ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

BOVINE BABESIOSIS AND ITS VECTORS AT KOBO AND GIRANA VALLEYS IN AMHARA NATIONAL REGIONAL STATE OF ETHIOPIA.

BY

SEYOUM ZENEBE

JUNE 2007
DEBRE ZEIT, ETHIOPIA

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

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LIST OF ABBREVIATIONS

ANRS	Amhara National Regional State
BOFED	Bureau of Finance & E. development
CACC	Central Agriculture Census Commission
CL	Confidence Interval
CSA	Central statistics Authority
EDS	Early dry season
ELISA	Enzyme- Linked Immuno Sorbent Assay
ERS	Early rainy season
FAO	Food Agriculture Organization
GDP	Grand domestic Product
IFT	Indirect Fluorescence Antibody
ILRI	International Livestock Research Institute
Km	Kilometer
LDS	Late dry Season
LRS	Late rainy season
PAS	Peasant Association
RH	Relative Humidity
SNNPRS	Southern Nation Nationality People Regional Stat
Spp.	Species
TBD	Tick borne disease

ABSTRACT

A study on bovine babesiosis and its vector was conducted at Kobo and Girana valleys of North Wollo Zone in Amhara National Regional state, Northern Ethiopia. The objective of the study was to determine the prevalence and identify at species level of bovine babesiosis and the vector and indicate the associating risk factor for *Boophilus decoloratus* tick through longitudinal study. A total of 370 cattle from both valleys were used for a cross sectional study while 60 cattle were also involved in longitudinal study during four consecutive seasons. The longitudinal study was conducted on October, December, January and March that represents late rain, early dry, late dry and early rain seasons respectively. The prevalence of *Babesia bigemina* in a cross sectional study of age groups <2, 2-4 and >4) was (20.3%, 24.3%, and 16.1%), respectively. The prevalence of B. bigemina among the three age groups was not statistically ($p>0.05$) significant. Sex (male and female) basis of *Babesia bigemina* infection rate were (12.7% and 7.6%) respectively and high infection rate (26.5%) were observed in animals with poor body condition than animals with good body condition (15.19%). The prevalence of sex and body condition wise was found statistically ($p<0.05$) significant. Animals that are found at Kobo valley (22.7%) and Girana valley (17.8%) were surveyed on disease prevalence during a cross sectional study. The prevalence of *Babesia bigemina* in the valleys was not statistically ($p>0.05$) significant. The infestation rate of *Boophilus decoloratus* in age groups (<2, 3-4 and >4) were 73.2%, 82.1% and 91.9%), respectively. And sex (male and female) wise infestation rate were (86.6% and 78.3%), respectively. Age and sex wise prevalence was found statistically ($p<0.001$ and $p<0.05$) significant, respectively. Older animals, males and animals with poor body condition were found to carry more ticks than others. Out of the whole *Boophilus decoloratus* collected, the proportion of females adult ticks were higher in number than males once, that is the ratio male to females was (0.41: 1) and *Boophilu decoloratus* from the whole tick counts was represent (24.4%), rank third next to *Amblyomma cohaerence* (24.9%) and *Amblyomma variegatum* (28.22%). Different tick species have their own preference of attachment sites on their host. Though *Boophilus decoloratus* has its own preference of predilection feeding sites, but in this study except ano-vulva the tick was collected from all over the host body. Mainly back (shoulder), dewlap, head and ear, but also from venter, tail and hoof. The prevalence of *Babesia bigemina* during longitudinal study in cattle sub populated by age, sex, and body condition were studied, in general the infection rates were lower, except animals with 3-4 age groups (30%) and sex wise

prevalence of longitudinal study was found higher than a cross sectional. The difference among age and sex wise were not statistically ($p>0.05$) significant. Body condition basis prevalence of *Babesia bigemina* result of longitudinal study was found similar with that of a cross sectional study result and the difference was ($p<0.05$) significant. The infestation rate of *Boophilus decoloratus* studied in longitudinal study during four consecutive seasons on animals sub populated by age, sex, body condition, valleys and season was generally lower than that of a cross sectional study and the difference statistically was not ($p>0.05$) significant except seasons which was found ($p<0.05$) significant. In all variables studied the prevalence of *Boophilus decoloratus* was found higher generally during rainy seasons than that of dry seasons. The risk of *Boophilus decoloratus* infestation was found lower in dry seasons as compared to rainy seasons (OR = 1.56, 95% Cl = 1.43 - 2.39) and the likelihood of acquiring infection was (OR = 3.56, 95% Cl =1.56 - 8.14) 3 times higher in rainy seasons than dry seasons. The main cause of high infestation and infection in the valleys were observed as a result of the unrestricted movement of animals from different part of the country that used to pass via the valleys to the Northern part the country, time less bounded usage of acaricide by different non-ethical individuals, inadequate dosage of acaricide and drought. Restriction of animals' movement, quarantine measures, strong and sustainable extension works should be done to create awareness of the livestock keepers.

***Key words*:** Bovine babesiosis, *Boophilus decoloratus*, Kobo and Girana valleys, North Wollo, Ethiopia.

1. INTRODUCTION

Ethiopia is located in the horn of Africa between latitude from 3^0N to 15^0N of the equator and longitude from 33^0E to 48^0E (CSA, 2003; Tamire, 2003), is an agrarian country with an estimated human population of more than 62.9 million and a total land area of 1,101,000 Km^2 (Tamire, 2003). The proportion of total population in agricultural sector is 82.4% (CSA, 2003). According to (CSA, 2003) and (FAO.AGAL, 2003) estimate the livestock population of Ethiopia is about 30 million head of cattle, 24 million sheep, 18 million goats, 7.2 million equine, one million camels, 25 thousand pigs and 55.6 million poultry. Livestock are an important and integral part of farming systems in Ethiopia. The Ethiopian livestock contribute about 18.8% of the total GDP (FAO.AGAL, 2003). Among livestock, cattle are a primary resource for the people and government of Ethiopia (ILRI, 1999).

Cattle are a source of high quality protein (meat, milk), they contribute for the economic welfare of the people by providing hide, skin, power and traction for agriculture purposes, fertilizer for increasing the productivity of small holding (Minjauw and Mc Leod, 2003). They are means of converting poor quality forage marginal land, surplus crops, crop residues and by-product of agriculture and industry to high value commodities, increase income and hence the economic viability of the farming system (Jajasuriya, 1999).

Among the major constraints of cattle production are Bovine Babesiosis and *Boophilus* tick (Tick and tick-borne protozoal disease) rank high. It was well established that parasitized animals perform less efficiently, food conversion adversely affected; carcass quality reduced, skin yield decline and consequent economic losses (Morris, 1988).

Babesiosis and *Boophilus* tick parasites of domesticated and wild animals occur through out the world and their prevalence show no tendency toward over all declines in tropical and subtropical region especially in developing countries. On contrary, their significant as causes of production losses increase in many areas; (FAO, 1984) and (Seifert, 1996) estimate that 80% of the world cattle populations are at risk from tick and tick-borne disease.

Bovine babesiosis is caused by protozoan intraerythrocytic parasites of the genus *Babesia* of the order *Piroplasmid* of the phylum Apicomplexa. Economically, bovine babesiosis, caused by *B.*

bovis, B. bigemina, B. major, and B. divergens forms a serious problem as it is widely distributed and threatens the health and safety of million cattle. Of the species affecting cattle *Babesia bovis* and *B. bigemina* are of major economic importance to livestock industry in tropical and subtropical region of the world since at least several million cattle globally at a risk. Both of these are widely distributed and are especially important in Africa, Asia, Australia, and central and south America. (Mc Cosker, 1981).

The vector of *Babesia* is tick (Friedhoff, 1988). The most important vector in the tropical and subtropical is *Boophilus* species. *Babesia bovis* is more pathogenic than *B. bigemina*, although the latter is more widespread and a higher rate of transmission. *Babesia bovis* infections result in high mortality rates among susceptible cattle, and cause a virulent disease and are characterized by high fever, ataxia, anorexia, and general circulatory shock, often accompanied by nervous symptoms as a result of sequestration of infected erythrocytes in cerebral capillaries. In *Babesia bigemina* infections, the major symptoms include fever, haemoglobinuria, haemoglobinaemia, and anaemia. Intravascular sequestration of infected erythrocytes does not occur with *B. bigemina* infection (Friedhoff, *et al*., 1989).

Once an animal has been infected with one of these species, it has life long immunity against subsequent re infection with the same species. There is also some evidence that *B. bigemina*-immune animals against subsequent *B. bovis* infection experience some degree of cross-protection. Young animals are more resistant to infection, calves that has been fed colostrums from an immune or a non- immune dam being equally resistant to *B. divergens* (Dalguesh, 1993). *Boophilus* tick, as other important ticks are undoubtedly the most important ecto-parasite of livestock on global scale. They transmit infective organism to animal and man; many of which cause diseases of major economically importance. They inflict great havoc by continual loss of blood, and by creating different grade of lesions on the skin, which may result in the formation of secondary infections and contribute to the formation of myiasis and cause down grading the quality of hide and skin (Normal *et a*l., 1988).

According to (Walker *et a*l., 2003) ticks that are considered to be most important to the health of domestic animals in Africa comprise about forty spp. Among these ticks, *Boophilus decoloratus*, (Pegram *et al.*, 1981); (Haile, 1987); (Mekonnen, 1998); (De Castro, 1994); and (Walker *et al*.,

2003) and *Boophilus annulatus* (Haile, 1987); (De Castro, 1994) are the most important ticks distributed world wide. *Boophilus decoloratus* is the most abundant and widespread tick species in Wollo (Seyoum, 2001). It predominates in broad –leaves and coniferous forest, but absent from drier area (De Castro, 1994).

Ticks are a cause of much ill health to domestic animals and a cause of considerable economic loss to their owners. In the world, an estimate of US$ 7, 000 million annual losses is caused by tick and tick-borne diseases (FAO, 1984), this estimate provides a guide to region of the world where *Boophilus microplus* is the only major tick infesting cattle and this figure may indeed be much higher in regions where different ticks species are present.

Currently Ethiopia has started exporting live animals; meat, skin and hides to different countries and this are enabling the earning of hard currency that is badly needed by the country. Such export market is highly influenced by the quality of meat, skin and hide produced in the country especially from diseases point of view. One of the risks that make the export vulnerable is the presence of various tick-borne and ticks. External parasites results in down grading of skin and hide that uses for exporting purpose at newly emerging export Tannery which have negative impacts for the exporters in particular and for the country in general.

Despite these problems, the achievement made on the study of tick *Boophilu*s and tick-borne disease (babesiosis) in Ethiopia in general (except some areas) is not significant and does not cover all regions especially those marginal and fertile valleys where suitable environment as well as un controlled cattle movement exist. Therefore, the objective of the study is in general assess the magnitude of bovine babesiosis and its vectors at Kobo and Girana valleys of North Wollo zone in eastern Amhara sub-region (ANRS) and specific objectives include:

- To determine the prevalence of bovine babesiosis at Kobo and Girana valleys.
- To study the prevalence and risk factors of *Boophilus* tick.
- To test the correlation between the prevalence of *Boophilus* tick and babesiosis.

2. LITERATURE REVIEW

2. 1. Bovine babesiosis

2. 1. 1. Definition, Etiology and Morphology

Bovine babesiosis is a febrile, tick-borne disease of cattle, caused by one or more protozoan parasites of the genus *Babesia.* The disease can spread between animals by the bite of an infected tick and ticks become infected by feeding on animals that are either sick from disease, or are healthy but have the parasite in their blood (carriers). Ticks infect animals when they feed on them, through their saliva, a single infected tick can pass disease on to an animal. Bovine babesiosis generally characterized by extensive erythrocytic lysis leading to anemia (pale to yellow eyes and gums), icterus, hemoglobinuria, and death. The two that are of most concern are *B. bigemina* and *B. bovis*, which are transmitted primarily by *Boophilus* ticks throughout the tropical and subtropical areas. *B. Bigemina* is a large *Babesia* that is pleomorphic but characteristically is seen and identified by the pear-shaped bodies joined at an acute angle within the mature erythrocyte. Round forms measure 2 µm and the pear-shaped, elongated ones are 4-5 µm (Annex-1) (Gonzales *et al*., 1971)

Babesia bovis is a small pleomorphic *Babesia* typically identified as a single body, as small round bodies, or as paired, pear-shaped bodies joined at an obtuse angle within the mature erythrocyte. The round forms measure 1-1.5 µm and the pear-shaped bodies 1.5 by 2.4 µm in size (Annex-1) (Mohoney, 1977).

2. 1. 2. Epidemiology

Both *Babesia bovis* and *Babesia bigemina* are transmitted transovarially (from one generation to the next via the egg) by *Boophilus* spp, a one-host tick, with transmission after final development in the salivary glands of the larval stage (*B. bovis*) or the nymphal and adult stages of the tick *(B. bigemina*). *Babesia bovis* infection does not persist in *Boophilus microplus* beyond the larval stage while *B. bigemina* can pass from one generation to the next even when the ticks feed on non- susceptible hosts (De vos *et al*., 2004).

The prepatent period after *B. bovis* infection is generally 6-12 days with peak parasitaemia reached about 3-5 days later. In the case of *B. bigemina* the prepatent period is 12- 18 days from the time ticks first attach but this can be shortened by transfer of ticks amongst cattle in close proximity (De vos *et al*, 2004).

Cattle between 3 and 9 months of age have higher innate resistance to most tick-borne diseases and consequently disease incidence and corresponding mortality are typically lower for this stock class (Goff *et al*, 2002). If a sufficiently high proportion of a herd is consistently exposed to *Babesia* spp as calves a state of endemic stability may develop in which clinical tick fever is rarely seen (Bock *et al*, 2004).

Babesia bovis infection rates in *B. microplus* are often very low. As a result, infection does not always follow exposure to ticks if infection levels are low. Droughts, dipping practices or use of tick- resistant breeds of cattle may delay transmission of *B. bovis* for months or years resulting in an unstable disease situation (Bock *et al*, 2004).

Babesia bigemina transmission rates are higher than *B. bovis* because more ticks carry it and therefore endemic stability is more likely to develop to *B. bigemina* than to *B. bovis* in regions where both are present (Bock *et al*, 2004).

Bos indicus breeds almost invariably experience milder signs of babesiosis than Bos taurus breeds. This phenomenon is thought to be a result of the evolutionary relationship between Bos indicus cattle, *Boophilus* spp. and *Babesia* spp. (Bock *et al*, 2004).

Babesia bovis infection lasts for at least four years in Bos taurus cattle and *B. bigemina* usually less than 6 months. Immunity to both parasites remains for at least four years regardless of the status of the infection (De vos *et al*, 2004). Most cattle with a significant B. indicus content lose *B. bovis* infection within 2 years, but immunity to *B. bovis* lasts at least 3 years (Johnston *et al*, 1978).

