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**EPIDEMIOLOGICAL STUDY ON HEMOPROTOZOAN PARASITES OF ZEBU
CATTLE IN AMBO AND TOKE KUTAYE DISTRICTS OF WEST SHEWA ZONE,
OROMIA, ETHIOPIA**

MVSc Thesis



BY

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY**

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**A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis
Ababa University in partial fulfillment of the requirements for Degree of Masters of
Veterinary Science**

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June, 2017

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As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by **Gudina Mekonnen Ayana** titled “**Epidemiological study on Major Hemoprotozoal Parasites of Zebu Cattle in Ambo and Toke Kutaye districts of west Shewa zone, Oromia, Ethiopia**” and recommend that it will be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Parasitology.

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DECLARATION

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

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LIST OF ABBREVIATIONS

AARDB	Ambo Agricultural and Rural Development Bureau
BW	Body Weight
CA	Capillary tube agglutination
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
IFAT	Immunofluorescent antibody test
IHA	Indirect Hemagglutination Assay
ILRI	International Livestock Research Institute
kg	kilogram
m.a.s.l	meters above sea level
mg	milligram
NTTCC	National Tsetse and Trypanosome control center
OIE	Office Epizootic International
PAs	Peasant Association
PCR	Polymerase Chain Reaction
spp	species
TKADB	Toke Kutaye Agricultural Development Bureau

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ABSTRACT

A cross sectional study was conducted from October, 2016 to May, 2017 to determine the prevalence of hemoprotozoan parasites, to detect the species of hemoprotozoa parasites, to identify the vectors of hemoprotozoal parasites and risk factors associated with the occurrence of hemoprotozoal parasites in cattle in Ambo and Toke Kutaye districts of West Shewa Zone. A total of 384 blood samples were collected from randomly selected cattle to assess the presence of hemoprotozoan parasites by using buffy coat and thin smear technique and stained using Giemsa stain and identified by using oil immersion objective lens light microscope. The three hemoprotozoan parasites identified in the study area were *Babesia bigemina*, *B. bovis* and *Trypanosoma vivax*. The overall prevalence of hemoprotozoan parasite in the study area was 9.4% (7.6% *Babesia bovis*, 1% *B. bigemina*, 0.8% *T. vivax*). Statistically significant difference was observed between groups of sex, age, body score condition and tick infestation of cattle in presence of hemoprotozoal parasite. Out of the total positive cattle with hemoprotozoal parasite 72% (26/36) of them were anemic (their packed cell volume values less than 25%) and the rest 28% (7/36) of them were not anemic (their packed cell volume value greater or equal to 25%) which is statistically significant. The major tick species identified in the study area include *Rhipicephalus* spp *Amblyomma* spp *Hylomma* spp and *Rhipicephalus (Boophilus)* spp *Tabanus*, *Stomoxys* and *Chrysops* were the biting fly species identified in the study area.

Key words: *Ambo, Cattle, Hemoprotozoa, Prevalence, Toke Kutaye, Vectors*

1. INTRODUCTION

Ethiopia has an enormous and diverse livestock population that plays an important role in the economy and livelihoods of farmers and pastoralists with a total contribution of 15% of Gross Domestic Product and 33% of the agricultural output. Estimates of livestock population show that there are about 53.99 million heads of cattle, 25.5 million sheep, 24.06 million goats, 9.01 million equines and 0.92 million camels in Ethiopia (CSA, 2013). From the total cattle population, 98.95% are local breeds while the remaining are hybrid and exotic breeds (Leta and Mesele, 2014). Despite the large animal population, their productivity is low due to poor nutrition, reproduction insufficiency, management constraints and prevailing livestock diseases (Bekele *et al.*, 2010). Livestock diseases are the major causes of economic losses to the peasant farmers and pastoralists in Ethiopia amounting to hundreds of millions of birr annually (UNDP, 1994).

Arthropod transmitted hemoparasitic diseases are economically important vector-borne diseases of tropical and subtropical parts of the world including Ethiopia (Sitotaw *et al.*, 2015). The infection is mainly transmitted by arthropod vectors, or through blood transfusion (Salih *et al.*, 2015). They are of great economic impact on livestock affecting 80% of the world cattle population and causes economic loss (Hamsho *et al.*, 2015). Hemoprotozoan diseases especially babesiosis, theileriosis and trypanosomosis are considered as major impediments in the health and productive performance of cattle (Rajput *et al.*, 2005). These disease have got a serious economic impact due to obvious reason of death, decreased productivity, lowered working efficiency (Uilenberg, 1995), increased cost for control measures (Makala *et al.*, 2003) and limiting introduction of genetically improved cattle in an area (Radostits *et al.*, 2000).

