significance ($P < 0.05$). This finding was in agreement with the work done in Chittagong by Karib *et al.* (2011). The most probable cause for the high count and burden of tick in female cattle could be hypothesized that some hormonal influences may be associated with this phenomenon. FAO (2004) reported that higher level of prolactin and progesterone hormones make the individual more susceptible to any infection. Moreover, stresses of production such as pregnancy and lactation make the female animals more susceptible to any infection.

The predominant tick species during both study seasons was *Rh. pulchellus*, but high total count and mean burden was observed in short rain season (29.39 ± 14.392) than major dry season (17.14 ± 7.294). In Ethiopia, *Rh. pulchellus*, “Zebra tick”, three host tick species, are the most predominant ticks inhabiting dry, semi-arid and bush land and are found to be most abundant with marked seasonal changes. This finding was inconsistent with the earlier studies

accomplished in the present study area (Solomon and Kaaya, 1998; Regassa, 2001) and in the Rift Valley and eastwards (Walker *et al.*, 2003), in the drier lowlands of Harar (Manueri and Tilahun, 1991) and in north eastern (Seyoum, 2001).

It is evident that tick burdens are further exacerbated by the availability of adequate numbers of hosts. Since, each and every family keeps as many as livestock which increases livestock density and promotes tick-host contact (Minjauw and McLeod, 2003). This might be the most probable factor for the high abundance of *Rh. pulchellus* throughout the study period, as both mature and immature stages of *R. pulchellus* feed on a wide variety of domestic and various large wildlife species hosts (Walker *et al.*, 2003). Therefore, in an area like Borena pastoral area, where extensive livestock herding practiced, the frequency and intensity of cattle contact with all livestock species is likely high and consequently influence abundance of this species (Regassa, 2001). A similar observation for *Rh.pulchellus* host variability was reported in Somali region (Zeleeke and Bekele, 2004) and in southern Ethiopia (Solomon *et al.*, 2004).

Amblyomma gemma, “Gem-like bont tick” (Susan, 1998); was identified as a second predominant species and highest burden was observed during rainy season. The statistical analysis of the result showed significant mean burden variation ($t=7.272$, 95% CI=0.561-0.976, $df=766$, $P<0.05$) between short rain and major dry season. A similar wider distribution of *A.gemma* in this study area was reported (Solomon *et al.*, 1998; Regassa, 2001; Walker *et al.*, 2003). Like the present study area which is characterized by lowland and dry types of vegetation, most of the previous studies conducted in the country revealed, as the distribution of *A.gemma* was clearly associated with dry types of vegetation or semi-arid rangelands (Morel, 1989; Pegram *et al.*, 1981) and in lowland areas (Seyoum, 2001; Desie, 2005).

In contrast, Sileshi *et al.* (2001) reported the decreasing order of *Amblyomma* species abundance in central Ethiopia as *A. variegatum*, *A. cohaerens*, *A. lepidum* and *A. gemma*. This abundance variation is possibly due to the difference in ecological, climatic, animal breed, farming practice etc of these two areas. Moreover, the predominant occurrence of *A. gemma* might be due to its high host seeking properties under the present study area environmental condition, type of animals and management system (Latif and Walker *et al.*, 2004). The preference of attachment site by *A.gemma* might be also one of important factor for its population abundance and survival (Solomon *et al.*, 2004).

This study has also revealed *B.decoloratus* as the third most abundant species in the area. *Boophilus decoloratus* was collected throughout the study period, but showed high significant variation in mean tick burden ($t=16.814$, 95% CI=1.557-1.969, $df=766$, $P<0.05$) during short rain than major dry season. In contrast, Solomon *et al.* (2003) at Sebeta; Bekele (2002) at Alemaya Agriculture University Campus; Abebaw *et al.* (2004) in southwestern Ethiopia and Desie (2005) at Wolayta observed its most abundant occurrence all at with approximately similar altitude and during the dry season. These the above mentioned regions in which *B.decoloratus* reported in high abundance during the dry season; received dry season mean maximum and minimum temperature as well as average dry season humidity of closely similar to that of the present study area short rain season.

According to the previous work done by de Castro (1994) in areas with bimodal rainfall, like Borena pastoralists, female *B. decoloratus* is abundant from end of rainfall peak to the beginning of the next rainy season. That is April-May and September-October of the present study area, respectively. Abebaw *et al.* (2004) has been reported the high dependence of *B.decoloratus* on moist microenvironments because of its great susceptibility to percentage losses of total body water, and drop in haemolymph volume at low humidity. This study illustrates that *B.decoloratus* perish rapidly when the humid protection is disrupted. All of this facts possibly justifies the over decreasing of *B.decoloratus* during major dry season.

During this study period, the collections of other tick species were too few too suggest their seasonal proportion and mean abundance variation. They were (in order of abundance) *A.variegatum*, *H.m.rufipes*, *H.truncatum*, *A.lepidium* and *A.cohearens*. All species were collected throughout study period with peak abundance in October of short rain season. The reason for such little abundance of these species might be associated with availability of suitable hosts or the climatic factor in the study area which could not be suitable for survival of unfed ticks or else the behavior of these ticks on finding their hosts might be lesser.

The study of tick species on the host population dynamics and their survival in diverse natural habitats might be useful to initiate formulating integrated, suitable, efficient and economical tick control measures (Luciana *et al.*, 2011). However, the currently available tool for tick control in many countries relaying on using acaricides, often without understanding of such

responsible factors. These leads to environmental pollution, development of resistant tick strains and escalating costs (Alan, 2011). Therefore, the FAO Working Group on Parasite Resistance recommends the modified AIT proposed by Drummond *et al.* (1973) for the detection of acaricide resistance in the field (FAO, 2004). Despite the high variation of AIT results obtained for SP, OP and Amitraz, the AIT is a valuable tool for rapid and cheap detection of loss of susceptibility to a certain acaricide (Jonsson *et al.*, 2007).

In view of the aforesaid facts, in the present study, the in-vitro effect of Amitraz 12.5% and Diazinon 60%EC at recommended field concentration against field population of engorged adult female *A.gemma* and *Rh.pulchellus* were evaluated using modified AIT. Hence, its result may give some clue on the ground for complains hoisted on acaricide efficacy failure by field animal health workers and livestock herders.

Accordingly, Amitraz and Diazinon at recommended concentration exhibited an overall mean percent oviposition control (%C) of 100% and 88.85% on *A.gemma*, respectively. This indicated that all engorged female *A.gemma* treated with Amitraz was unable to laid eggs after 7 days of incubation. Although, Amitraz showed evidence of greatest effect on oviposition of *A.gemma*, the statistical comparison between the mean %C results of Amitraz and Diazinon revealed no significant differences ($t=2.438$, $df=4$, 95% IC= -1.5498-23.8498, $P>0.05$). The highest oviposition inhibition effect of Amitraz (%C= 100%) was achieved most likely due to the sensitivity of *A.gemma* that had no so long prior exposure to such group of acaricides.