Because the *B. bovis* infection does not persist in ticks beyond the larval stage, infected cattle are the main reservoir of infection. However, in the case of *B. bigemina* where the infection can persist from one generation of ticks to the next with out reinfection and the carrier state in cattle

is often short- lived; ticks play an important role not only as vectors, but also as reservoirs of infection (Johnston *et al*, 1978).

2. 1. 3. Developmental cycle

Life cycle in cattle: The typical life cycle of *Babesia* spp. is presented in Figure 1. Following attachment of an infected tick, *Babesia* spp. trophozoites are released into the blood, infecting erythrocytes. Within the erythrocytes, the parasite multiplies by binary fission, an asexual form of schizogony. Naïve ticks attach to the animal and become infected with *Babesia* spp. when they ingest a blood meal (Gardiner *et al*, 1988). Infective forms called small merozoites are produced in large numbers in the salivary glands of feeding ticks. The infective forms of *B. bovis* are injected into cattle by larval ticks, and those of *B. bigemina*, by nymphal and adult ticks. In infected cattle, the parasites invade red blood cells, where they undergo development and division and destroy the red cell. More parasites are thus released into the blood stream and invade other red blood cells. The destruction of blood cells releases the red pigment, haemoglobin. In severe infections, sufficient haemoglobin is excreted by the kidneys to discolour the urine - hence the common name 'red-water'. The blood cell cycle is repeated until the animal dies or until its immune system is able to control the infection. Parasites may remain in the blood stream in very small numbers, perhaps for years, without causing sickness (a carrier of the disease).

Life cycle in tick: In the tick, the developmental cycle is more complex, with the parasites undergoing many changes. The parasites invade and multiply in various tissues of the developing embryo, and of the new generation of larvae. When a female tick feeds on an infected animal, Babesial parasites present in the red blood cells enter the gut lumen of the tick. In the gut lumen, the parasites escape from the red cells and invade gut epithelial cells where they undergo massive multiplication. The end result is the production of large parasites called large merozoites, which are released into the haemolymph, that is, the tick's 'blood'.

These are motile and able to swim and some enter the oviduct and invade the developing eggs. Here, the parasites multiply again and then remain dormant until the eggs hatch and the larval progeny infest a suitable host. After attachment of infected seed ticks (larvae), the *Babesia* is activated and development recommences. The main stimulus for this seems to be heat, provided by proximity of the larvae to the warm-blooded animal (Brian 1997).

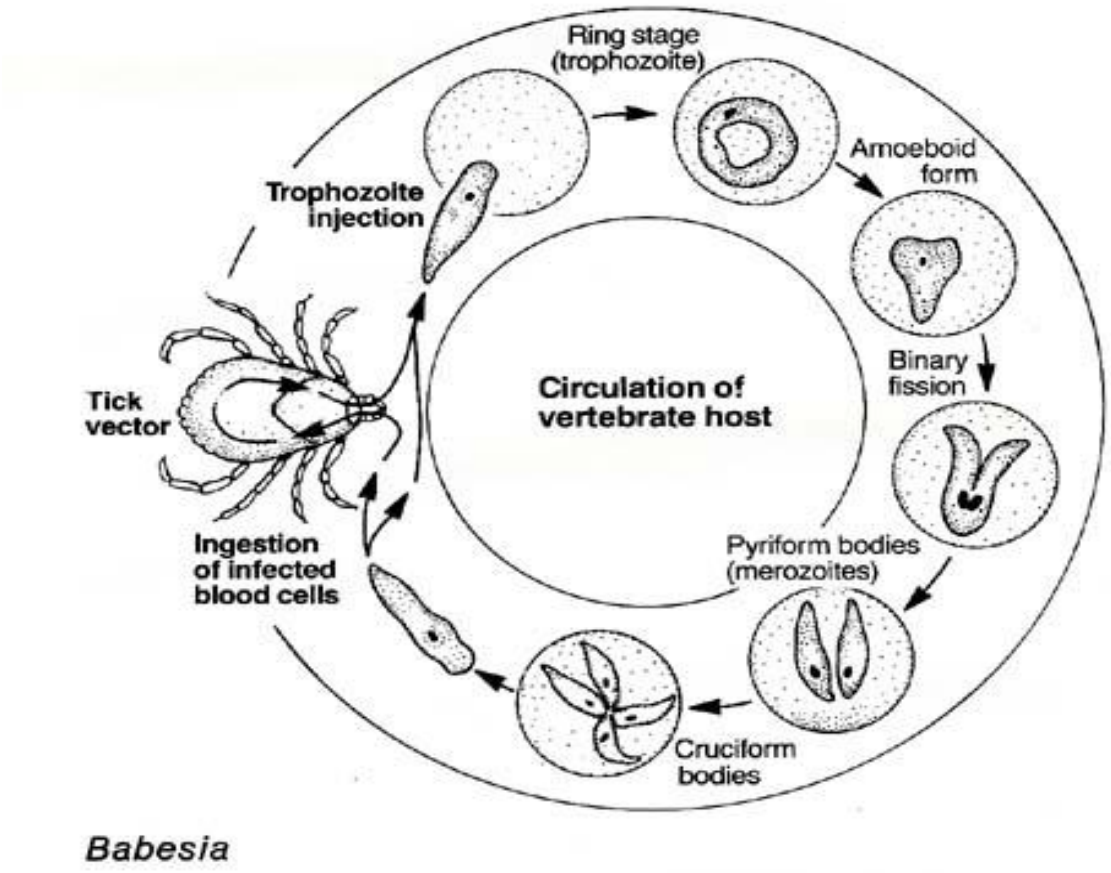

Fig. 1. Life cycle of *Babesia* spp. Source: (Gardiner *et al*.,1988)

2. 1. 4. Host range

Cattle are the principal hosts, but it is reported that the water buffalo and African buffalo may also become infected (Pumell, 1981). It is possible that other ungulates are infected, but from a practical point of view, these infections are nominal and, except under unusual conditions, rare. Such hosts are probably not significant reservoirs of infection (Pumell, 1981).

Babesia bigemina is widespread in cattle and occurs wherever *Boophilus* ticks are encountered, which includes North and South America, Southern Europe, Africa, Asia, and Australia (Mc

Cosker, 1981). Babesiosis also occurs on the Caribbean and South Pacific islands. Cattle and the invertebrate tick hosts provide the major reservoir of infection. Wildlife and no bovine hosts generally have not been incriminated (Mc Cosker, 1981).

2. 1. 5. Transmission

Ticks acquire *Babesia* infection during their feeding on infected animals. The infection is then passed to the ovaries, and thus the emerging larvae carry the infection. The *Babesias* continue to develop within the larvae, and transmission usually occurs in the new host during the nymphal and adult stages. *Boophilus annulatus*, *B. microplus*, and *B. decoloratus* are the principal vector of *B. bigemina*. Mechanical transmission is possible, but it is not efficient enough to maintain infection in the absence of specific tick vectors (Mohoney, 1977)..

The same ticks (*B. annulatus*, *B. microplus*) that transmit *B. bigemina* are usually capable of transmitting *B. bovis*. The tick *B. decoloratus*, which is widely distributed in Africa, does not appear to transmit *B. bovis* even though it readily transmits *B. bigemina* (Kuttler, 1984).
Other factors that influence the infection rate in larval ticks are temperature, and time after hatching. Studies suggest that larvae that spend 2 to 4 weeks on the ground before infesting cattle are more likely to spread babesiosis than newly hatched larvae. It seems that *Babesia* needs a dormant period within non-parasitic larvae to develop fully. Cool weather (about 14°C) appears to enhance this development. On average about 1 in 10,000 ticks are infected with *B. bovis* and 1 in 3,000 infected with *B. bigemina* (Brian, 1997).

2. 1. 6. Incubation Period and Clinical signs

Natural transmission occurs by the feeding of infected nymphal and adult ticks, and evidence of infection occurs 2-3 weeks after tick infestation. Following blood inoculation, the incubation time may be 4-5 days or less, depending on the size of the exposing inoculum. *B. bovis* has a longer incubation time than does *B. bigemina*, but since *B. bovis* is transmitted by the larval stage of the vector rather than by the nymphal and adult stages, *B. bovis* prepatency (measured from the time of tick infestation) is only slightly longer than that of *B. bigemina*. The incubation time is usually

10-14 days; however, this can be shortened by large inoculums. Infection with *B. bigemina* is usually accompanied by the presence of *Boophilus* ticks. Calves normally are reasonably resistant to *Babesia*, for the infection does not usually result in clinical disease. In older animals, clinical signs can be very severe; however, differences in pathogenicity may occur with various *B. bigemina* isolates associated with different geographic areas. Mahoney observed that the Australian *B. bigemina* rarely causes disease, whereas *B. bigemina* in Africa is highly pathogenic. Personal experience by the author suggests that *B. bigemina* as seen in the Western Hemisphere is highly pathogenic; however, it is probably less so than *B. bovis* (Mohoney, 1977).

The first sign is usually a high fever with rectal temperatures reaching 41.5^{o} C (106.7^{o} F). There is anorexia, and ruminal atony. Often the first visible appearance of infection is that the animal isolates itself from the herd, becomes uneasy, seeks shade, and may lie down. Cattle may stand with an arched back, have a roughened hair coat, and show evidence of dyspnea and tachycardia. The mucous membranes are first injected and reddened, but as erythrocytic lysis occurs, the color changes to the pallor of anemia. Anemia is a contributory factor to the weakness and loss of condition seen in cattle that survive the acute phase of the disease. The anemia may occur very rapidly, with 75 percent or more of the erythrocytes being destroyed in just a few days. This is usually associated with severe hemoglobinemia and hemoglobinuria. After onset of fever, the crisis will usually pass within a week, and if the animal survives, there is usually severe weight loss, drop in milk production, possible abortion, and a protracted recovery. Mortality is extremely variable and may reach 50 percent or higher, but in the absence of undue stress most animals will survive (Mohoney, 1977).

Infections of *B. bovis* resemble, in many respects, those seen with *B. bigemina*, but there are some characteristic differences. Hemoglobinuria and hemoglobinemia are not as consistently seen in infections with *B. bovis*, although they may occur. The level of anemia is frequently less severe, but central nervous system involvement (high fever, anorexia, depression, ataxia, circulatory shock and sequestration of erythrocytes in brain capillaries which may result in in-coordination, teeth grinding, and mania) is major clinical signs of *B. bovis*. It is generally conceded that *B. bovis* is the more virulent of the two organisms (De vos *et al*., 2000).
Animals commonly develop in coordination and depression and go down with the head extended but later thrown back and have involuntary movement of the legs. These signs are followed by

death. Whereas the packed cell volume (PCV) in most fatal infections with *B. bigemina* will be well below 10 percent, death commonly occurs with *B. bovis* when the PCV is 12 percent or higher. When hemoglobinuria is seen, the color is not so dark, nor is the plasma following PCV determination so red. The observed parasitemias in peripheral blood are usually much lower with *B. bovis* than with *B. bigemina* (De vos *et al*., 2000).

2. 1. 7. Gross Lesions and Histopathology

The lungs may be edematous and congested in cattle that have died early in the course of infection. The pericardial sac may contain serosanguineous fluid and subepicardial and subendocardial petechial hemorrhages. The liver is enlarged and icteric, and the gallbladder, which may have hemorrhage on the mucous surface, is distended with thick, dark green bile. The spleen is markedly enlarged, and has a dark pulpy consistency. The abomasal and intestinal mucosa may be icteric with patches of subserosal hemorrhages. The blood is thin and watery. The urinary bladder is frequently distended, with dark, reddish-brown urine. Jaundice is commonly distributed in the connective tissue. The lymph nodes are edematous and often have petechiation (Wright *et al*., 1989).

In cattle that have suffered a more prolonged illness, acute lesions are much less conspicuous. Subepicardial petechial hemorrhages may be present, the carcass is usually emaciated and icteric, the blood is thin and watery, the intermuscular fascia is edematous, the liver yellowish-brown, and the bile may contain flakes of semisolid material. The kidneys are pale and often edematous, and the bladder may contain normal urine, depending on how long past the hemolytic crisis the necropsy is performed. Although the spleen is enlarged, the pulp is firmer than in acute Babesiosis (Mahoney, 1977).

The pathogenesis of Babesia bigemina infection is almost entirely related to parasite induced intravascular haemolysis. Anaemia, jaundice, haemoglobinuria and excess thick granular bile are commonly seen in fatal cases but not congestion of the brain and visceral organs. Cardiac haemorrhages and splenic enlargement are not as marked as after *B. bovis* infection, but pulmonary oedema is a more regular feature (De vos *et al*, 2004).

The most striking microscopic change seen in acute *B. bovis* infections is sequestration of parasitised erythrocytes and resultant congestion in capillary and visceral organs, especially in the brain and kidneys (De vos *et al*, 2004). Sequestration is not a feature of *B. bigemina* infections with parasitsed cells evenly distributed throughout the circulatory system and little or no congestion (De vos *et al*, 2004).

Variable degrees of centrilobular necrosis of the liver are seen in *B. bovis* and *B. bigemina* infections. In both infections, but particularly with *B. bovis*, there are marked accumulations of macrophages, neutrophils, lymphocytes and plasma cells within the sinusoids and central veins of the liver. Pulmonary oedema is seen more frequently in *B. bigemina* than in *B. bovis* cases, as is extensive necrosis of the pulp of the spleen. Phagocytosis of erythrocytes and cell debris is most marked in the hepatic and renal lymph nodes, but can also be detected in the liver and spleen. In the spleen there is congestion and a marked reduction in the ratio of white to red pulp (De vos *et al*, 2004).

2. 1. 8. Diagnosis

Based on clinical signs: Fever, anemia, jaundice, and haemoglobinuria are suggestive clinical signs of Babesiosis in cattle found in enzootic areas where *Boophilus* ticks occur. Cattle infected with *B. bigemina* may also exhibit irritation and aggressive, but signs of cerebral derangement such as circling, head pressing mania and convulsion have only been reported in cases of *B. bovis* infection. If these signs are also associated with erythrocytic destruction, the diagnosis of babesiosis is strengthened. Clinically, babesiosis can be confused with anaplasmosis, trypanosomosis, theileriosis, bacillary hemoglobinuria, hemobartonellosis and leptospirosis. Rabies and toxins must also be considered in cattle exhibiting nervous signs. A positive diagnosis requires the identification of the *Babesia* on blood smears or either positive serologic tests or transmission experiments, or both (Bose *et al.*, 1995).

Blood smears examination: The traditional method of identifying Haemoparasite in infected animals is by microscopic examination of thick and thin blood films stained with, for example, Giemsa. Thick blood films are 10 times more sensitive than thin blood films and are therefore very useful for the detection of low-level *Babesia* spp infections. The sensitivity of this technique is such that it can detect parasitaemia as low as 1 parasite in 10^6 - 10^7 red blood cells (Bose *et al.*,

1995). Species differentiation is good in thin films but poor in the more sensitive thick films. This technique is usually adequate for detection of acute infections, but is not suitable for detection of carriers where the parasitaemias are usually well below levels detectable by this method (Bose *et al.,* 1995).