In another part of the trial, the oviposition response of field collected engorged adult female *Rh.pulchellus* was assessed to evaluate the efficacy of Amitraz 12.5% and Diazinon 60%EC. Amitraz revealed a mean %C of 90.94%, while Diazinon achieved a mean %C of 71.41%. However, *Rh.pulchellus* treated in each tested acaricide laid eggs, differences was observed in the number of oviposited ticks, in mean weight of laid eggs and %C achieved between *Rh.pulchellus* treated with Amitraz and Diazinon. The mean %C result statistical analysis showed no significant variation ($t=2.26$, $df=4$, 95% IC= -4.47969-43.70635, $P>0.05$) between the oviposition inhibition effect of the two acaricides. However, Amitraz has high effect on oviposition of *Rh.pulchellus* than Diazinon when applied at field recommended dose. Such efficacy status variation between Amitraz than Diazinon might be associated with the high sterilization effect of Amitraz than Diazinon at field recommended concentration. As an

effective acaricide prevents the female ticks from laying eggs or the eggs do not hatch (Sileshi *et al.*, 2003).

The efficacies of Amitraz 12.5% and Diazinon 60% EC varied from 78.38% to 100% and 61.11% to 97.06% respectively. The analysis of variance, revealed significant differences between the overall mean %C of the two acaricides, in which Amitraz 12.5% presented higher mean efficacy (%C= 95.47%) than Diazinon 60% EC (%C=80.09%).

Several authors, however, had studied the efficacy of Amitraz 12.5% on different tick populations using AIT. The results have shown different susceptibility levels. In most of the studies Amitraz 12.5% revealed high degree of tickicidal efficacy that agrees with the present finding. Similarly, a closely comparable finding (98-100%) was reported by Sileshi (2001) at Sebeta, Ethiopia. In South Africa, Sileshi *et al.* (2002) also reported 100% efficacy for Amitraz 12.5%. Souza *et al.* (2003) in Southeast Brazil obtained mean Amitraz efficacy of above 95%. The minimum mean efficacy of Amitraz in the present result (78.38%) agrees with that of Mendes *et al.* (2001) finding and he found an average efficacy of 77.44%.

In contrast, Furlong *et al.* (2007) found mean efficacy of 47.9% for Amitraz, which is greatly different from the present result. Camilo *et al.* (2009) also observed the presence of resistance or the low efficacy of Amitraz in the majority of populations was analyzed. In Northeast region of Brazil, low efficacy of Amitraz 40.5% and 30.95% was reported by Santana (2000) and Campos and Oliveira (2005), respectively.

In this study, the extent at which both *Rh.pulchellus* and *A.gemma* oviposited was greater when treated with Diazinon compared to treatment with Amitraz. This oviposition extent variation was most probably associated with prolonged prior exposure of this two tick species to Diazinon 60%EC. As majority (84.6%) of interviewed responds for the questionnaire, Diazinon 60%EC usage has begun over the past 10 years in the area. Similarly, Sileshi *et al.* (2003) observed relatively higher level of resistance to organophosphorous chemical than Amitraz 12.5% in South Africa due to its utilization for over 10 years. Silva *et al.* (2000), in addition, strongly supports this finding and observed sequential use of the same chemical group for long periods has favored the development of resistance.

In addition, the differences in the efficacy of these two evaluated acaricides were most likely attributed from the widespread, frequency, irregular application, inadequate spraying, and improper mixing of acaricides and on the use of acaricides stored for long days after dilution; which was the common feature of tick control situation in the study area. Such acaricides had loss efficacies and which may lead to failure to maintain the adequate lethal concentration. In a manual acaricide spraying, a common acaricide application method in the study area, there are predilection sites for certain tick species which are not effectively treated. The use of an acaricide at incorrect concentration is one of the prime factors which affect the efficacy of an acaricide and causes of tick control failure (Natala *et al.*, 2005; Kirby, 2010; Alan, 2011). Similar investigation which agree with the present finding was observed in Ethiopia (Sileshi, 2001; Yilma, *et al.*, 2001) and abroad the country (Bianchi *et al.*, 2003; Furlong *et al.*, 2007).

Moreover, the pastoralists in the study area believed that they needed to increase the concentration of acaricides during the peak tick season to control the excessive tick burdens infesting their cattle or decrease the concentration to apply acaricides to all of their cattle. This type of increased acaricide concentration would undoubtedly have lead to a higher selection pressure for tick resistance (Luciana *et al.*, 2011) as the high acaricide concentration would effectively kill all susceptible ticks leaving only a residue of highly resistant individuals in the population (Pegram *et al.*, 2000). Each successive application would be a selective process that would concentrate the genes responsible for the resistance and eventually the majority of the ticks in the population would be resistant to the acaricide being applied against them (Rosario-Cruz *et al.*, 2009).

During this study, almost all pastoralists complained to failure of acaricides to kill ticks after being treated and concluded that the acaricide was not working. Especially, this coincided with the period during which heavy tick burdens were present on cattle. In vitro laboratory tests, however, indicated that acaricides those were mostly used for tick control in the area had relatively high (%C= 95.47% in case of Amitraz) or relative efficacy (80.09% in case of Diazinon) even for the ticks collected predominantly at the study area.

6. CONCLUSIONS AND RECOMMENDATIONS

During the study, eight tick species belonging to genera of *Amblyomma*, *Rhipicephalus*, *Boophilus* and *Hyalomma* were identified. *Rhipicephalus pulchellus* and *A. gemma* were the most abundantly distributed species on cattle of Borena pastoral areas, and contributed more than 70% of total collected ticks. The seasonal (short rain and major dry seasons) was play great role for tick species distribution and abundance dynamics in the area. Age and sex of cattle were also found to be important determinant for tick population dynamics. Irrespective of the seasons, high total tick count and mean tick burden was found on adult cattle than youngest; and on females than males.

The in vitro trial indicated that most of engorged female *Rh. pulchellus* and *A.gemma* ticks treated with Amitraz 12.5% at field recommended dose were unable to lay eggs and the eggs of the few laid were smaller batch and had significantly less mean weight than the control groups. Whiles, those *Rh. pulchellus* and *A.gemma* immersed in Diazinon 60%EC revealed relatively more number of oviposited ticks and laid eggs with higher mean weight than those ticks immersed in Amitraz. In the overall of the trial, a mean %C of 95.47% for Amitraz and 80.09% for Diazinon was achieved. Thus, Amitraz at field recommended dose provide effective oviposition inhibition of field engorged *Rh. pulchellus* and *A.gemma* than Diazinon.

The tick control situation in the study area was entirely depends on drug usage, mainly application of Amitraz 12.5% and Diazinon 60%EC; and featured by several constraints such as irregular acaricide application, under or over dose concentration, a long time usage of one acaricide type and improper mixing of acaricides, which may lead to failure to maintain the adequate lethal concentration. Moreover, there was no effective acaricide marketing and monitoring.