Parasite identification and differentiation can be improved by using a fluorescent dye, such as acridine orange instead of Giemsa. A quantitative buffy coat method using acridine orange to stain parasites in capillary tubes was developed to demonstrate *Plasmodium* in human blood and could potentially also detect low *Babesia* parasitaemias, but differentiation is likely to be poor (Bose *et al.,* 1995).

Samples from live animals should preferably be taken from capillaries, such as those in the tip of the ear or tip of the tail, as *B. bovis* is more common in capillary blood. *Babesia bigemina* and *B. divergens* parasites are uniformly distributed through the vasculature. If it is not possible to make fresh smears from capillary blood, sterile jugular blood should be collected into an anticoagulant such as ethylene diamine tetra-acetic acid (EDTA) (e.g. 1 mg/ml). Heparin may affect the colour characteristics of the staining and is not recommended (Bose *et al.,* 1995). The sample should be kept cool, preferably at 5°C, until delivery to the laboratory.

Thin blood films will be air-dried, fixed in absolute methanol for 1 minute, and stained in 10% Giemsa stain for 20-30 minutes. It is preferable to stain blood films as soon as possible after preparation to ensure proper staining definition (Bose *et al.,* 1995) .

Examination of impression smears: Samples from dead animals should consist of thin blood films, as well as smears from (in order of preference), cerebral cortex, kidney, liver, lung, and bone marrow. Organ smears are made by pressing a clean slide on to a freshly cut surface of the organ or by crushing a small sample of the tissue between two clean microscope slides drawn lengthwise to leave a film of tissue on each slide. The smear is then air-dried (assisted by gentle warming in humid climates), fixed for 5 minutes in absolute methanol, and stained for 20-30 minutes in 10% Giemsa. This method is especially suitable for the diagnosis of B. bovis infections, but is unreliable if death has occurred over 24 hours previously. However, parasites can often be detected in blood taken from veins in the lower limb region one or more days after death (Anon, 1984).

Serological tests: The indirect fluorescent antibody (IFA) test is widely used to detect antibodies to *Babesia* spp., but the *B. bigemina* test has poor specificity. Cross-reactions with antibodies to *B. bovis* in the *B. bigemina* IFA test are a particular problem in areas where the two parasites coexist. An internationally validated ELISA for the diagnosis of *B. bovis* infection has been developed (Waltisbuhl *et al.,* 1987) but, despite the efforts of several investigators in different laboratories, there is still no similarly validated ELISA for *B. bigemina.* ELISAs for antibodies to *B. bigemina* typically have poor specificity. In one study (El-gaysh *et al.,* 1996), *B. bigemina* antiserum appeared to react nonspecifically with fibrinogen. However, an ELISA recently developed and validated in Australia (Molloy *et al.,* 1998), shows considerable promise. ELISAs have also been developed for *B. divergens* (Chaavin *et al*., 1995), using antigen derived from culture, but there does not appear to be one that has been validated internationally

2. 1. 9. Treatment and Control

Successful treatment of *B. bigemina* depends on early diagnosis and the prompt administration of effective drugs. There is less likelihood of success if treatment is delayed until the animal has been weakened by fever and anemia. If medication is administered early, however, success is the rule, for there are several effective compounds (Kuttler, 1981).

One of the first successful treatments was trypan blue. This treatment may be used to determine the type of infection present. *B. Bigemina* is susceptible to trypan blue treatment, whereas *B. bovis,* is not. Generally, the small babesiass are more resistant to chemotherapy (Severe cases of cerebral babesiosis are refractory to treatment). The most commonly used compounds for the treatment of babesiosis are diminazene diaceturate (3-5 mg/kg), imidocarb (1-3 mg/kg), and amicarbalide (5-10 mg/kg). At a dosage of 3.0 mg/kg, imidocarb provides protection from babesiosis for ~4 wk and will also eliminate

B. bovis and *B. bigemina* from carrier animals. Long-acting tetracycline (20 mg/kg) may reduce the severity of babesiosis if treatment begins before or soon after infection. However, the quinuronium and acridine derivatives are also effective. Treatment of *B. bigemina* is so effective in some instances that radical cures occur that will eventually leave the animal susceptible to

reinfection. For this reason, reduced drug levels are sometimes indicated. Imidocarb has been successfully used as a chemoprophylactic that will prevent clinical infection for as long as 2 months but allow mild subclinical infection to occur as the drug level wanes resulting in premunition and immunity (Kuttler *et al*., 1985).

Ticks can be controlled by using a wide variety of improved compounds, including the chlorinated hydrocarbons, carbamates, organophosphates, and natural and synthetic pyrethrins. Animals should preferably be exposed to the parasites at a young age so that they can develop natural immunity in areas where the diseases occur. In some tropical countries, tick control rather than eradication is the goal. This approach attempts to establish an equilibrium in which the tick numbers are sufficient to maintain low-level infection in the cattle and hence immunity to acute babesiosis. In addition to estimation of cattle serum reactivity to *B. bigemina* and *B. bovis*, the knowledge of infestation levels and the infection rates in the ticks are essential information for a comprehensive epidemiological study on bovine babesiosis (Alonso *et al.,* 1992).

Treatment responds and control mechanism of *Boophilus* and babesiosis is based on tick occurrence, abundance, distribution and enzootic stability. *Babesia* is distributed through worldwide especially in tropical and subtropical region. *Babesia* as cattle disease has been reported in Ethiopia (Solomon *et al.,* 1998). However, the exact prevalence in different parts of the country and its impact on livestock production is not very well known. (Existing tick-borne diseases in different region of Ethiopia are shown in (Table-1).

Table 1. Existing bovine babesiosis on different regions of Ethiopia.

Regions	Bovine Babesiosis (TBD)	Samples	Diagnostic test	Reported prevalece	Authors
Amhara	Babesia bigemina Babesia spp.	Blood smears Impression	Microscopic	38.5%	(Seyoum, 2001)
SNNPRS	Babesia spp.	Impression Blood smear	Microscopic	-	(Jewaro, 1986)
Central Ethiopia	Babesiosis	Blood smear	Microscopic	8.85%	(Wallaga, 1997)
Oromiya	Babesia bigemina	Blood smear Serum	Microscopic Serology (ELISA)	17.39% 72.10%	Solomon et al, (1998)

2. 2. *Boophilus* ticks

2. 2. 1. Morphology and Classification

The mouth of *Boophilus* ticks is short the dentition on the hypostome is arranged in to two columns each consisting of numerous rows of which consists of three denticles (3/3) and (4/4) dentition. The internal margin of the first segment of each palp has a bristle bearing protuberance. The capituli is hexagonal in shape. The scutum of the male yellowish in clour and often so poorly sclerotized that the out line of the gut can be seen through it. There are numerous fin hairs on the conscutum of the males and scutum of females. The eyes are difficult to see, and in the females two distinct groove divide the scutum into a central yellow area and two lateral areas that are reddish- brown colour (*B. decoloratus*), there are no festoons. *Boophilus* tick as Ixodidae is grouped under the phylum Arthropod, class Arachnida, order Acarina, sub-order Ixodida and family Ixodidae (Jongejan and Uilenberg, 1994) and (Walker et al., 2003).

2. 2. 2. Life cycle

Boophilus tick is dioecious thus having separate sexes and posses a tremendous reproductive capacity. The life cycle of one-host ticks is usually rapid, for *Boophilus* it takes three weeks for the feedings on one host and two months for egg laying and larval development (Bowman, 1999).

The adult female tick on the host sucks up considerable amount of blood and copulates on the host with the male while feeding (Morel, 1989 and Seifert, 1996). After attachment the female is inseminated once, before it is ready to fully engorge with blood and subsequently once, compels its single large blood meal, but the male feed intermittently and mate with females repeatedly. During mating the male crawls under the female and after manipulating the female genital opening with mouth parts, transfer the spermatic, a sac contain the spermatozoa, into the opening, most likely with the aid of his front legs (Urquhart *et al.,* 1996 and Walker *et al.,* 2003). Oviposition occurs after a period of digestion and oogenesis in natural shelter, under protective cover of grass, stone or in plant litter. Under suitable conditions (humid and hot), the egg hatches and six-legged larva emerges (Seifert, 1996). After a period of maturation, it begins to seek the first host, either lying in wait on the grass blade or moving to hunt actively.

Generally there are three active stages in the life cycle of a hard tick (Sonenshine, 1993). (Fig. 2): larva, nymph and adult tick (Jongejan and Uilenberg, 1994). Based on the number of hosts required to complete the development during their life cycle, *Boophilus* is classified as one-host tick. All three feedings of any individual tick occur on the same individual host, (Urquhart *et al.*, 1996 and Waker *et al.,* 2003).

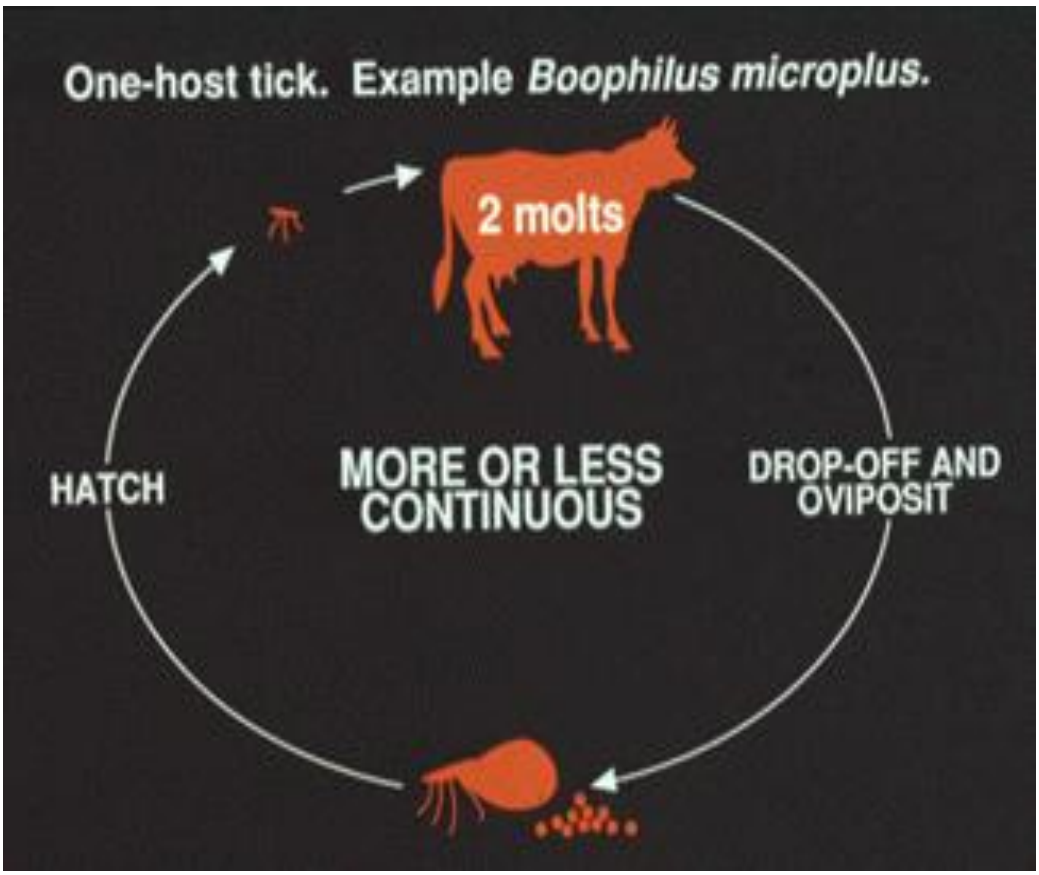

Fig. 2. Life cycle of one host tick. Source: (Soneshine, 1993)

2. 2. 3. Feeding

As other ticks, *Boophilus* tick feed only on the blood of their host. All *Boophilus* species are adapted to feed on cattle, but some may survive by feeding on sheep or antelope. Because *Boophilus* are one-host ticks all stages must be able to feed on the same species of host. According to (Radostits and Gay, 1994) all species are bloodsuckers and may cause death from anemia. All species of *Boophilus* tick at each stage of the life cycle are parasitic feeding solely on blood, liquefied tissue. The tick crawls on to their host and attach to the skin with their mouthparts. These consist of a tube called the hypostome and palps only hypostome penetrates the skin. Often a material (cement) is secreted in the saliva that glues the palps to the outer epidermis and the hypostome to the dermis. The hypostome contains moveable rods called chelicerae, which cut a hole in the dermis and break the capillary blood vessels very close to the surface of the skin, forming a feed lesion. Bite is relatively painless, but invasion by large numbers are debilitating (Radostits and Gay, 1994).

Larvae and nymphs feed on the blood of small wild animals and birds. Adult feed on bigger usually domestic animals. *Boophilus* tick at different stage feed on the same host usually domestic animal (Jongejan and Uilenberg, 1994). Larvae take typically 3 to 5 days to fully engage with blood, nymphs 4 to 8 days and females 5 to 20 days. When the tick has fully engorged with blood they detach from the hosts skin and drop to the ground (Jongejan and Uilenberg, 1994).

2. 2. 4. Distribution of *Boophilus* tick in Ethiopia

Boophilus decoloratus which is also known as the blue tick, require moisture warmth and distributed between 32^0 N and 40^0 S, it is the commonest, widespread and frequent one host cattle tick in Africa. Its distribution pattern is similar to that of *A. variegatum*. It is abundant in wetter highlands and sub-highlands of Ethiopia (Feseha, 1983). (Seleshi *et al.,* 2001) have found this species in nearly all districts except Zone-3 of Afar Regional state. This tick species occurs in regions with savanna and temperate climates, typically in grasslands and woodened areas used as cattle pasture. Studies conducted by externship students from the FVM have shown the existence of *B. decoloratus* widely through out the country, which implies that it is the commonest tick species in Ethiopia Tables 2 and 3.

(Jongean and Uilenberg 1994) indicated the confinement and restrictiveness of *B. decoloratus* to Africa continent and its competitiveness with *B. microplus*, which has excluded from larger areas of Africa. The African *B. decoloratus* is a relatively inefficient vector of *B. bovis* and cannot transmit *B. bovis* (FAO, 1984).