Based on the above conclusions, the following recommendations are forwarded:

- ❖ Strategic control of ticks using acaricides based on seasonal dynamics of ticks is required
- ❖ Awareness creation of pastoralists on proper use of acaricides to avoid drug resistance

- ❖ It would be valuable to conduct this efficacy evaluation test using a larger number of adult ticks, and/or other in-vitro efficacy evaluation methods involving larval and nymphal stage up on the result of this study.
- ❖ Further In vivo trial should be conducted to assess the residual effect of these acaricides.
- ❖ From the government part, attentions should be given on effective legislation for acaricide importation, marketing and monitoring.
- ❖ Strengthening veterinary service delivery in the study area
- ❖ Tick-borne diseases in the area should be studied.
- ❖ The AIT with ELT method still needs to be validated and standardized for use in Ethiopia and indeed Africa. As it is probably the most rapid method for detecting field resistance.

7. REFERENCES

- Abebaw, G. (2004): Seasonal dynamics and host preference of *Boophilus decoloratus* (Koch, 1944) on naturally infested cattle in Jimma zone, south western Ethiopia. *Ethiopian Veterinary Journal*, **18** (1): 19-28.
- Abebaw, G. and Tamiru, T. (2010):- Prevalence of ticks on local and crossbred cattle in and around Asella town, Southeast Ethiopia. *Ethiopian Veterinary J.*, **14** (2): 79-89.
- Abunna, F., Kasasa, D., Shelima, B., Megersa, B., Regassa, A. and Amenu, K. (2009): Survey of tick infestation in small ruminants of Miesso district, West Harargie, Oromia region, Ethiopia. *Trop. Anim. Health Prod.* **41** (6): 969-972.
- Adamu, G. (1996): Bioassay of acaricide resistance on three common cattle tick species (Ixodidae) a case study in Holetta area central highland. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debrezeit, Ehtiopia.
- Alanr, W. (2011): Eradication and control of livestock ticks: biological, economic and social perspectives. Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall Place, Edinburgh EH9 1QH, UK.
- Angus, B. M. (2003): Tick Fever and the Cattle Tick in Australia. Beverley M. Angus, (compact disc ISBN 0 9750104 17) Brisbane. Pp 1829–1996.
- Assefa, B. (2004): A survey of ticks and tick-borne blood protista in cattle at Asela, Arssi Zone. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.
- Barker, S. C. and Murrell, A. (2004): Systematic and evaluation of ticks with a list of valid genus and species names. *J. Parasitol.*, **129** (7): 15-36.
- Bekele, T. (2002): Studies on seasonal dynamics of ticks of Ogaden cattle and individual variation in resistance to ticks in eastern Ethiopia. *J. of Vet. Medicine*, **49** (6): 285-288.
- Belay, S. (2004): A survey of cattle tick species in and around Mizan-Teferi, Bench Maji Zone, and SNNPRS. DVM thesis, Addis Ababa University, FVM, Debre Zeit, Ethiopia.
- Belew, T. and Mekonnen, A. (2011): Distribution of Ixodid Ticks on Cattle in and Around Holeta Town, Ethiopia. *Global Veterinaria*, **7** (6): 527-531.

- Berhane, M. (2004): Distribution of livestock tick species in Awassa area. DVM thesis, Addis Ababa University, Faculty veterinary Medicine, Debre-Zeit. Pp 1-16.
- Borena Zone Pastoral Area Development Office (2009/10): Annual Livestock Population and Diseases Report of Borena Zones Pastoral Districts. Borena Zone Livestock Population Report, Cattle population and diseases.
- Camillo, G., F. F. Vogel, L. A. Sangioni, G. C. Cadore and R. Ferrari (2009): In vitro evaluations of acaricides efficiency to bovine's ticks of RioGrande do Sul State, Brazil. *Ciência Rural*, **39** (2): 490–495.
- Campos, D. A. and P. R. Oliveira (2005): “In vitro valuation of acaricides efficiency to *Boophilus microplus* (Canestrini, 1887) Acari: Ixodidae) from bovines at the region of Ilh'eus, Bahia, Brazil.” *Ciência Rural*, **35** (6): 386–1392
- CARE-Ethiopia, (2009): Value Chain Analysis of Milk and Milk products in Borena Pastoralist Area. Regional Resilience Enhancement against Drought Project, Yonas Business Promotion and Consultancy P LC, Addis Ababa, Ethiopia. Pp 12-25.
- Catsro-Janer, E., Rifran, L., Piaggio, J., Gil, A., Miller, R. J. and Schumaker, T.T.S. (2009): In vitro tests to establish LC50 and discriminating concentrations for fipronil against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and their standardization. *Veterinary Parasitology J.*, **162** (31):120-128.
- de Castro, J. J. (1994): A survey of the tick species in western Ethiopia including the previous findings and recommendation, for further tick survey in Ethiopia. Technical report AG: DP/ETH/83/023, FAO, Rome, pp.1-83.
- Desie, S. (2005): Cattle Tick Dynamics in Different Agro-Ecological Zones of Wolayta, Southern Ethiopia. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. Pp 18-83.
- Dioli, M., Jean-Baptiste, S. and Fox, M. (2001): Ticks (Acari:Ixodidae) of the one-humped camel in Kenya and Southern Ethiopia: Species composition, attachment sites, sex ratio and seasonal incidence. *Revu Elev. Med. Vet. Pays trop.*, **54** (2): 115
- Drummond, R. O., S. E. Ernst, J. L. Trevino, W. J. Gladney, and O. H. Graham (1973): “*Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides”. *Journal of Economic Entomology*, **66** (1):130–133.
- Farmer, E. (2010): End Market Analysis of Ethiopian Livestock and Meat. A Desk Study Micro report # 164.

- Food and Agricultural Organization (1971): Recommended methods for the detection and measurement of resistance of agricultural pest to pesticides-tentative method for larvae of cattle ticks, *Boophilus microplus* spp. *FAO Plant Prot. Bull.*, **19**:15–18.
- Food and Agricultural Organization (1984): Ticks and tick-borne disease control. A practical field manual, Volume-1, Tick control. FAO, Rome, pp 1-299.
- Food and Agricultural Organization (2004): “Acaricide resistance: diagnosis, management and prevention,” in *Guidelines Resistance Management and Integrated Parasite Control in Ruminants*, Animal Production and Health Division, Agriculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy, pp 25–77.
- Furlong, J., J. R. Martins, and M. C. A. Prata (2000): The tick of cattle and resistance: we have to celebrate. *A Hora Veterin'aria*, **27** (4): 1–7.
- Furlong, J., Martins, J. R. and Prata, M. C. A. (2007): The tick of cattle and resistance: we have to celebrate. *A Hora Veterin'aria J.*, **27** (7): 27-34.
- Garcia-Garcia, J. C., Montero, C., Redondo, M., Vargas, M., Canales, M., Boue, O., Rodriguez, M., Joglar, M., Machado, H., Gonzalez, I. L. (2000): Control of ticks resistant to immunization with Bm86 in cattle vaccinated with the recombinant antigen Bm95 isolated from the cattle tick, *Boophilus microplus* Vaccine, **18** (21): 2275-2287.
- George, J. E., Pound, J. M. and Davey, R. B. (2008): Acaricides for controlling ticks on cattle and the problem of acaricide resistance. In *Ticks: Biology, Disease and Control* (ed. Bowman, A. S. and Nuttall, P. A.), Cambridge University Press, UK, pp 408–423.
- Getachew, Y. (2004): Tick species infesting livestock in Jimma area, Southwest Ethiopia. DVM thesis, Addis Ababa University, F. V. Medicine, Debre Zeit, Ethiopia. Pp 1-19.
- Graf, J. F., Gogolewski, R., Leach-Bing, N., Sabatini, G. A., Molento, M. B., Bordin, E. L. and Arantes, G. J. (2004): Tick control: an industry point of view. *Parasitology* **129**, (Suppl.).
- Haile, B. (1987): Study of the topographical distribution of ticks on economically important domestic animals in Illubabor. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. Pp 1-46.
- Hassan, C. (2003): Animal Health Challenges During Drought: Implications, Opportunities and Managements. Proceedings of the 17th Annual Conference of Ethiopia Veterinary Association, Addis Ababa, June 5-6, 2003, Ethiopia.

- Hoogstraal, H. (1956): African Ixodoidea Volume I Ticks of Sudan. Department of the Navy, Washington D. C.
- Horak, I., Camicas, J. and Keirans, J. (2002): The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): A world list of valid tick names. *Exper. Applied Acarology*, **28**: 27-54
- Jonsson, N. N. (2006): The productivity effects of cattle tick infestation on cattle, with particular reference to *Bos indicus* cattle and their crosses. *Vet. Parasitol*, **137** (2): 1–10.
- Jonsson, N. N., Miller, R. J. and Robertson, J. L. (2007): Critical evaluation of the modified-adult immersion test with discriminating dose bioassay for *Boophilus microplus* using American and Australian isolates. *Vet. Parasitol.*, **146** (7): 307–315.
- Kabir, M. H. B., M. M. H. Mondal, M. Eliyas, M. A. Mannan, M. A. Hashem and N. C. Debnath (2011): An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. *African J. of Microbiology Research*, **5** (4): 346-352.
- Kaiser, M. N. (2004): Report on tick taxonomy and biology. AG: DP Eth/83/023 Tick survey. Consultant Report, FAO, Rome, pp 92.
- Keirans, J. E. and Robbins, R. G. (1999): A world checklist of genera, subgenera and species of ticks (Acari: Ixodida). *J. Vector Ecol.*, **24** (23):115-129.
- Kirby, C. (2010): Tick Management Hand book. Biological tick Control, 2nd Edit. The Connecticut Agricultural Experimentation Station, New Haven, pp 70-71.
- Landim, V. C., E. Silva and V. Paes (2006): Diagnosis of the situation of resistance to acaricides in *Boophilus microplus* in beef and dairy cattle in the Uberaba region. *FAZU Journal*, **3**: 63.
- Latif, A. A. and Walker, A. R. (2004): An introduction to the biology and control of ticks in Africa. ICTTD-2 project, pp 1-29.
- Lodos, J., Boue, O., de la Fuente, J. A. (2004): Model to simulate the effect of vaccination against *Boophilus* ticks on cattle. *Vet. Parasitol.* **87** (4): 315-326.
- 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, Pp 16-21.

- Luciana, G., Brito, F., S. Barbieri, Rodrigo, B., Rocha, M. C., Oliveira, S. and Elisana, 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, *Veterinary Medicine International*, **201** (6): 2-13.
- Manueri, K. K. and Tilahun, J. (1991): A survey of ectoparasites of cattle in Harar and Dire Dawa districts, Hararghe administrative region of Ethiopia. *Bull. Anim. Hlth. and Prod. in Afr.*, **39**: 15-24.
- Matthewson M. D. and Blackmann G. G. (1980): Resistance to certain organophosphorus ixodocides in strains of *Boophilus decoloratus* from Zambia. *Vet. Rec.*, **107**: 491.
- Mendes, M. C., C. J. Verissimo and C. N. Kaneto (2001): "Bioassays for measuring the acaricides susceptibility of cattle tick *Boophilus microplus* in São Paulo state,
- Minjauw, B. and de Castro, J. J (2000): Host resistance to ticks and tick-borne diseases: Its role in integrated control. *Breeding for Diseases Resistance in Farm Animals*. CAB International, pp153-169.
- Minjauw, B. and McLeod, A. (2003): Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, center for Tropical Veterinary Medicine, University of Edinburgh, UK. Pp 1-116.
- Morel, P. (1989): Study on Ethiopian ticks (Acarida, Ixodida). Republic of France, Ministry of Foreign Affairs, French Vet. Mission, Addis, C. J. E. M. V. T., Pp 7-332.
- Natala, A. J., Agyei, A. D. and Awumbila, B. (2005): Susceptibility of *Amblyomma variegatum* ticks to acaricides in Ghana. *Experimental and Applied Acarology*, **35** (1): 259-268
- Okello-Onen, J., Hassan, S. M. and Essuman, S. (1999): Taxonomy of African ticks, an identification manual. International Center for Insect Physiology and Ecology press, Nairobi, Kenya, Pp 1-124.
- Pegram, R. G., Hoogstraal, H. and Wassef, H. P. (1981): Ticks (Acari: Ixodidae) of Ethiopia. Distribution, Ecology and Host relationship of tick species infecting livestock. *Bulletin of Entomology Research*, **71** (9): 339-359.
- Pegram, R. G., Wilson, D. D. and Hansen, J. W. (2000): Past and present national tick control programs: why they succeed or fail. *Annals of the New York Academy of Scies*, pp 546