Boophilus annulatus often found together with *B. decoloratus*. Cattle are the main hosts of *B. annulatus* but also occasionally sheep, goats, and wild ungulates can support successful completion of the life cycle. It transmits the protozoan *Babesia bigemina* and *Babesia bovis* to cattle. Heavy infections cause damage to hides and probably lead to a reduction in the rate of growth of cattle (Walker *et al.,* 2003 and (Hall, 1985). Except a single record made by (Bekele 1987) there is no any notification of the existence and distribution of this ticks species in different region. This tick is well established in the Eastern part of neighboring Sudan, and so poses a major risk to the Ethiopian cattle population that has no immunity to *Babesia bovis* (Tick modeling workshop, 1997). *Boophilus decoloratus* is also the most widespread in Africa

stretching from West Africa through central Africa to east Africa and extending into the Sudan and Southern Africa (Pegram *et al*., 1986). This wide geographical spread of *B. decoloratus* within Africa is probably due to its tolerance of a wide range of temperature. The species will survive and its adults will oviposit at 15 and 37^{0c} with low mortality of the eggs at both temperatures; consequently, *B. decoloratus* was the only tick species found on cattle throughout the ecological zones of most African countries. The distributions also are known to vary through space and time due to interactions of many factors, including climate, host diversity, levels of resistance of hosts, absence of tick control measures and management practices that affect host behaviors (Latif, 1986).

Table 2. Existing *Boophilus* tick reported in different regions.

Region	Genus and Species	Authors
SNNPRS	B. decoloratus	(Sebsibe, 1989)
		(Tesfanesh, 1993)
Amhara	B. decoloratus	(Mesele, 1989)
Oromia	B. decoloratus	(Tadele, 1989)
	B. annulatus	(Wallaga, 1997)
Tigray	Boophilus ssp.	(Hagos and Markos, 1996)
	B. decoloratus	(Surafel, 1996)
Harari	B. decoloratus	(Gulilat, 1987)

Table 3. *Boophilus* tick reported from different ranches and Research centers of Ethiopia

Research center	Genus and Species	Authors
Abernosa ranch	B. decoloratus	(Solomon, 1993)
Didtuyura	B. decoloratus	(Solomon, 1994)
Bako Research center	B. decoloratus	(Teshome, 1985)
Metekel ranch	B. decoloratus	(Alekaw, 2000)
Jimma state farm	B. decoloratus	(Kassaye, 1994)
Ghibe ILRI (lowland)	B. decoloratus Boophilus spp.	(Adey, 2004) and Zelalem, 2003)

2. 2. 5. Tick collection (vector)

Tick collection is important for establishing a laboratory tick colony and identification. Ticks can be collected from live, dead and free-living hosts (Spickett *et al*., 1991). However, care should be taken to avoid and exposure of the collected ticks to acaricide. For collection of specific lifecycle stages of a particular tick species, knowledge of the distribution, seasonal abundance, host preference and preferred attachment sites of the different instars can save much time and effort (Spickett *et al*., 1991).

Tick collection from live hosts: To determine the exact predilection sites of tick of cattle, the animals body can be divided into 18 areas (Baker and Ducasse, 1987), each of the sites is handled separately for the purpose of tick collection. However, total tick count of the sort undertaken by Baker and Ducasse, are not always possible for practical reasons. Instead, eight clearly defined sites on each host animals can be selected for study because of their importance as feeding sites for the different stages of the commoner ticks of cattle (Kaiser *et al*., 1982). The sites are (ear, head, dewlap, back, abdomen, udder/ scrotum, ano-vulva and hoof).

There are different sites for example; *Rhipicephalus* can be collected from head, anus; *Amblyomma*, from ventral, dewlap, axillae; *Boophilus* from back, shoulder and dewlap and

Hyalomma from tail and dewlap (Londt *et al.,* 1979). To remove ticks it is necessary to use strong steel forceps designed for ticks' collection.

Tick collection from dead hosts: Ticks for identification purpose can also be collected from dead animals. Usually after the animals have been killed for whatever reason, they are transported to the laboratory. There the carcass of each animal is skinned and half the skin of the head, half the skin of the body and upper legs, the whole skin of the tail as well as one lower front leg and one lower back leg with skins in the bags that are tightly secured and stored over night. The following days, the skins are thoroughly scrubbed with brushes with bristles and washed. The tick-detaching agent (10% of NaOH) remaining in the plastic bags and the material obtained from scrubbing and washing the skins is sieved on sieves with 150um aperture (Horak *et al*., 1992). The residues in the sieves are collected, preserved in 10% formalin and stored.

In this method, the skin is subjected to 10% NaOH digestion, and the operator must wear protective clothing. The respective portions of skin of the animals are immersed in separate baths of 10% NaOH for 3 to 7 days until the hair is sufficiently loosened from the skin portions are discarded The sludge containing the ticks is left in the NaOH solution until all the hair is dissolved, there after the solution is sieved and the residue on the sieve is collected and preserved with 10% formalin (Van Dyk and Mc Knzie, 1992)..

Drag and vegetation sampling for free living adult ticks: Many adult Ixodid ticks can be collected whilst questing for hosts from vegetation. This can be done by dragging sampling technique a distance of 250m (Spickett *et al.,* 1991). In this method, ten-1000 by 100 mm flannel strips are attached adjacent to one another by means of Velcro tape on a 1200 mm-long wooden spar. Each collection is made by an operator pulling this spar, with the flannel strips attached, by means of a string or twin harness attached to its end, for a distance of 250 m over the vegetation (Spickett *et al.,* 1991)

Other dragging methods consists of a cloth of one meter square which is dragged slowly across the vegetation for 5m to 10m (for approximately 30 seconds of walking: or (a longer distance depending on local knowledge). Larvae, nymph and adults will grip onto the dragging cloth temporarily and can be collected with a forceps. A white cloth of cotton toweling is effective. It is fitted with a bar at the front and accord for pulling it. These method works well for larvae and

nymphs of questing species but is less efficient for adults and hunting species (Latif and Walker, 2004). Traps using carbon dioxide as an attractant can be used to collect hunting ticks. In the same way adult ticks can be collected by hand from the tips and stems of grass with in a specified measured area or a long side a measured length of road or path (Spickett *et al.,* 1991).

2. 2. 6. Tick identification

The important taxonomic key used in the identification of different tick species at genera and species level are based on the following morphological features: Pulps (short, long, and medium), Enameled pattern (Present / absent), Eye (Present / absent, orbited and hemispherical), Eleven festoon (present / absent), Anal groove (anterior / posterior), Adanal plate, scutum (present/ absent), Porose areas shape (in female), Hypostome teeth numbers, Cornua (shape), and legs are also important taxonomic keys among others used for identification of ticks (Baker, 1999) and (Walker *et al*., 2003). (Annexes 2 - 10)

3. MATERIALS AND METHODS

3. 1. Description of the study area and Study population.

3. 1. 1. Study area.

The study was conducted at Kobo and Girana valleys, which is found in Kobo, Gubalaftu and Habru districts and the valleys have got 36 lowland Kebeles. Kobo valley is bounded in the North with Tigray Region, in the East with Afar Region, in the West with Gidan district and in the South with Gubalaftu district. Girana valley is bounded in the North with Gubalaftu district, in the East with afar Region, in the West with Ambassel district and in the South with Tewledre district. The valleys are vast stretched North to South a long these three districts (figure-3). Kobo and Girana valleys are 575 km far from Addis Ababa. The altitudinal variation of Kobo valley ranges from 1260-3000 m above sea level and is divided into three major agro- ecological locations that is 3% Dega, 38% Weynadega and 59% Kola. Has annual rainfall 500- 800 mm, and its annual average temperature ranges from 12.31^{0c} - 33.07^{0c} and 48% Relative humidity. The altitudinal variation of Girana valley ranges from 1200- 2350 masl and is divided into three major agro- ecological locations that is 3.5% Dega, 40% Weynadega and 56.5% Kola. Has annual rainfall 750 - 1000 mm, and its annual average temperature ranges from 15^{0c}- 28.6^{0c} and 54% Relative humidity. The valleys experience a bimodal rainfall pattern. A long rainy season that extends form June to September, and a short rainy season from February to May. The land escape is marked by the presence of undulating terrain. The vegetation until fairly recently it was covered by bushes and broad leaved deciduous trees. Besides, scattered woodland and savanna grassland type occurs in the lowlands (Seyoum, 2001).

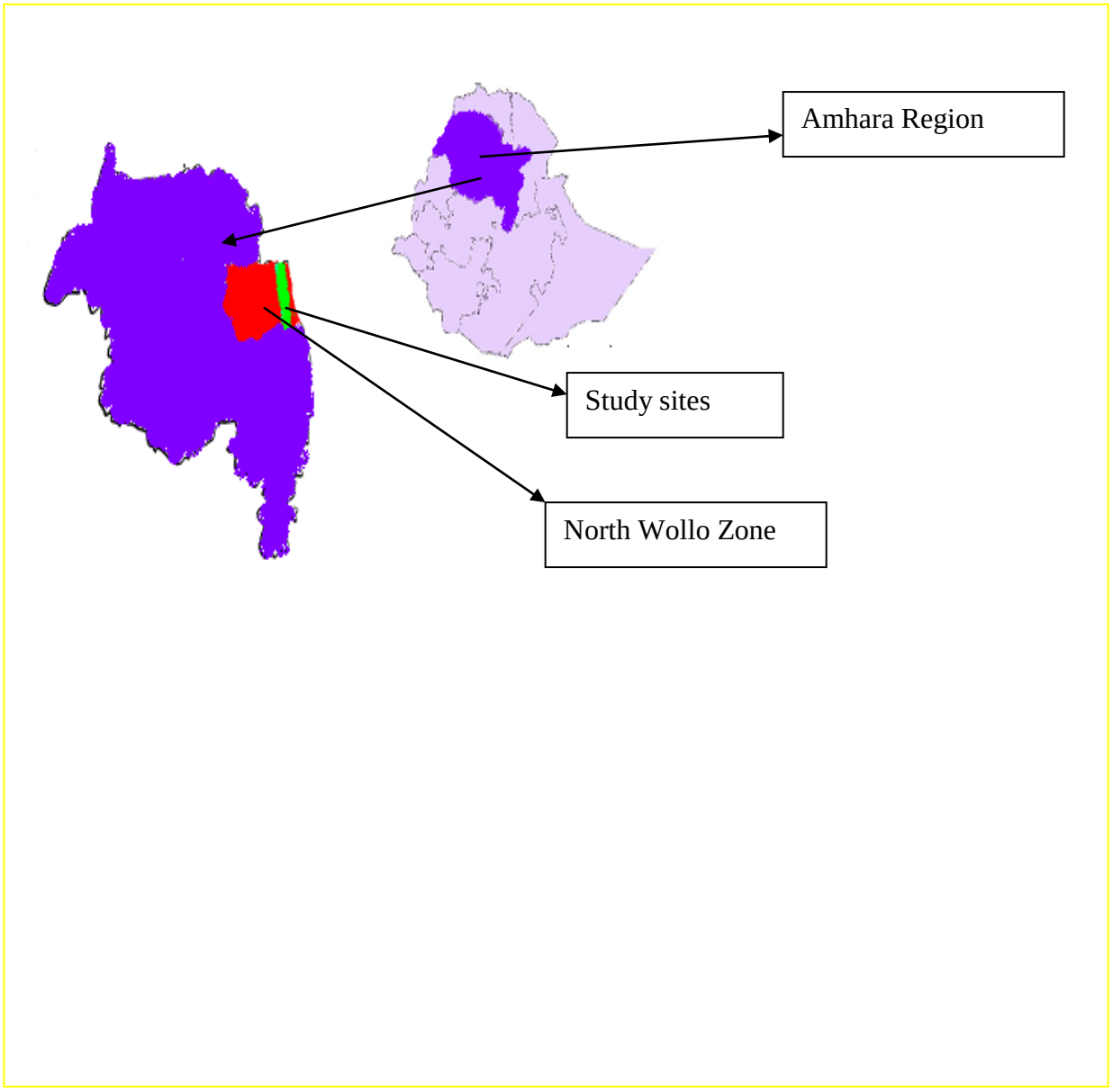

Figure 3. Map of Ethiopia showing Amhara National Regional State and the study sites. Source: BOFED (2006)

3. 1. 2. Study populations

In the ANRS, the livestock comprises of cattle, sheep, goats, equine and camels raised as integrated part of the crop agriculture. Backyard poultry and apiculture are also practiced and have significant contribution in supplementing the small holders' subsistence economy. Now days there are small private dairy and fattening farms, which show dramatic sweeping movement increase the livelihood of the people in the area. Sheep and equines are dominant species in the high land, while cattle, goats and camels are mainly reared in the mid to lowland areas of the region. The livestock populations in the region were estimated to be 10.5 million cattle, 5.3 million sheep, 3.8 million goats, 1.88 million equines, 18,186 camels and 13.4 million chickens and cattle population in the three districts was estimated to be 394, 061 (CACC, 2003). The major livestock production system in the region is mixed crop- livestock farming.

The study population constituted 430 (among which 370 for cross sectional and 60 animals for seasonal follow up studies) indigenous cattle managed under smallholder mixed crop-livestock farming system. There are different wild animals such as, wild cat, hyena, fox, monkey, tortoise, rabbit, antelope, warthog and many different types of birds were observed commonly in the study areas (Seyoum, 2001).

3. 2. Study design

3. 2. 1. Sampling methods.

The study of bovine babesiosis and the vector (*Boophilus)* was conducted at Kobo and Girana valleys. Kobo and Girana valleys are found in Kobo, Gubalaftu and Habru districts. Different Peasant association (PA'S) and cattle that were found in the valley were as sampling unit. The valleys were selected purposively due to road availability and its importance and the Peasant associations (PA'S) were selected randomly (lottery system) from the total of each corresponding districts in the valleys. The cattle were also selected randomly using systemic random sampling, from indigenous breeds of Bos indicus managed under extensive production system.

3. 2. 2. Sample size determination

The sample size for the present study was determined using the formula indicated by (Pfeiffer, 2002). To determine the level of disease occurrence taking Previous estimate of prevalence of 38.5% (Seyoum, 2001), accepted error 5% and 95% confidence level.

$$n = \frac{1.96^2 \, P \, exp \, (1-P)}{d^2}$$

Where:

n - Required sample size

P exp- expected prevalence

d- Desired absolute precision

Accordingly, a minimum of 363 cattle has to be sampled for microscopic examination (bovine babesiosis) and for the vector, *Boophilus* study. However for the sake of convenience 7 cattle were added and a total 370 animals were sampled. From the total of 370 animals, 60 negative animals were reselected randomly for longitudinal study. These animals were identified by their name, color, by their owners name and with ear tag and not treated with any acaricide during the study period.

3. 3. Study type

A cross-sectional study type was employed to estimate the prevalence of bovine babesiosis and *Boophilus.* A longitudinal study type was used to assess the seasonal occurrence of the vectors and the corresponding etiological agents (*Babesia bigemina* and *Babesia bovis*).

3. 3. 1. Cross- sectional study

A random sample of individuals of a population (herd, group) was taken to estimate the prevalence of babesiosis and the vector. The study was conducted on 370 animals that were selected randomly in the three districts of the valleys. Available data such as body condition, age and sex of the animals were recorded prior to blood sampling and tick collection. Age

determination was conducted according to the description provided by FAO (1983) for zebu cattle (Annex- 11).