- Radostits, O. M., Gray, C. C., Hincheliff, K. W. and Constable, P. D. (2006): Veterinary Medicine. A text book of the Disease of Cattle, horses, sheep, pigs and goats; 10th Edition Oxford, New York, pp 1462-1468.
- Rahmeto, A., Thedrous, F., Mesele, A. and Jemere, B. (2010): Survey of ticks infesting cattle in two districts of Somali Regional State, Ethiopia. *Veterinary World*, **3** (12):539-543.
- Regassa, A. (2001): Tick infestation of Boran cattle in the Boran Province of Ethiopia. *Onderstepoort Journal of Veterinary Research*, **68** (8): 41- 45.
- Rodríguez-Vivas, R. I., Mata, M. Y., Pérez, G. E. and Wagner, W. (2004): The effect of management factors on the seroprevalence of *Anaplasma marginale* in *Bos indicus* cattle in the Mexican tropics. *Tropical Animal Health Production*, **36** (4): 135-143.
- Rosario-Cruz, R., C. Almazan-Garcia, R. Miller, D. Dominguez-Garcia, O. R. Hernandez and J. De La Funte (2009): Genetic basis and impact of tick acaricide resistance. *Front. Biosci.*, **14**: 2657–2665.
- Salih, D. A., I. I. Julla, S. M. Hassan, A. M. EL Hussein and F. Jongejan (2008): Preliminary survey of ticks (Acari: Ixodidae) on cattle in Central Equatoria State, Southern Sudan. *Journal of Veterinary Research*, **28** (75): 47–53.
- Samish, M. and Alekseev, E. (2001): Arthropods as predators of ticks. *J. Med. Entomol.*, **38** (21):1-11.
- Santana, V. L. A. (2000): Situation of chemical control of *Boophilus microplus* the sub-regions in the area of forest and wasteland in the state of Pernambuco, based on in vitro efficacy of acaricides in engorged females. Dissertation in Veterinary Science, Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil.
- Sathaporn, J. A, Weeraphol Jansawan, A, Omar, O., Barriga, B. and Roger, W. (2004): Reduced Incidence of *Babesia bigemina* Infection in Cattle Immunized against the Cattle Tick, *Boophilus microplus*. Depart. of Parasit., FVM, Kasetsart University, Bangkok, Thailand.
- Seyoum, Z. (2001): Study of ticks and tick-borne diseases on cattle at Girana valley in the North Wollo Zone. Proceeding of the Ethiopian Veterinary Association, Vol.15.
- Shanahan, G. J. and Hart, R. J. (1966): Change in response of *Lucilia cuprina* (Weid.) to organophosphorous insecticides in Australia. *Nature*, **212** (5069):1466-1467.

- Shaw, R. D. (1966): Culture of an organo-phosphorus resistant strain of *Boophilus microplus* (Canestrini) and assessment of its resistance spectrum. *Bull. Entomol. Res.*, **56**:398-405.
- Shaw, R. D. and Malcom, A. A. (1969): Resistance of *Booph. microplus* to Organophosphorus insecticides. *Vet. Res.*, **76**: 210-211.
- Sileshi, M. (2001): *In vivo* evaluation of Amitraz against ticks under field conditions in Ethiopia. *J. S. Afr. Vet. Asso.*, **72**: 44-45.
- Sileshi, M., A. Kgasi, W. Mureithi, Getachew, Z., Tilahun, T., Solomon, G., Yilma, J. and N. R. Bryson (2004): In Vivo And In Vitro Evaluation Of The Efficacy Of Cypermethin High-Cis (Ecotomin®) Against Cattle Ticks In Ethiopia. *Ethiop. Vet. J.*, **8** (1): 29-38
- Sileshi, M., Bryson, N. R., Fourie, L. J., Peter, R. J., Spickett, A. M., Taylor, R. J., Strydom, T., Kemp, D. H. and Horak, I. G. (2003): Comparison of 3 tests to detect acaricide resistance in *Rhipicephalus (Boophilus) decoloratus* on dairy farms in the Eastern Cape Province, South Africa. *J. S. Afr. Vet. Asso.*, **74** (9): 41-44.
- Sileshi, M., Hussein, I. and Bedane, B. (2001): The distribution of ixodid ticks (Acari: Ixodidae) in central Ethiopia. *Onderstepoort J. Vet. Res.*, **68** (4): 243-251.
- Sileshi, M., N. R. Bryson, L. J. Fourie, R. J. Peter, A. M. Spickett, R. J. Taylor, T. Strydom, D. H. Kemp and I. G. Horak (2002): Acaricide Resistance profiles of single and multi - host ticks from communal and commercial farming areas in the Eastern Cape and North West Provinces of South Africa. *Onderstepoort Journal of Veterinary Research (in press)*
- Sileshi, M., Pegram, L. 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**(2): 1-24.
- Sileshi, S. (1998): Ticks, tick-borne diseases and control strategies in Ethiopia, Proceedings of the second International conference on tick-borne pathogens at the host-vector interface: a global perspective. August 20- September 1, 1995, Kruger National Park, South Africa. Pp 441-446.
- Silk, P. (2009): Occurrence of tick borne haemoparasites Kwazulu-Natal and Eastern Cape Provines, South Africa. MSc thesis in Veterinary Tropical Diseases, F.V.Science, University of Pretoria, South Africa. Pp 6-7.