The body condition of the study animals was estimated by visual inspection and palpation of the loin muscle and tail head of the animal. The degree of fatness over these areas was studied, and a score of good and poor was given. The method of scoring was adapted by (Radostitis and Blood, 1985). The degree of fatness was marked from 0 to 1 as Poor and from 2 to 4 as Good (Annex-12).

3. 3. 2. Longitudinal study

A total of 60 animals were randomly reselected from those animals, which already selected for cross-sectional study at Kobo and Girana valleys and these animals were studied for their tick burden, relative abundance, male to female and adult to immature ticks' ratio of *Boophilus.* Tick infestations were also studied on different seasons of the year, age groups, sex and body conditions. Bovine babesiosis was studied on the basis of age groups, sex and body conditions. The correlation between the disease and the vector were also tested.

3. 4. Study Methodology

3. 4. 1. Blood smear examination

Blood samples were collected from 370 animals for cross-sectional study and 60 for longitudinal study and thick as well as thin blood smears prepared (Annex- 13). The samples were obtained from blood capillaries, in the tip of the ear. *Babesia bigemina* parasite is uniformly distributed through the vasculature (Annex- 14). Thin blood films were air- dried, fixed in absolute methanol and stained in 10% Giemsa. The prepared blood films were stained as soon as possible to ensure proper staining definition. Thick films were made by placing a small drop (approximately 50µl) of blood onto a clean glass slide. The droplet blood was air- dried, fixed in absolute acetone and stained in 5% Giemsa. Then the slides were washed with tap water, dried and examined under oil immersion objective of compound microscope for the presence of *Babesia* organism as described by (Sloss *et al*., 1994).

Two oxen were died during the study period showing clinical symptoms such as high temperature (41.9^{0c}), haemoglobinuria, anemia, and stand with an arched back and during post mortem, the liver was enlarged and icteric, petechial hemorrhage on pericardial sack, gallbladder was distended and pronounced pulmonary oedema was observed. Brain and visceral organs were not congested. Tentatively bovine babesiosis was suspected.

Appropriate samples were collected in animals found dead during the study period. The collected samples include thin blood films, samples from cerebral cortex, kidney, liver, lung and bone marrow. Organ smear were made by pressing a clean slide on to freshly cut surface of the organ or by crushing a small sample of the tissue between two clean microscopic slides drawn lengthwise to leave a film of tissue on each slide. The smears were air-dried, fixed in absolute methanol, and stained in 10% of Giemsa. This method was especially suitable for the diagnosis of *B. bovis* infections (Anon, 1984).

3. 4. 2. Tick collection

Ticks were collected at once from the whole body of 370 animals for determination of prevalence. Similarly for seasonal longitudinal study 60 animals randomly reselected from the total of 370 animals. These animals were considered in order to differentiate one another using ear tag on their neck. Removal of feeding ticks from the animals were carried out at four different times (early and late rainy season i.e. spring (March to April) and autumn (September to October) early and late dry season i.e. autumn to winter (November to December) and winter (January to February). In this way the first round sample was collected on September, 2006 corresponding to late rainy, the second was sampled on November, 2006 representing early dry season, the third round sample was conducted in January, 2007 which represent the late dry season and lastly, the fourth round sample was collected on March, 2007 represent early rainy season.

The animals to be sampled were casted down or make to stand in the crush; tick collection was made on alternative body sides (Annex-15). All visible attached adult, nymph and larvae of all tick species from the eight predilection sites: (ear, head, dewlap, back, abdomen, Venter (udder/ scrotum), ano-vulval and hoof (feet) were collected as described by (Kaiser *et al.,* 1982). The tick samples from each animal were placed into pre-labeled screw cap universal bottle corresponding

by their body site and preserved with 68% of methanol. Identification of ticks was conducted after each collection ends using taxonomic key provided by (Walker *et al*., 2003).

3. 5. Data analysis

Data collected from the study area was recorded in computer. Microsoft Excel was used for data entry and management. The data was analyzed using STATA 7.0 soft ware. The prevalence of *Boophilus* tick and babesiosis were computed. The infection and infestation rate were computed and analyzed for different, age groups, sex, body condition, areas and seasons. Person's Chi-square (X^2), the Fisher exact and T-tests statistics were used to test the association and existence of differences in prevalence between age groups, sex, body condition and areas of *Boophilus* tick and babesiosis. Multivariate logistic regression model was fitted to the data to establish the interaction of different risk factors such as sex, age groups, body condition and season on the prevalence of *Boophilus* tick.

Descriptive statistics: such as percentage, confidence intervals (Cl) were used to summarize the proportion. A statistically significant association between variables was established if the computed p- value is less than ($p< 0.05$).

4. RESULTS

4. 1. Cross-sectional study

4. 1. 1. Blood smear examination study results

Babesia bigemina was the only babesiosis identified in Kobo-Girana valleys during the study period. From the total of 370 blood samples examined the over all prevalence of *Babesia bigemina* in the valleys was 20.3% in animals sub populated by age, sex, body condition and areas (Table 4).

The prevalence of *Babesia bigemina* in age groups (<2, 2-4 and >4) in the study areas was found to be 20.3%, 24.3% and 16.1%, respectively. Those animals examined in 3-4 age groups slightly more infected with the disease than other group' of animals. However, analysis of age wise prevalence among the different age groups was not statistically significant ($p>0.05$) difference.

The infection rates of *Babesia bigemina* on sex (female and male) basis at Kobo and Girana valleys were compared and the result revealed that 7.6% and 12.7%, respectively. The infection rate was higher in male than the female animals and there was statistically ($p<0.05$) significant difference in the prevalence between female and male.

During the study period animals with poor body condition and with good body condition were observed and the prevalence of the disease was 26.5% (151) and 15.19% (219), respectively. The prevalence of bovine babesiosis in animals with poor and good body was found to be statistically significant ($p<0.05$)

The prevalence of bovine babesiosis at Kobo and Girana valleys was found to be 22.7% and 17.8%, respectively. The prevalence of *Babesia bigemina* at the valleys was not statistically ($p>0.05$) significant.

During microscopic examination equal number of thick and thin blood films were taken and strictly examined. The repeated examination result indicated that thick blood films were found more sensitive than thin blood films (Annex- 14).

Table 4. Prevalence of *Babesia bigemina* in cattle sub populated by age, sex, body condition and area.

Variables	No of animals examined	No of infected	Infection rate (%)	P-value
Age				
<2	123	25	20.3	
2-4	123	30	24.3	0. 271
>4	124	20	16.1	
Sex				
Female	185	28	7.6	0. 014
Male	185	47	12.7	
Body condition				
Poor	151	40	26.5	0. 013
Good	219	35	15.19	
Valleys				
Kobo	185	42	22.7	0. 244
Girana	185	33	17.8	

4. 1. 2. Tick collection study findings.

A total of 8,534 adult and immature ticks (14 tick spp) were collected during the study period. Among which only *Boophilus* ticks were the objective of the present study. The rest 5,634 ticks (13 tick spp) were collected and identified only for the purpose of comparison. All 2,900 collected *Boophilus* ticks in the study areas were identified as *Boophilus decoloratus* (Annex-16). This tick species was the only vector of *Babesia bigemina*, which was identified among *Boophilus* genus in the study areas.

The result found in the present study shows that all examined cattle in the study areas were positive for *Boophilus decoloratus*. The over all prevalence was 82.4% in cattle sub populated by age, sex, body condition and areas (Table 5).

Table 5. Prevalence of *Boophilus decoloratus* in cattle sub populated by age, sex, body condition and area.

Variables	No of animals examined	No of infected	Infestation rate (%)	P-value
Age				
<2	123	90	73.2	
2-4	123	101	82.1	0. 001
>4	124	114	91.9	
Sex				
Female	184	144	78.3	0.036
Male	186	161	86.6	
Body condition				
Poor	151	126	83.4	
Good	219	180	82.2	0. 492
Valleys				
Kobo	185	152	82.2	0. 891
Girana	185	153	82.7	

The prevalence of *Boophilus decoloratu*s in age groups (<2, 2-4 and >4) was 73.2%, 82.1% and 91.9%, respectively (Table 5). All age groups of animals were found to carry relatively high number of *Boophilus decoloratus* with mean tick burden, (8.3, 9.5, and 10.4 ticks), respectively (figure 4). Age wise prevalence of *Boophilus decoloratus* among the three age groups were statistically significant ($p< 0.001$) difference at Kobo-Girana valleys.

The infestation rates of *Boophilus decoloratus* on sex (female and male) basis were (78.3% and 86.6%) (Table 5) and their mean tick burden was (8.3 and 10.6 ticks) respectively. Male animals carry relatively high number of ticks than female (Figure 5) and the difference was found to be statistically ($p < 0.05$) significant.

Boophilus decoloratus infested animals appear to be higher in animals having poor body condition and the infestation rate was (83.4%) and the mean tick burden was (10.7 ticks) than those in good body condition (82.2%) and with mean ticks burden of (8.7 ticks) Figure 6. However no statistically significant ($p > 0.05$) difference was observed. The infestation rate at Kobo valley was (82.2%) and that of Girana valley was (82.7%). The infestation rate area wise was almost the same and the difference was not significant ($p > 0.05$) at kobo and Girana valleys.

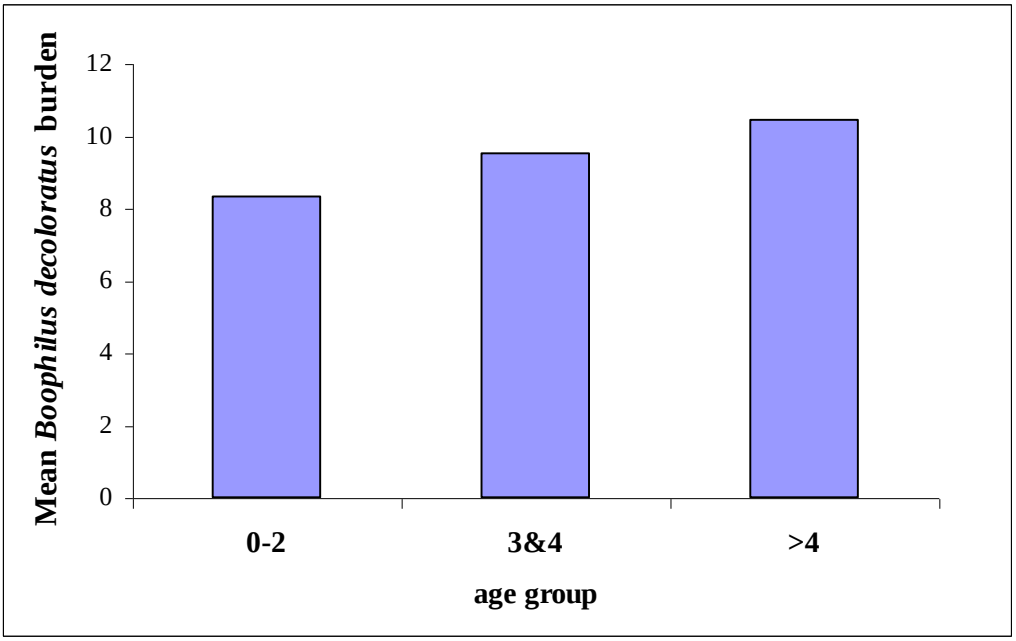

Figure 4. Mean *Boophilus decoloratus* burden on age groups basis.

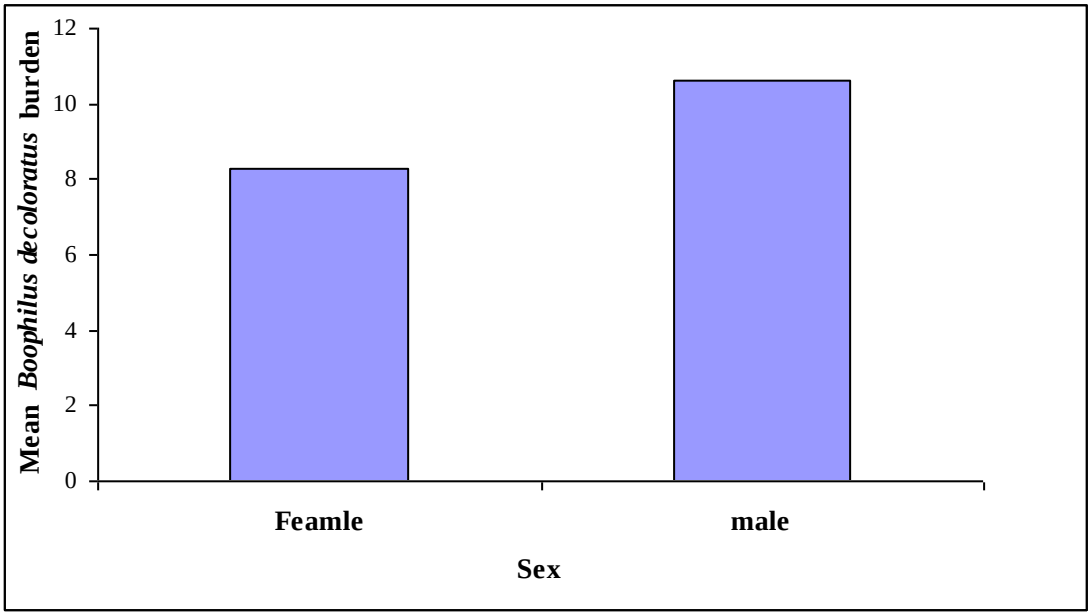

Figure 5. Mean *Boophilus decoloratus* burden on sex basis

Figure 6. *Boophilus decoloratus* burden on body condition basis.

Fourteen tick species belonging to four genera (*Amblyomma, Rhipicephalus, Hyalomma* and *Haemaphysalis* were collected and identified during cross sectional study (Table 6). Among the genus mentioned the objective of this study protocol is to study only the sub genus *Boophilus decoloratus*. Other tick species here were used only for the purpose of comparison. The genus *Amblyomma* represented the largest proportion (56.45%) followed by *Rhipicephalus* (42.68%) and the genus *Hyalomma* (0.68%) and *Haemaphysalis* (0.20%) were represented the lowest number of ticks at Kobo- Girana valleys. The most prevalent and abundant tick species from the total tick count were *A. variegatum* (28.22%), *A. cohaerence* (24.9%) and *Boophilus decoloratus* (24.4%). Other tick species also collected and identified, but were small count. During tick collection immature ticks were also included and were represented (12.11%) from the total count. There was a relative prevalence and abundance difference; however there was no variation in genera and species type encountered in both valleys. *Boophilus decoloratus* count at Kobo (n=1461) and Girana (n=1439) almost it seems similar except slight prevalence difference.

Table 6. Relative abundance of *Boophilus* tick species at Kobo and Girana valleys.