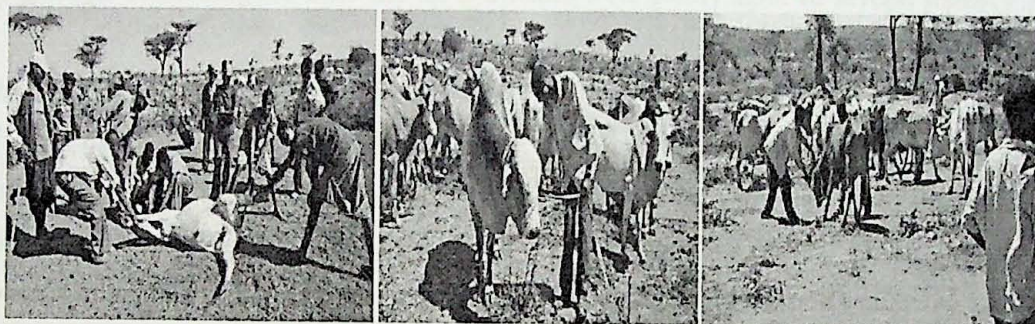
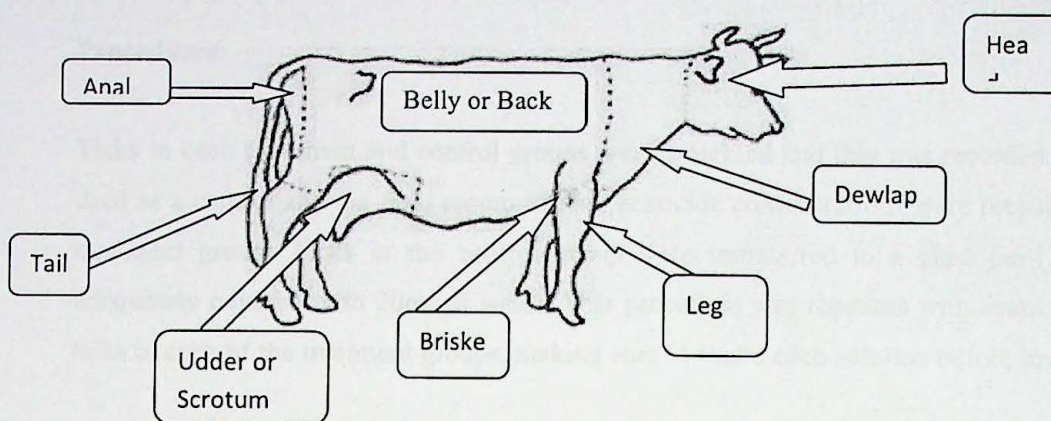
- Silva, M. C. L, R. Neves Sobrinho and G. F. C. Linhares (2000): *In vitro* evaluation of effectiveness of chlorfenvinphos and cyhalothrin for the control of *Booph. microplus* in dairy farms of the microregion of Goiânia—Goiás. *Brazilian Anaml. Sci*, **1**(2): 43–148.
- Sinshaw, S. (2000): Distribution of ticks and tick-borne diseases at Metekel Ranch. *Ethiopian Veterinary Journal* **1** (4): 40-59.
- Solomon, G. (2001): Problem of Acaricide resistance for the control of ticks. *Proceeding of the Ethiopian Society of Animal production 9th Conference. (In press)*
- Solomon, G. and G. P. Kaaya (2001): Studies on the development and survival of *Rh. pulchellus* and *A. gemma* under field condition in Ethiopia. *Ethiopian Vet. J.*, **5** (1): 28-48.
- Solomon, G. and Kaaya, G. P. (2002): Evaluation of two Neem products for the control of major Arid land tick species on livestock. Ethiopian Pharmacuetical Assoc 22 Annual scientific conf. Aug. 2-3, 2002, pp 13.
- Solomon, G. and Kaaya, G.P. (1998): Comparison of resistance in three breeds of cattle against African Ixodid ticks. *Experimental and applied acarology*, **2** (4): 223-230.
- Solomon, G., Kaaya, G. P., F. Gebreab, T. Gemetchu and G. Tilahun (1998): Ticks and tick borne parasites associated with indigenous cattle in Did-tuyura ranch, Southern Ethiopia. *Ins. Sci. Applic.* **18**, 59-66.
- Solomon, G., Nigist, M. and Kassa, B. (2003): Seasonal Variation of ticks on Calves at Sebeta in Western Shewa Zone. *Ethiopian Veterinaty J.*, **7** (1and 2):17-30.
- Solomon, G., Sileshi, M., Kaaya, G. P., Tilahun, T., Yilma, J. (2004): Prevalence of Ixodid ticks and Trypanosomosis in camels in southern Ethiopia. *Ethio. Vet. J.*, **8** (2): 23
- Solomon, N. (2004): Capacity development for promotion of livestock export from the pastoral area of Ethiopia. Preceeding of the 18th annual conference of the Ethiopian Veterinary Association, held in Addis Ababa, June 9-10, 2004, Ethiopia, pp 117.
- Souza, A. P., A. A. Sartor and V. Bellato (2003): Efficacy of acaricides in dairy cattle herds in southern region of Parana state, Brazil,” *J. of Agronomy and Vet. Sciences*, **2** (1): 131.
- Susan, E. Aiello (1998): The Merck Veterinary Manual, 8th edit, Merck and Co. Inc., USA, 67.

- Tadesse, M. (1994): Survey on mange mites and ticks of camel and small ruminants in Dire-Dawa Region. DVM thesis, Addis Ababa University Faculty of Veterinary Medicine, Debre-Zeit. PP. 1-25.
- Teshale, S. (2004): Ethno veterinary practice of Borana Pastoralist and their possible contribution to livestock production and management. National Veterinary Institute (NVI), Debrezeit, Ethiopia.
- Thrusfield, M. (1995): Veterinary Epidemiology, Second edition. U. K. Black well science Ltd. Pp 182-198.
- Torr, S., Eisler, M., Coleman, P., Morton, J. and Machila, N. (2003): Integrated control of ticks and tsetse. A report for the DFID advisory and support service contract, project ZV 0151; NRI code V 0160, pp 1-135.
- Torres, D. F. (2008): The brown dog tick *Rhipicephalus sanguinus* (Acari: Ixodidae): From taxonomy to control. *Veterinary Parasitology*, **152** (9):173-185.
- Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Pena, A., Horak, I. G., Latif, A. A., Pegram, R. G. and Preston, P. M. (2003): Ticks of domestic animals in Africa: a guide to identification of species, Bioscience report. Pp 1-221.
- Walker, J. B., Keirans, J. E. and Horak, I. G. (2000): The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. Cambridge University Press, Cambridge.
- Wharton, R. H. (1967): Acaricide resistance and cattle tick control. *Aust Vet J.*, **43** (9): 394-398.
- Xu, Y, Bruno, J. F. and Luft, B. J. (2005): Identification of Novel ticks salivary gland proteins for vaccine development. *Biochem B. Res Commun.* Department of Medicine, Staat University of New York at Stony Brook, Stony Brook, NY 11794, USA, **326** (4): 4-901.
- Yakhchali, M. and Hasanzadehzarza. H.S. (2004): Study on some ecological aspects and prevalence of different species of hard ticks (Acarina: Ixodidae) on cattle, buffalo and sheep in Oshnavieh suburb. Pajouhesh-va-Sazandegi in *Anim. Fish Sci.*, **63**: 30-35.
- Yilma, J., Adamu, G. and Zerbini, E. (2001): Bioassay of acaricide resistance on three common cattle tick species at Holeta, central Ethiopia. *Revue Med. Vet.*, **152** (7): 385-390.

- Yismashewa, W. (2005): Epidemiology of ticks and TBDs of cattle in Decha district, Southern Ethiopia. MSc Thesis, Addis Ababa University, FVM, Debre Zeit, Ethiopia. Pp 1-8.
- Zahid, I. A., Latif, M. and Baloch, K. (2005): Incidence and Treatment of Theileriosis and Babesiosis. Research Institute for Physiology of Animal Reproduction Bhunikey, (Pattoki), District Kasur, Pakistan. *Pakistan, Vet. J.*, **25** (3):137-141.
- 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.

8. ANNEXES

Annex 1: Cattle body regions used for tick collection (Modified from Kaiser, 2004)



Restraining and casting of cattle during adult tick collection at Borena pastoral area

Annex 2: Laboratory Methodology for Modified Adult Immersion Test (AIT)

This test procedure was originally described by Drummond *et al.* (1973) and modified by South Africa Bureau of Standards in East London (Strydom, pers. Comm., 2000 and FAO, 2004).

Procedures:

Ticks in each treatment and control groups were weighted and this was recorded. Water was used as a control and the field recommended acaricide concentrations were prepared for each treatment group. Ticks in the control group were transferred to a glass jar (100ml) and adequately covered with 20ml of water. This procedure was repeated with acaricides for the ticks in each of the treatment groups, making sure to shake each solution before immersion.

Each consecutive treatment group was then immersed at exactly 30 second intervals and a stop watch was used to ensure proper timing. During the remainder of the 30 minutes period, the glass jar containing the ticks and the acaricides were gently agitated several times.