Ticks species	Study sites/ Valleys		Over all prevalence
	Kobo	Girana	(%)
B. decoloratus	1045 (24. 6%)	1035 (24. 21%)	2080 (24. 4%)
Boophilus spp*	416 (9. 8%)	404 (9. 5%)	820 (9. 6%)
A. Variegatum	1211 (28. 4%)	1197 (28. 0%)	2408 (28. 22%)
A. cohaerence	1051 (24.7%)	1078 (25. 22%)	2129 (24. 9%)
A. lepidium	21 (0.51%)	23 (0. 54%)	44 (0. 52%)
A. gemma	11 (0. 26%)	13 (0. 3%)	24 (0. 3%)
Amblyomma spp*	103 (0. 51%)	111 (2. 6%)	214 (2. 51%)
R. e. evertsi	171 (4. 02%)	169 (3. 95%)	340 (3. 98%)
R. pulchellus	183 (4. 3%)	187 (4. 4%)	370 (4. 34%)
R. lunulatus	5 (0. 12%)	4 (0. 1%)	9 (0. 11%)
R. praetextatus	5 (0. 12%)	6 (0. 14%)	11 (0. 13%)
R. simus	3 (0. 07%)	7 (0. 16%)	10 (0. 12%)
Hy. m. rufipes	19 (0. 45%)	21 (0. 5%)	40 (0. 47%)
Hy. truncatum	7 (0. 16%)	11 (0. 26%)	18 (0. 21%)
H. aciculifer	3 (0. 07%)	2 (0. 05%)	5 (0. 06%)
H. parmata	5 (0. 12%)	7 (0. 16%)	12 (0. 14%)
Total	4259 (49. 91%)	4275 (50. 1%0	100%

Key:

B: Boophilus *A: Amblyomma* *R: Rhipicephalus* *Hy: Hyalomma*

H: Haemophysalis *H.m: Hyalomma marginatum*

*Immature

Generally out of the whole tick population collected and identified, the proportion of males' adult ticks was higher than adult females. Even though, in the case of *Boophilus decoloratus* sex ratio female ticks were higher in number than the males once (Table 7).

Table 7. Sex ratio in different adult tick species.

Species	Adult ticks		Sex ratio
	M	F	M : F
B. decoloratus*	600	1480	0.41: 1
A. variegatum	804	300	2.68: 1
A. cohaerens	1529	600	2.55: 1
A. gemma	15	9	1.67: 1
A. lepidium	27	17	1.59: 1
R. e. evertsi	227	113	2.01: 1
H. m. rufipes	27	13	2.01: 1
H. parmata	8	4	2: 1

***** =The objective of this work is to study *Boophilus*; other ticks here were used only for the purpose of comparison.

Different tick species have their own preference of attachment sites on their respective hosts. Despite the fact that *Boophilus decoloratus* has its own preference predilection body sites, in this study except ano- vulva the tick was virtually collected from all over the host body. Mainly

predominates on back (shoulder), dewlap, head, ear, venter, tail and hoof (Table 8). Immature *Boophilus* showed affinity of attachment all over the host body sites..

Amblyomma variegatum and *A. coharence* were collected from all eight-attachment body sites, predominates mainly on venter (udder/ scrotum and belly), and dewlap. *Rhipicephalus evertsi evertsi* have shown preference of attachment mainly to ano-vulva and small numbers to ear and venter.

Table 8. Distribution of tick species on different predilection body sites of attachment

Sites of attachment	n= 2080	B.d (%)	n= 2408	A.v (%)	n= 2129	A.c (%)	n =24	A.g (%)	n= 44	A.l (%)	n= 340	R.e.e (%)	n= 820	B. spp (%)	n= 214	A. spp (%)
Ear	220	10.8	3	0.12	17	0.8	-	0	0	0	2	0.6	87	10.6	2	0.93
Head	311	14.9	10	0.42	31	1.5	-	0	0	0	-	-	71	8.7	15	7.01
Dewlap	651	31.3	271	11.3	281	13.2	5	20.8	7	15.9	-	-	191	23.3	33	15.4
Back	890	42.8	56	2.33	79	3.7	3	12.5	5	11.4	-	-	364	44.4	21	9.8
Venter	5	0.24	1613	66.9	1340	62.9	12	5.0	30	68.2	1	2.3	89	10.9	72	33.6
Ano-vulva	-	-	79	3..3	51	2.4	1	4.2	1	2.3	337	99.1	15	1.8	11	5.14
Hoof	1	0.05	215	8.9	219	10.3	2	8.33	1	2.3	-	-	2	0.24	31	14.5
Tail	2	0.1	161	6.7	111	5.21	1	4.2	0	0	-	-	1	0.12	29	13.6

Key:
B. d: Boophilus decoloratus *A. g: Amblyomma gemm* *A.c. Amblyomma cohaerence* *R. e. e.: Rh. e. evertsi*
A. v: Amblyomma variegatum *A. l: Amblyomma lepidium* *B. spp: Boophilus immature*
A.spp: Amblyomma immature

4. 2. Longitudinal study

4. 2. 1. Blood smear examination study findings.

To determine the types of bovine babesiosis and indicate the respective prevalence 60 animals were randomly selected and studied across four consecutive seasons. The only bovine babesiosis encountered (identified) at Kobo and Girana valleys was *Babesia bigemina* and the over all prevalence during longitudinal study was indicated to be (21.7%) in the animals sub populated by age, sex and body condition (Table 9). The age groups (<2, 2-4 and >4) wise study prevalence across the four consecutive seasons was (15%, 30% and 20%) respectively. The infection of *Babesia bigemina* was higher during early rainy and late dry seasons where as during early dry and late rainy seasons the infection of the parasite was too low. There was not significant ($p>0.05$) difference in *Babesia bigemina* infection between different age groups.

Sex (Male and female) basis comparison of this intra-erythrocytic protozoa parasite infection rate finding was indicated to be (24.1% and 19.1%) respectively. However, statistically there was no significant ($p>0.05$) difference between sex groups. The infection rates of animals with poor body condition (33.3%) were more aggravated than animals with good body conditions (13.9%) and statistically there was a significant ($p<0.05$) difference across four seasons studied (Table 9).

Table 9. Prevalence of *Babesia bigemina* in cattle sub populated by age, sex and body condition.

Variables	No of animals examined	No of infected	Infection rate (%)	P-value
Age				
<2	20	3	15	
2-4	20	6	30	0.530
>4	20	4	20	
Sex				
Female	31	6	19.1	0.683
Male	29	7	24.1	
Body condition				
Poor	24	8	33.3	0. 013
Good	36	5	13.9	

The infection rate of *Babesia bigemina* and *Boophilus decoloratus* infestation rate was compared during the study period at Kobo-Girana valleys across different seasons, the result revealed that during early rainy season ($r=0.1091$; $p<0.05$) in general both were increased, even though, *Babesia bigemina* infection was decreased during late rain to early dry seasons where as *Boophilus decoloratus* was decreased during early to late dry seasons (Figure 7).

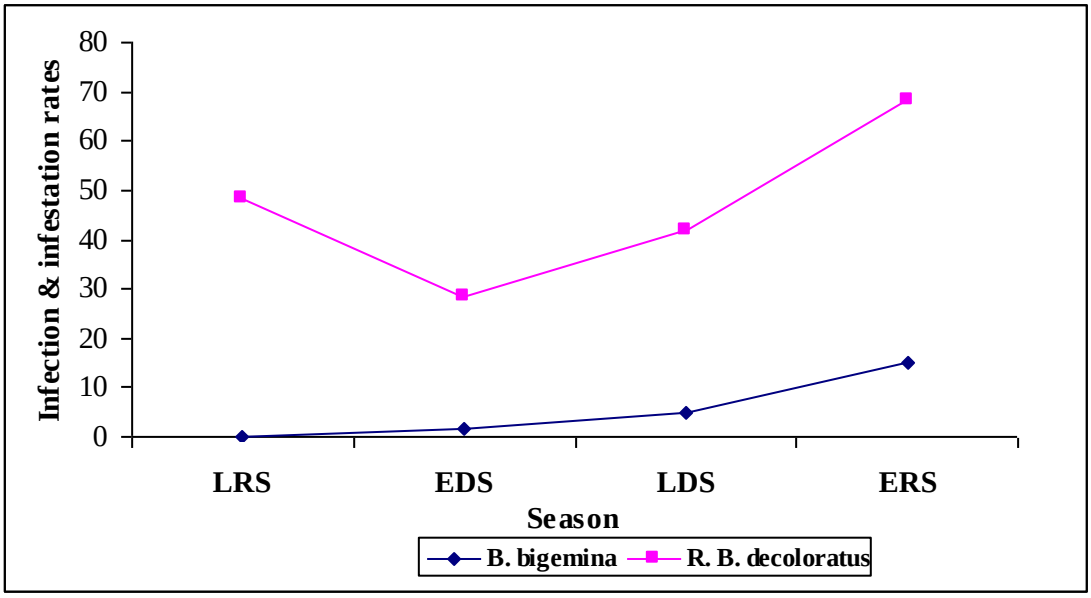

Figure 7. *B. Bigemina* infection Vs *Boophilus decoloratus* infestation rates on seasonal basis

4. 2. 2. Tick collection study findings

To assess the infestation rate and identify the respective *Boophilus* tick a total of 1,520 adult and immature ticks were collected from 60 randomly selected animals across four consecutive different seasons (Table 10).

The only *Boophilus* identified at kobo and Girana valleys was *Boophilus decoloratus* (Annex-15) and the respective prevalence was (41.3%) in the animals sub populated by age, sex, body condition, valleys and seasons. The infestation rate of *Boophilus decoloratus* on age groups (<2, 2-4, and >4) basis was (32.5%, 47.4%) and 43.9%), respectively. *Boophilus decoloratus* infestation was relatively

Table 10. Proportion of infested animals in different risk factors

variables	No of animals examined	No of infected	Infestation rate (%)	P-value
Age				
<2		26	32.5	
2-4	60	37	47.4	0.136
>4		36	43.9	
Sex				
Female		45	36.3	0.107
Male	60	54	46.6	
Body condition				
Poor		43	44.8	0.363
Good	60	56	38.9	
Valleys				
Kobo		43	35.8	0.088
Girana	60	56	46.7	
Seasons				
Late rainy season		29	48.3	
Early rainy season		32	53.3	0.014
Early dry season	60	16	26.7	OR=1.56
Late dry season		22	36.7	

high in 2-4 and >4 age groups than other age groups. This shows there was slight infestation rates variation among the age groups, but the difference was not statistically ($p > 0.05$) significant.

Sex wise study of infestation rate shows that males with (46.6%) were highly infested than females (36.3%) and animals with poor body condition (44.8%) more infested than animals having good body condition (38.9%). In both cases the difference was statistically ($p > 0.05$) not significant.

During longitudinal study *Boophilus decoloratus* infestation was assessed area wise (Kobo and Girana valleys) and the result revealed that Animals studied at Girana valley (46.7%) were found slightly more infested than animals at Kobo valley (35.8%), though the difference was not significant ($p > 0.05$).

Across the four study seasons, early rainy (53.3%), late rainy (48.3%) and late dry (36.7%) seasons were with high *Boophilus decoloratus* infestation and early dry season with (26.7%) was the lowest tick infestation season among the four seasons studied. The difference between seasons was statistically ($p < 0.05$) significant.

The multivariate analysis performed using logistic regression model adjusted for assumed risk factors (sex, age, body condition, area and season) for *Boophilus decoloratus* infestation indicate that the over all model for the assumed risk factors was statistically not significant ($p > 0.05$) for the occurrence of *Boophilus decoloratus* with the exception of season. The risk of *Boophilus decoloratus* infestation was lower in dry season as compared to rainy season (OR= 1.56, 95% Cl = 1.43 - 2.39) (Table 11) and the likelihood of contracting the disease was (OR = 3.56, 95% Cl = 1.56- 8.14) more than 3 times higher in rainy season than dry season.

Table 11. Multivariate logistic regression for the interaction of risk factors in cattle infested by *Boophilus decoloratus* in Kobo and Girana valleys.

Risk factors	Odds ratio	Std. Err	P-value	95% Cl
Sex	1.50	0.41	0.137	0.88- 2.56
Age	1.21	0.20	0.253	0.87- 1.68
Body condition	0.82	0.22	0.471	0.48- 1.40
Area	1.66	0.45	0.060	0.98- 2.81
Season	1.56	0.67	0.014	1. 43- 2.39

* Season was found to be the major risk factor in cattle infestation.

5. DISCUSSION

Tick transmitted bovine babesiosis and its vector have crucial effect and importance from economic point of view on newly emerging dairy husbandry and bull fattening in particular in the study areas and in general in the Region. The present study revealed that the vector is spreading to animals in the valleys from cattle and camels, which are not native of the study areas, but uses the valleys to passes via the study areas to the Northern part of the country. Vectors control action, as a strategy option is not widely implemented in the area. For this very reason bovine babesiosis control methods basically remained dependent on the widely use of curative and some time prophylactic drugs.

The coexistence of indigenous cattle in relative harmony with tick and tick-borne diseases for eternity may not expected in the low and mid land border areas (kobo and Girana valleys) where even enzootic stability seems exists. The reason behind for the death and apparent symptoms might be the exacerbated by other concomitant new infection caused by uncontrolled animals' movement from different corner of the country that passes mainly via fertile valleys of Kobo-Girana to the Northern part of the country. The main protocol of this study was to identify the species of Bovine babesiosis and the vector at kobo and Girana valleys in Kobo, Gubalftu and Habru districts, and to determine their respective prevalence in animals sub populated by age, sex, body condition, and area based on cross sectional and longitudinal studies. The study was aimed to provide an authentic analysis, to impart base line data for future studies and to point out the direction of crucial control option of *Boophilus decoloratus* /vector/ based on concrete circumstance.

The over all bovine babesiosis prevalence in Kobo and Girana valleys (20.3%) in cross sectional study type while in longitudinal study registered was (21.7%), the aforementioned prevalence of indicated disease in both study protocol type seems similar except very slight difference. This slight infection rates difference was may be resulted, because the samples for cross sectional study has been taken at one point in time, where as in the case of longitudinal study the samples have taken on four consecutive seasons. Time difference sampling may directly or indirectly correlate with abundance and load of tick infestation on host and at the same time *Babesia bigemina* infection difference may occur.

The prevalence of *Babesia bigemina* obtained in this study was lower than reported by (Seyoum, 2001) and higher than the, one reported by (Solomon *et al*., 1998); (Wallaga, 1997.

The prevalence by age groups revealed higher infection rates in animals with the age of 2-4 years (24.3%) followed by those between <2 years of age (20.3%), and the lowest prevalence was indicated in animals greater than 4 years of age (16.1%). In longitudinal study also animals with 2-4 age groups have got higher infection rates (30%) and followed by those animals with the age of greater than 4 years with (20%) of prevalence which were indeed (16.1%) in cross sectional study, but animals of age <2 years have shown lower prevalence (15%) than that of a cross sectional (20.3%) type of study. Though the difference on age wise in both study protocol types was not statistically ($p>0.05$) significant. The infection rate difference in the two-study types may be, because of tick parasite load on cattle and the infection rate in the ticks, but generally young calves possess a strong immunity.

Sex wise longitudinal study prevalence (24.1% and 19.1%) was higher than a cross sectional (12.7% and 7.6%) study, respectively. Sex wise difference in longitudinal study type was not ($p>0.05$) significant where as in a cross sectional study the difference was statistically ($p<0.05$) significant. Similar result that coincides with longitudinal study was reported by (Seyoum, 2001). During both study protocol type animals with poor and good body conditions were compared for bovine babesiosis infection (Tables 4 and 9) and the result was revealed that the difference was statistically ($p<0.05$) significant. Animals with poor body condition have got higher infection rate than animals with good body conditions. The reason might be the immunity of emaciated animals (poor body condition) can be exacerbated by others concomitant new infections than animals with good body conditions.

Areas basis a cross sectional study prevalence of *Babesia bigemina* finding indicate that kobo valley with (22.7%) of prevalence was higher than Girana valley (17.8%), even though, slight infection rate difference the result was not statistically ($p>0.05$) significant. Because the aforementioned valleys are found in the same agro climatic zone and more over the orresponding samples were collection at the same time and season.

The *Babesia bigemina* infection rate was compared with *Boophilus decoloratus* during the study period at Kobo and Girana valleys across different seasons, the result revealed generally both

were increased, ($p<0.05$) during early rainy season, even if, the infection and infestation rates were varies. *Babesia bigemina* infection decreased during late rainy to early dry seasons where as *Boophilus decoloratus* was declined during early to late dry seasons (figure 7).

During microscopic examination thick and thin blood films were compared on samples, which were taken from the same animals, the result revealed that thick films was found better to use in order to get positive cases than using thin blood smear. In view of the fact that thick films were more sensitive than thin blood films, but it was observed that during microscopic examination thin blood films were better in species differentiation than thick blood films.

Boophilus decoloratus prevalence study was conducted in a cross sectional and longitudinal type of study at Kobo and Girana valleys. High tick infestation was identified during a cross sectional study than in longitudinal study, almost in both valleys animals examined were found harboring at least more than three tick species. A similar finding on number of tick species harboring result has been reported in cattle of Ethiopia by (Mohamed, 1985), (Bekele, 1987), (Tedla, 1991).

During both types of study protocol four genera and fourteen tick species, which include *Amblyomma, Rhipicephalus, Hyalomma* and *Haemaphysalis* and *A. variegatum, A. cohaerence* and *Boophilus decoloratus* among others (Table 6) respectively. Similar findings was reported in the same study area by (Seyoum, 2001) and other researchers, particularly those who worked in the Southwest part of the country were reported, indicating *Boophilus decoloratus* as a common and abundant tick species of cattle; (Seleshi *et al*., 1992), (De Castro, 1994), (Kassaye, 1994), (Teshome *et al*., 1995).

In the present study, *Boophilus decoloratus* (24.4%) was found to be the third most predominant tick species next to *Amblyomma variegatum* (28.22% and *A. coharence* (24.9%) from the total count during a cross sectional and longitudinal study. The tick was distributed evenly at Kobo and Girana valleys, almost with the same pattern in different seasons studied, even though; it was mainly recorded in early and late rainy seasons. This finding was not coinciding with the result achieved and reported by (Teshome *et al*., 1995).

Boophilus decoloratus was collected from all animals examined during a cross sectional and longitudinal studies, even in dry seasons. This mode of collection throughout the study period may be in agreement with the findings of different researchers like, (Hoogstraal, 1956) in his early study has been described that the wide distribution of *Boophilus decoloratus* throughout

most of the Ethiopian faunal region currently known as Afro tropical zoogeographical region, with in its range it occurs even where except in more open dry areas and in tropical forest. (Morel, 1980) also ratified that this tick species is the Ethiopian proper and its distribution only disquiet tropical Africa. In deed, of its parasitic importance has been quoted on cattle in many instances. He indicated that it might naturally can infest wild ungulates on parts of its distribution area, mainly predominate on ruminants less commonly infest carnivorous mammals.

The immature *Boophilus decoloratus* in both protocol study types at Kobo (9.8%) and Girana (9.5%) were the most predominating immature ticks in number more than *Amblyomma* immature ticks collection. The over all prevalence was (9.6%), these species were widely and equally distributed in the study areas and with peak number during late rainy season and in early rainy season low count was observed. This finding was not coinciding with the result obtained and reported by (Yitbarek, 2004).

Evnthough, other tick species were not the objective of this study, but used only for the purpose of comparison with *Boophilus decoloratus* were also observed. Among, which *Amblyomma variegatum* (28.22%) was found to be the most predominate and common tick species in the valleys. High count in number of this ticks were observed in a cross sectional than in longitudinal study in early rainy season. This result not coincides with the result reported by (Seyoum, 2001) at Girana valley in which A. cohaerence was the most common tick species was noted during previous study. This infestation rate variation could probably was attributed to different seasonal change of microclimate especially temperature and the respective humidity.

In the present study all animals examined were found positive with *Boophilus decoloratus*, however high infestation rate was observed during early and late rainy season. This might be enlightened that humidity and temperature are crucial factors that influence the seasonal variation of ticks. According to (Solomon *et al*., 2003) that the prevalence rate and intensity of infestation were generally low during the dry season and high in rainy season. Different species could have different microclimate requirement, as a general rule tick activity adjusted so that tick reproduction will be greater during the rain season. As indicated by (Hunter, 1994) the importance of rainfall as limiting factor in tropical and subtropical climates where temperature is suitable for tick activity.

In cross sectional study older animals (> 4 age) were found to carry an average of 10.44 ticks per animal (91.9%) of *Boophilus decoloratus* followed by 2-4 age with an average of 9.5 ticks per animal (82.1%) and an average of 8.33 ticks per animal (73.2%) were collected from ≤2 age groups. And male animals (86.6%) where highly infested than females (78.3%) once. Age and sex wise prevalence were found statistically ($p<0.001$ and $p<0.05$) significant respectively. In the same way, the highest prevalence of tick infestation was observed in animals with poor body condition (83.3%) than those with good body condition (80.2%) and mean tick burden was also higher in animals with poor body condition compared to animals with good body condition. Nevertheless, the difference was ($p>0.05$) not significant. A similar result on age group basis was reported by (Seyoum, 2001), but this result achieved was not coinciding on sex wise prevalence.

Area basis of *Boophilus decoloratus* prevalence in a cross sectional study type under taken in Kobo (82.2%) and Girana (82.7%) valleys result achieved seems was similar and the difference was not ($p>0.05$) significant. The reason for similar result obtained might be that the corresponding samples were collected at the same time and season and probably favorable microclimatic conditions were equally induced in both valleys.

In longitudinal study the infestation rate was lower than that of a cross sectional study. Age wise prevalence shows that animals with 2-4 age groups were found to carry an average of 13.5 ticks per animals (47.4%), which was higher than that of a cross sectional study, but the respective prevalence was found lower. And followed by older animals greater than four years of age having an average of 20.1 ticks per animals, which was found higher, but lower in infestation (43.9%) rates than a cross sectional study. Lower infestation (32.5%) rates and with mean burden (11.3 ticks) per animals was indicated on animals with <2 of age (young animals) among the age groups studied in longitudinal type of study.

Sex wise prevalence was found also lower than that of a cross sectional study, males (46.6%) and females (36.3%), but mean tick burden per animals was found higher than that of a cross sectional study. In the same way animals with poor body condition (44.8%) have higher tick infestation than animals with good body condition (38.9%) the result seems was similar with a cross sectional study type. Age and sex wise prevalence of longitudinal study result was found statistically ($p>0.05$) not significant, this result obtained in longitudinal study was different form the result achieved in a cross sectional study type. In contrast to this finding, (Tesfanesh, 1993) stated that age and sex differences of the host did not affect or influence the burden and species

of ticks. (Omen *et al.*, 1999) confirmed that generally more ticks were observed on cow than calves, very low tick count were observed on calves age ≤6 month than other age groups. During his previous study at Girana valley, (Seyoum, 2001) found that the number of ticks attached to animals increased with their respective age and females' animals were found with more tick infestation than the males' once. In contrast in the present study, in both study protocol males' animals were found to carry more ticks than the females' animals. The difference on tick burden (infestation) in different age and sex groups might be associated to grazing practices since in almost all parts of the country calves do not go to pasture for grazing with their dams and males once often use to graze separately form females animals during different time of the day because of their hard work in the farm, as a result the infestation chance will be higher than the female animals.

Area basis of longitudinal study of *Boophilus decoloratus* prevalence of kobo (35.8%) and Girana (46.7%) valleys was lower than the result obtained in a cross sectional study, even though, the difference was not statistically ($p>0.05$) significant in both protocol study types. The difference might be related to seasonal variation that temperature, humidity and rainfall are essential for determining the multiplication of ticks on the body of the host and pasture are the most important factors which may quantifies the season.

Boophilus decoloratus host attachment (infestation) was studied on four different seasons. The seasonal basis result revealed that during early rainy season (53.3%) and late rainy season (48.3%) was higher than that of late dry (36.7%) and early dry (26.7%) seasons. The differences among seasons were statistically ($p<0.05$) significant. The difference could be explained that the cycle of season determines the alternation of appearance, reduction, and disappearance of tick, thus as a general rule tick activity is adjusted so that tick reproduction is greatest during the rain season and at the end of rainy season there is a marked decrease with a progressive fall to almost zero in dry season (Morel, 1989).

The multivariate analysis using logistic regression model fitted to the assumed risk factors for *Boophilus decoloratus* infestation in longitudinal study signified was season. The risk of *Boophilus decoloratus* infestation seems was lower in dry season as compared to the rainy season (OR = 1.56, 95% Cl = 1.43-2.39) and the likelihood of acquiring the disease was more than 3 times higher (OR = 3.56, 95%Cl =1.56-8.14) in rainy season than dry season.

The prevalence of *Babesia bigemina* infection was ($p<0.05$) positively correlated with *Boophilus decoloratus* and was found statistically significant. In both cases (parasite and vector) were increased during the rainy seasons than that of dry seasons, because temperature, humidity and number of ticks were correlated during rainy seasons when especially temperature and humidity were maximum. The result of this study finding of *Boophilus decoloratus* was not agrees with the result reported by (Seyoum, 2001). The main reason might be an introduction of foreigner ticks to the study areas with uncontrolled animals' movement form different part of the country that often uses the valleys to pass to the Northern part of the country and also may be seasonal variations.

During both study protocol high count of *Boophilus decoloratus* was collected from Back/ shoulder, dewlap, head, ear and relatively low numbers were from venter, tail and hoof. A male to females of the aforementioned ticks' ratio was (0.41:1) respectively. Females ticks were more in number than the males once. A similar result of *Boophilus decoloratus* sex ratio was reported by (Seyoum, 2001) at Girana valley.

6. CONCLUSIONS AND RECOMMENDATIONS

The current study on bovine babesiosis and its vector at Kobo and Girana valleys of North Wollo Zone in Amhara Region, gave some vital information on current situation of the disease in the area under taken. Generally high prevalence of *Babesia bigemina* and its vector were observed mainly during early rainy season and seasons were also found to be the risk factors. *Babesia bigemina* and *Boophilus decolorats* were positively correlated during rainy season.

Large count in number of *Boophilus decoloratus* and relatively high number of animals were infected with *Babesia bigemina* in both study types were detected both clinically and microscopically. It is there fore important to under stand that in a situation of Kobo and Girana valleys it has not been suggested that enzootic stability might have established, which according to different researchers is not enviable and economically not acceptable as the condition where an outbreak of bovine babesiosis is occurs.

Boophilus decoloratus and indeed other tick species are too important not only of disease transmission but also they are excellent in blood sucking and cause anemia, tick worry and skin damage. *Babesia bigemina* is also one of the most important blood parasites that were observed during the study period, causing anemia, emaciation and animals die if not treated in time. The main cause of high infestation and infection in the valleys was observed as a result of the unrestricted movement of animals from different part of the country that used to pass via the valleys to the Northern part the country, time less bounded usage of acaricide by different non-ethical individuals, inadequate dosage of acaricide and drought.

Based on the current finding and the special concern of *Boophilus decoloratus* and the respective disease (*Babesia bigemina*) and indeed, other tick species also in the study area the following recommendations are indicated:

- Applications of acaricide especially during early rainy season stir up the tick population to manageable level with out disturbing the enzootic stability.
- Creation of awareness among livestock owners to avoid the use of non- ethical and inadequate dose of acaricide treatment, which may develop acaricide-resistance.
- Sustainable *Babesia bigemina* control could be instituted in the study area by manipulating the enzootic stability and the resistance capacity of local cattle in the area.
- Restriction of animals' movement and quarantine measures from marginal or bordering and especially across the country into the study areas.

- Strong and sustainable extension works should be done to create awareness of the livestock keepers on the potential effect of tick and tick-borne diseases *Boophilus decoloratus* and *Babesia bigemina*) and on means of improving the traditional management system in general and the nutritional status of their animals in particular should be directed.
- Constant epidemiological study and surveillance of tick and tick- borne diseases and the usage of new technology (sensitive diagnosis) like serology is quit important.