Thirty minutes after the immersion of the ticks in the control group, the liquid was decanted off, and the ticks were inverted onto a clean, dry piece of filter paper in an aluminium foil dish. All the ticks were placed on their ventral sides, each on a dry portion of the filter paper. The above procedure was then repeated with the ticks in the treated group at exactly 30 seconds intervals. They were then air dried at room temperature for an hour. The ticks were then pasted onto double sided adhesive strips on glass petridish with their ventral sides facing upwards, keeping their capituli clear of the tape. The glass petridish were then placed in larger, plastic boxes containing a moistened sponge for seven days in incubator maintained at temperature between 27⁰C and 30⁰C with a photoperiod of 12:12 (L:D) hour. Humidity was not measured but the sponge was freshly moistened every 24 hours.

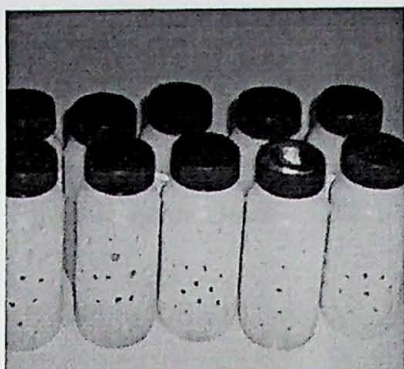
The ticks were then examined after seven days and the number of dead ticks that is those which had turned black was recorded and this was not seen as mortality due to the acaricides but rather natural died off. The eggs laid in each treatment group were then weighed and the results were then recorded on relevant forms.

To estimate the efficacy of acaricide, both groups (treated and control) of each tick species were tested either using the egg laying test (ELT) method which involves the comparison of the egg mass of ticks acaricide treated with the egg mass of untreated ticks and finally estimate the percentage control value, as if they represented a number of ticks dead by the test acaricide, using the following formula:

$$\text{Percent control} = \frac{\text{MEC} - \text{MET}}{\text{MEC}} \times 100$$

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

Annex 3: Some materials and activities during In-vitro efficacy trials



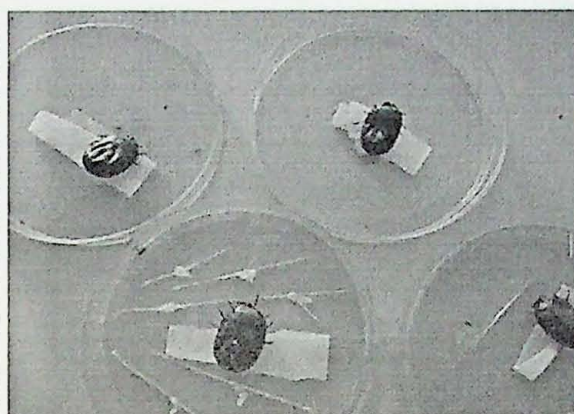
Plastic flasks with small holes



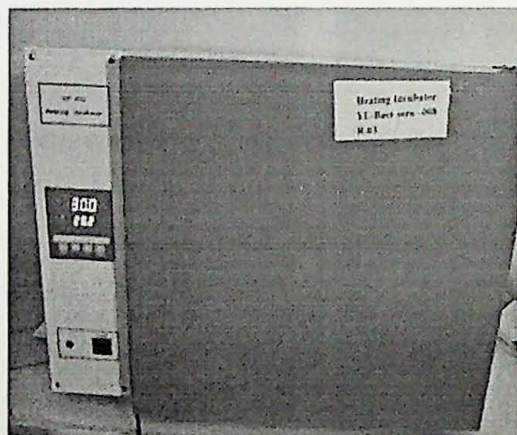
Size wise grouping of engorged adult females tick



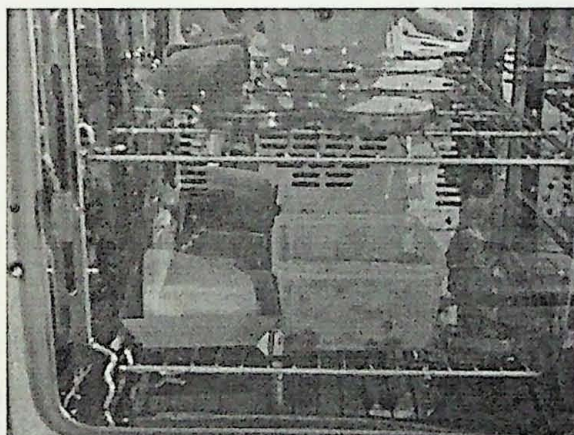
During weighting of eggs laid by each tick



Adult ticks pasted on double sided adhesive strips
With ventral side upward



Incubator adjusted at 28°C for 30min



Each pasted ticks put in to large plastic and incubated

Annex-4: Questionnaire set interview pastoralists on herd structure, tick infestation constraints, acaricide delivery and usage and tick control practice

Region _____ Zone _____ District _____ PA _____ Date _____

Livestock structure and management system

1. How many animals do you have?
A/ Cattle: Oxen _____ Cow _____ Bulls _____ Heifers _____ Calves _____
B/ Small ruminants: Sheep _____ Goat _____
C/Equines: Donkey _____ Horse _____ Mules _____
2. How do you manage your animals in different seasons of the year?
A/ During Long rainy season: Free grazing _____ Tether _____ Stall feeding _____
B/ During cool dry season: Free grazing _____ Tether _____ Stall feeding _____
C/ During short rainy season: Free grazing _____ Tether _____ Stall feeding _____
D/ During major dry season: Free grazing _____ Tether _____ Stall feeding _____
3. What is the main importance of keeping cattle?
A/Milk production B/ manure C/ social prestige and status D/ paying do ironic

Cattle tick infestation and tick control

1. What are the most five common diseases affecting your cattle?
A/ _____ B/ _____ C/ _____
D/ _____ E/ _____
2. Do you know ticks? A/ Yes B/ No
If yes, what do you call ticks by local name? _____
3. Have you seen livestock infested with ticks in this area? A/Yes B/ No
If yes, which livestock species infested most by ticks? A/ Cattle B/ Caprine C/Equine
4. Do you know that tick transmit diseases? A/ Yes B/No
5. What is/are the most importance of tick infestation on cattle production in this area?
A/ Losses B/ Pathogenic role C/ Transmission of disease D/ others ...
6. In which season of the year does tick infestation more common?
A/ Short rainy B/ Major dry C/ Long rain D/ Cool dry
7. How did you treat cattle infested with ticks? A/With drugs/acaricides B/ Culturally
If culturally, how? _____ specify _____

8. If you used drug, when did you start to use (in year)? A/1-4 B/5-8 C/> 9
9. Which type (color) of drug/acaricide do you know commonly used?
 A/Amitraz 12.5% B/Diazinon 60% EC
 C/Cypermethrin 2% D/Ivermectin
10. Why do you use this drug commonly? A/ Low cost B/ Easy available C/ High efficacy
11. Where did you get these drugs/acaricides? A/ Government vet. clinic
 B/CAHWs C/Private veterinary Pharmacy D/Local market
12. Who are giving the treatment? A/ CAHWs B/ Yourself C/ Veterinary personnel's
13. What method of acaricide application do you commonly used? A/Manual spraying
 B/ Dipping C/ Hand dressing D/ Spray races
14. Did you observe with drawl period and expired date? A/ Yes B/No
15. Do you have regular tick treatment/control programme? A/ Yes B/ No
16. What is your basis for acaricide dilution and dosage determination? A/Body weight
 B/Number of animals C/Age of animals D/ Type of acaricide application
17. Do you use acaricide that are diluted and stored for a day/s, week/s? A/ Yes B/No
18. Is there any situation that you treat your animal separately (based on sex, age, species, herd or other) against ticks? A/Yes B/No
 If yes, based on what? A/Sex: M____ or F____ B/Age: young _____ or adult _____
 C/Animal Species: _____ D/Herd/flock: _____
19. Did you practice any rotational usage of acaricides? A/Yes B/No
 If yes, what and what type of acaricides?
20. Do you aware of proper usage and handling of acaricides; like storage condition, observing withdrawal period and expired date, wearing gloves during preparation and application, previously? A/Yes B/ No
21. Do you have any awareness on the development of acaricide resistance by ticks and its consequences for the livestock production? A/ Yes B/ No

Thank you!!!!

Name of interviewer: _____

Signature: _____

Date _____

Annex 5: Ovipositing response of engorged adult female *Rh.pulchellus* and *A.gemma* after immersion in Amitraz 12.5% and Diazinon 60%EC and 7 days' incubation.

Trail	Tick species	Acaricides	N	Eng.wght (gm)	S	No.LE	Egg M (gm)	%C
Trail-I	<i>Rh.pulchellus</i>	Amitraz	10	2.77	1	1	0.02	94.44
	<i>Rh.pulchellus</i>	Diazinon	10	2.83	2	2	0.14	61.11
	<i>Rh.pulchellus</i>	Control	10	2.78	10	9	0.36	0.00
	<i>A.gemma</i>	Amitraz	10	2.82	0	0	0.00	100.00
	<i>A.gemma</i>	Diazinon	10	2.60	1	1	0.06	81.25
	<i>A.gemma</i>	Control	10	2.37	9	8	0.32	0.00
Trail-II	<i>Rh.pulchellus</i>	Amitraz	10	2.61	1	1	0.08	78.38
	<i>Rh.pulchellus</i>	Diazinon	10	2.81	1	1	0.07	81.08
	<i>Rh.pulchellus</i>	Control	10	2.62	9	9	0.37	0.00
	<i>A.gemma</i>	Amitraz	10	2.56	0	0	0.00	100.00
	<i>A.gemma</i>	Diazinon	10	2.38	1	1	0.01	97.06
	<i>A.gemma</i>	Control	10	2.44	8	8	0.34	0.00
Trail-III	<i>Rh.pulchellus</i>	Amitraz	10	3.10	0	0	0.00	100.00
	<i>Rh.pulchellus</i>	Diazinon	10	2.74	1	1	0.11	71.79
	<i>Rh.pulchellus</i>	Control	10	2.86	10	10	0.39	0.00
	<i>A.gemma</i>	Amitraz	10	2.50	0	0	0.00	100.00
	<i>A.gemma</i>	Diazinon	10	2.71	1	1	0.04	88.24
	<i>A.gemma</i>	Control	10	2.55	9	9	0.34	0.00

N=Number of female tick immersed and incubated; Eng.wght (gm) = Engorgement weight (gm); S= Number of tick survived after immersion and 7 days incubation; No.LE =Number of laid eggs; Egg M (gm) = Average egg mass per treatment group (gm); %C=Percent Control

9. CURRICULUM VITAE

1. PERSONAL DATA

Full name: - Dr. Eyob Eshetu Bekele

Place of birth: - Oromia, Bale, Ginnir

Date of birth: - January 26, 1984

Sex: - Male

Marital status- Single

Religion- Christian (Orthodox Tewahido)

Nationality- Ethiopian

Current address: - Mobile 0913028538 (Private) Tel. 046-4-46-03-89 Office,

E-mail: eyobeshetu@ymail.com, P.O.Box 169, Yabello, Borena

Occupation- Parasitology Department Head, Yabello Regional Veterinary Laboratory

2. EDUCATIONS

I. MSc in Tropical Veterinary Parasitology from Addis Ababa University College of Veterinary Medicine and Agriculture, 2010 – 2012.

II. DVM from Jimma University Faculty of Veterinary Medicine, 2003 - 2008.

III. Secondary School- Ginnir Comprehensive Secondary School, 1999– 2002.

IV. Junior Secondary School- Ginnir Mokuria Tesema Junior Secondary School, 1995-1998.

V. Elementary School- Ginnir Turge Elementary School, 1991– 1994.

3. WARDS RECEIVED

I. Diploma Certificate in Computer training and report card (transcript) from Yabello Senior and Secondary and Preparatory school, January 10, 2009.

II. Certificate for the Participation and Completion on ELISA, AGID, RBPT, CFT, Sample collection, storage and media preparation, Bacterial isolation and identification,

Helminthes, Protozoan and Ticks identification techniques from NAHDIC (National Animal Health Diagnostic and Investigation Center), 2009 and 2011; Sebeta, Ethiopia.

III. Certificate for the Training on Principles and Techniques of Serology and Bacteriology from MoRD and NVI, 2009; Debreziet, Ethiopia.

4. WORK EXPERIENCE

- Head of Parasitology Department, Yabello Regional Veterinary Laboratory, 2009-2012.
- Animal Health Team Leader, Rayitu District, July, 2008 – January, 2009.

5. RESEARCH PAPERS

- In-Vitro Efficacy Evaluation of Selected Acaricides and Survey of Ixodidae Ticks on Cattle in Borena Pastoral Area, Ethiopia, MSc thesis, Addis Ababa University College of Veterinary Medicine and Agriculture, 2012.
- Prevalence of Ovine Lungworms in and around Asella, DVM thesis, Jimma University Faculty of Veterinary Medicine, 2008.

6. LANGUAGE

Mother tongue: - Afan Oromo

Other languages:- Good skill of writing and speaking: English and Amharic

7. REFERENCE

• Dr. Dinka Ayana (DVM, MSc, Associate Prof.)

Addis Ababa University College of Veterinary Medicine and College of Agriculture

P.O.Box 34; Email :) dinka_ayana@yahoo.com

Fax 251-1-339933, Tel. 251-1-26 11 28 Debre-Zeit, Ethiopia

I the under signed, declare that the thesis is my original work and has not been presented for a degree in any university.

Name: Eyob Eshetu Bekele

Signature  _____

Date of submission 03/07/2012

This thesis has been submitted for examination with my approval as University Advisor

Dr. Dinka Ayana _____