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8. ANNEXES

Annex-1. Morphology, animals affected and vectors of bovine babesiosis

Organism	Animals affected	Morphology of organism	Vectors
Babesia bigemina	Cattle	4.5 by 2.5 µm (large, round and pryform, acute angle)	*Boophilus annulatus, Boophilus decoloratus* and *Boophilus microplus*
Babesia bovis	Cattle	2.4 by 1.5 µm (Small, more rounded obtuse angle)	Boophilus annulatus, Boophilus microplus
Babesia divergens	Cattle	1.5 by 0.4 µm (Small narrow and obtuse angle)	Ixodes ricinus
Babesia major	Cattle	2.6 by 1.5 µm (Large round and pyriform)	Haemaphysalis punctata
Babesia jakimovi	Cattle	Similar to B. major	Ixodes ricinus
Babesia ovata	cattle	similar to B. bigemina	Haemaphysalis longicornis

Annex- 2. How to identify genera of ticks

Group	Size	Mouth parts	Other feature	Genera
Group 1	Large	Ventral and short	Scutum absent Pulvilli absent	*Argas* *Ornithodoros* *Otobius*
Group 2	Large	Anterior and long	Pale rings on legs, eyes present and large	*Amblyomma* *Hyalomma*
Group 3	Medium	Anterior and long Eyes absent	Plain dark legs	*Ixodes*
Group 4	Medium	Anterior and short	Eyes present and large Coxae-1 with paired spurs	*Dermacenter* *Rhipicephalus*
Group 5	Small	Anterior and short	Eyes absent or small Coxae-1 with small paired spurs or single spur	*Boophilus* *Margaropus* *Haemaphysalis*

Annex-3. *Boophilus annulatus* female (Species identification)

Porose areas	Hypostomal teeth	Palp articles internal margins	Coxa-1	Coxa-2 and 3	Genital aperture
Broad oval	4+4 columns	Have no protuberance and long slightly concave	Spurs are indistinct	Spurs are absent	Have a broad U- shape

Annex-4. *Boophilus annulatus* male (Species identification)

Mouth parts	Cornua	Coax-1 spurs length	Ventral plate spurs (Accessary adnal plate)	Ventral plate spurs (adanal plate)	Caudal appendage	Ventral plate spurs
Similar to female	Distinct	Short (faintly sclerotized)	Indistinct	Indistinct	Absent from fed male	No visible dorsally

Annex-5. *Boophilus decoloratus* female (Species identification)

Porose areas	Hypostomal teeth	Palp articles internal margins	Coxa-1 spurs	Coxa-2 and 3 spurs	Genital aperture Posterior lips
Narrow oval	3+3 columns	Has a protuberance with pectinate setae	Distinct	Present	Have a narrow U- shape

Annex-6. *Boophilus decoloratus* male (Species identification)

Mouth parts	Cornua	Coax-1 spurs length	Ventral plate spurs (Accessary adnal plate)	Ventral plate spurs (adanal plate)	Caudal appendage	Ventral plate spurs
Similar to female	Distinct	Short	Distinct	Distinct	Narrow in fed male	Visible dorsally

Annex-7. *Boophilus geigyi* female (Species identification)

Porose areas	Hypostomal teeth	Palp articles internal margins	Coxa-1 spurs	Coxa-2 and 3 spurs	Genital aperture Posterior lips
Narrow oval	4+4 columns	Has a protuberance with a pectinate setae	Distinct	Present	Have a narrow V- shape

Annex-8. *Boophilus geigyi* male (Species identification)

Mouth parts	Cornua	Coax-1 spurs length	Ventral plate spurs (Accessary adnal plate)	Ventral plate spurs (adanal plate)	Caudal appendage	Ventral plate spurs
Similar to female	Indistinct	Short	Distinct	Distinct	Narrow in fed male	Visible dorsally

Annex-9. *Boophilus microplus* female (Species identification)

Porose areas	Hypostomal teeth	Palp articles internal margins	Coxa-1 spurs	Coxa-2 and 3 spurs	Genital aperture Posterior lips
Broad oval	4+4 columns	Has a protuberance and is short and distinctly concave	Distinct	Present	Have a narrow U- shape

Annex-10. *Boophilus microplus* male (Species identification)

Mouth parts	Cornua	Coax-1 spurs length	Ventral plate spurs (Accessory adnal plate)	Ventral plate spurs (adanal plate)	Caudal appendage	Ventral plate spurs
Similar to female	Indistinct	Long (anterior spurs are conspicuous Dorsally)	Indistinct	Indistinct	Narrow in fed males	Visible Not visible dorsally

Annex-11. Age determination adapted by FAO (1983) for Zebu cattle (estimation in month).

< 24	Cattle with out pair permanent incisor
27	First pair permanent incisor
36	Second pair permanent incisor
42	Third pair permanent incisor
48	Forth pair permanent incisor
> 48	Four pair permanent incisor in wear

Annex- 12. Scores for measurement of body condition adapted from Radostits and Blood, 1985.

0 No fatty tissue is felt, shape of transverse processes clearly visible, deep cavity under tail and around tail head. Animal appears emaciated.

1 Deep depression in loin ends of transverse process sharp to touch and upper surface can be felt easily, cavity present around tail head.

2 Some fatty tissue felt under the skin, pelvis felt easily, ends of transverse processes feel rounded and upper surfaces felt only with pressure, visible depression in the loin.

3 Fatty tissue easily felt over the whole area, skin appears smooth, slight depression visible in loin., end of transverse processes can be felt with pressure, but thick layers of tissue on top.

4 Transverse processes cannot be felt even with firm pressure, no depression visible in loin between back bone and hip bones, patches of fat apparent under the skin, pelvis felt only with firm pressure.

Annex-13. Blood films data collection Format

Region……..

Zone ……

Districts**.....**

Keble (PA'S)……...

Animal's owners name ……..**..**

Animal species…….

Age……...

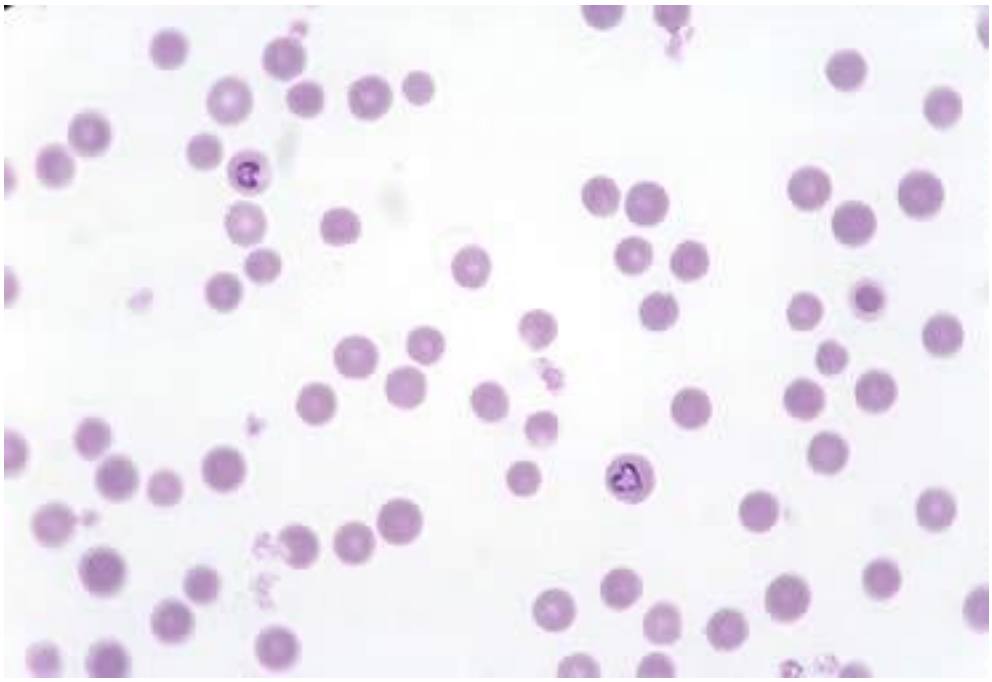
Sex……

Animals' identification…….

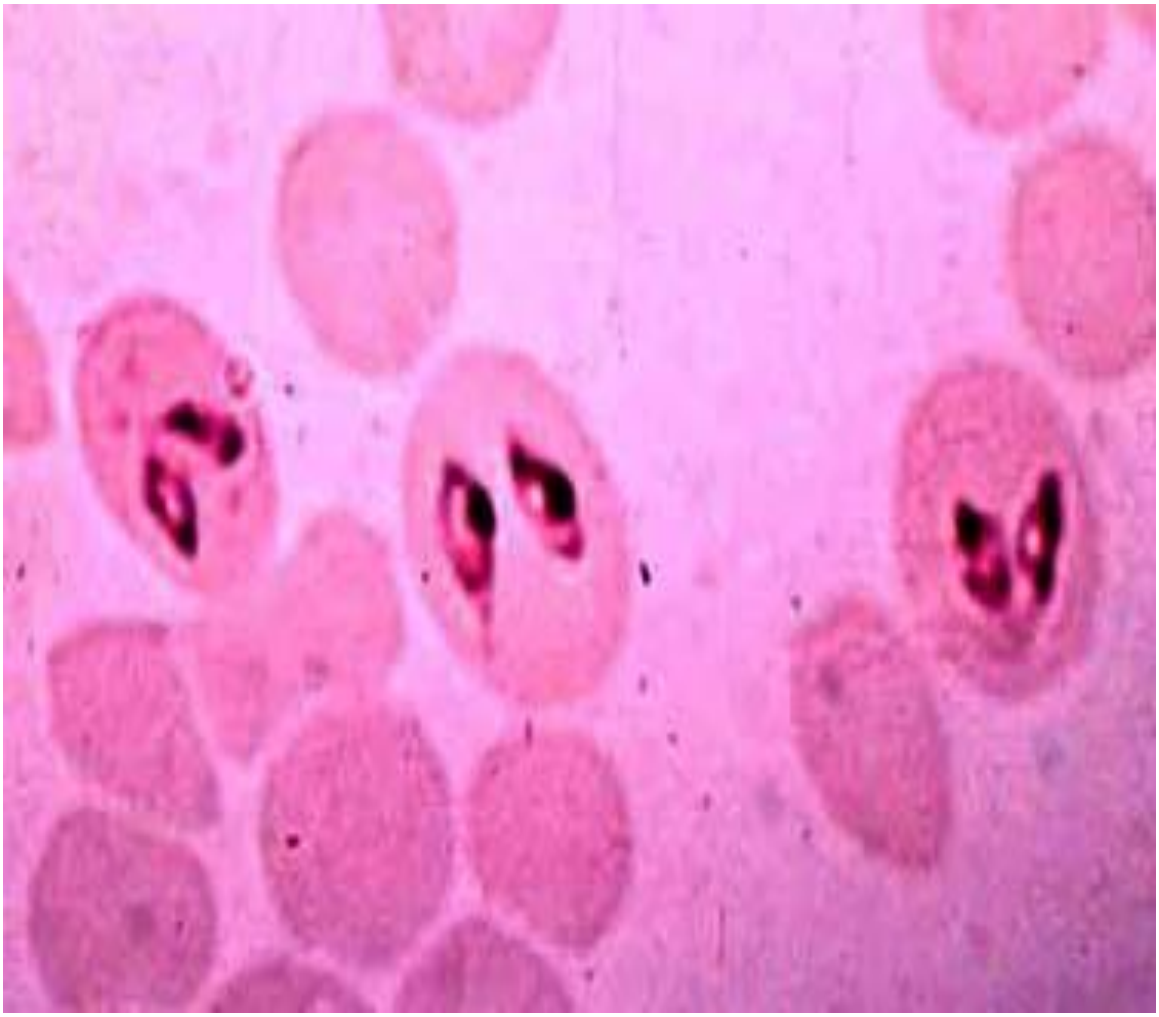
Body condition…….

Slide numbers……..

Annex-14. Pictures of the parasite (*Babesia bigemina*) detected in a cow



Babesia bigemina - blood film stain



Babesia bigemina – blood film smear

Annex-15 Tick collection predilection sites format

Region…….

Zone ……

Districts ……..

Keble (PA'S)……

Animal species…….

Age……

Sex……

Animals identification……..

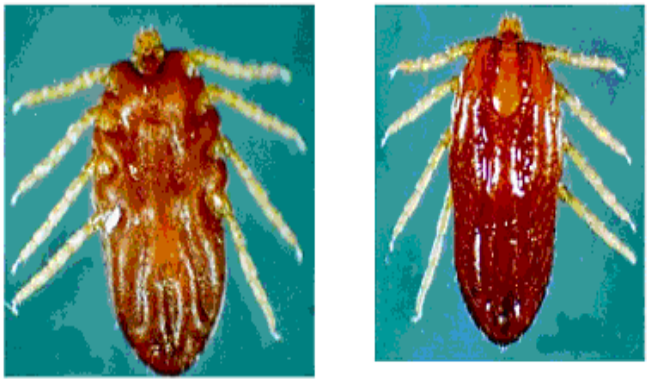
Body condition……

Tick predilection sites…….

- Ear
- Head
- Dewlap
- Back
- Abdomen
- Udder / Scrotum
- Aon-vulva
- Hoof / feet
- Universal bottle number

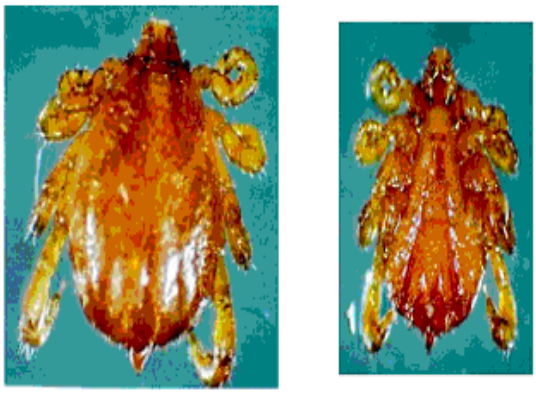
Annex- 16. Picture of the vector *Boophilus decoloratus* collected from cattle

Female *Boophilus decoloratus*



Ventral view dorsal view

Male *Boophilus decoloratus*



Dorsal view Ventral view

Boophilus decoloratus collected from cattle.

9. CURRICULUM VITAE

1. Personal data

Name:	Seyoum Zenebe
Date of Birth:	Sept. 16, 1960
Place of birth:	Golla (Oromia)
Language skill:	Amharic, Oromic, English and Spanish
Nationality:	Ethiopia
Marital status:	Single
Children:	2 aged 19 and 17 years
Religion:	Orthodox Christian
Position:	Team leader, Kombolcha regional Veterinary Laboratory, BOA
Membership:	Ethiopian Veterinary Association
Office contact:	Kombolcha Regional Veterinary Laboratory P.O. Box, 9, Kombolcha Mobil Tel. 0911758101

2. Educational Background

Sept. 1967 - July 1969	Primary school, Bale
Sept. 1970 - July 1971	junior school, Bale

Sept. 1972 - July 1973	Senior secondary school, Bale
Sept. 1974 - July 1975	Jimma T.T.I Achievement: Teacher Diploma And ESLCE
Sept.1981 - July 1986	Bayamo higher Agricultural Institute (CUBA) Achievement: Doctor of Veterinary Medicine (DVM)

3.Work Experience

July 1976 - July 1980	Teacher: In elementary, Junior and Senior high school
Oct. 1987 - Dec. 1987	Field Vet. Officer, Ministry of Agriculture
Dec. 1987 - Sept.2005	Team leader, Kombolcha Regional Veterinary Laboratory

4. Research papers

1. Study on Biometric and Economical aspect of *Fasciola hepatic*, DVM thesis (1985), BHAI, CUBA.

2. Study of ticks and tick-borne diseases on Zebu cattle at Girana valley in North Wollo Zone. Proceeding of 16th Ethiopian Veterinary Association Annual Conferaence, 2001

3. Preliminary study of Trypanosomiasis on cattle in three woredas of North Shewa Zone. Proceeding of 17th Ethiopian Veterinary Association Annual Conferaence, 2003

4. Distribution and host-parasite relationship of Ixodid ticks in eastern Amhara. Ethiopia Veterinary journal, Volume: 9, No-1, 2005.

5. Additional short trainings:

1. VET. Epidemiolgy and Economics, (1997), Germany

2. VET. Parasitology (2002), South Africa, Pretoria University

3. Computer literacy: Word processing, spread sheet and data base management (March, 2001)

10. SIGNED DECLARATION SHEET

"This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged"

Name Seyoum Zenebe

Signature _____

Date of submission _____

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

Advisors:

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