The occurrence and importance of hemoprotozoan disease is a reflection of complex interaction involving the causative organisms, vector, vertebrate hosts and environment (Akande *et al.*, 2010). The presence of hemoprotozoan disease of cattle broadly related to the presence and distribution of their arthropod vectors (ticks and flies) (Hamsho *et al.*, 2015). According to Walker *et al.*, (2003) ticks which are considered to be most important to

health of domestic animal in Africa comprise about seven genera. Among these genera, the main tick genera found in Ethiopia include *Amblyomma*, *Haemaphysalis*, *Hylomma*, *Rhipicephalus* and sub genus *Rhipicephalus* (*Boophilus*). The genus *Amblyomma* and *Rhipicephalus* are predominating in many parts of the country. In Ethiopia, there are about 47 species of ticks found on livestock and most of them have importance as vector and disease causing agent and also have damaging effect on skin and hide production (Tadesse and Sultan, 2014). With exception of *T. equiperdium*, which is a venereally transmitting disease, all *Trypanosoma* spp have arthropod vectors in which transmission is either cyclical or noncyclical (Urquart *et al.*, 1996). Tsetse-borne trypanosomiasis occurs only in Africa, south of the Sahara where there are tsetse flies (Abebe, 2005).

In Ethiopia, various surveys have been carried out on distribution, abundance and prevalence of hemoprotozoa species on livestock (trypanosomosis of ruminants, babesiosis in donkeys, and babesiosis in canine) in different regions of the country by various investigators (Tolossa, 2010). Bovine tropical Theileriosis is reported in Ethiopia (Humera area) for the first time in recent study by Gebrekidan *et al.* (2014) in which four species of *Theileria* were detected in cattle: *Theileria velifera* (*T. velifera*), *T. mutans*, and *T. orientalis complex* and *T. annulata*. The most prevalent trypanosome species in Ethiopia are *T. congolense* and *T. vivax* (Rowland *et al.*, 1993) reported that the prevalence rate of 37% for *T. congolense* in South west Ethiopia. However, the detailed status of the hemoprotozoan parasites in cattle is not thoroughly studied in Ambo and Toke Kutaye districts of West Shewa zone and information is so far scanty. Based on this background the research was initiated with the following objectives.

- To assess the prevalence of hemoprotozoa of cattle in the study area
- To identify the species/genera of hemoprotozoa of cattle in the study area
- To identify the risk factors associated with the occurrence of hemoprotozoal parasites

2. LITERATURE REVIEW

2.1 Major Hemoprotozoan Diseases of Cattle

2.1.1 Causative agents and their taxonomic classification

The most important hemoprotozoan diseases of cattle are caused by several species of *Babesia*, *Theileria* and *Trypanosoma* (Schnittger *et al.*, 2012). Babesiosis is a hemolytic disease caused by intraerythrocytic protozoa of the phylum *Apicomplexa*, order *Piroplasmida*, family *Babesiidae*, within the genus *Babesia* (EI-Ashker, 2015).

Theileriosis on the other hand is also an important diseases affecting cattle population worldwide. They are phylogenetically most closely related to members of the *Babesia* genus and fall in the order *Piroplasmida* under the phylum *Apicomplexa* (Lack *et al.*, 2012). According to Tarimo (2013) taxonomy of genus *Theileria* can be summarized as follows; *Theileria* belong to Kingdom: Protista, Subkingdom: Protozoa, Phylum: *Apicomplexa*, Class: *Sporozoa*, Subclass: *Piroplasmia* (piroform, round, rod-shaped parasites), Order: *Piroplasmida*, and Family: *Theileriidae*, Genus: *Theileria*. *Theileria parva*, *T. annulata* and *T. lestoquardi* feature prominently among pathogenic species of *Theileria* that affect domesticated ruminants. They share a unique biology of being able to transform a subset of infected mononuclear host cells, which can be cultured in vitro as persistently infected cell lines. Not all species of *Theileria* can transform mononuclear host cells, e.g., *T. orientalis*, *T. mutans* and *T. velifera*. If such species cause disease it is mainly due to multiplication of the parasite life-cycle stage within red blood cells (Norval *et al.*, 1992).

Trypanosomosis is most important protozoa disease of cattle that is caused by trypanosome species, a unicellular protozoan parasite of the phylum *Sarcomastigophora*, order *Kinetoplastida*, family *Trypanosomatidae* and genus *Trypanosome* (Magona *et al.*, 2003)

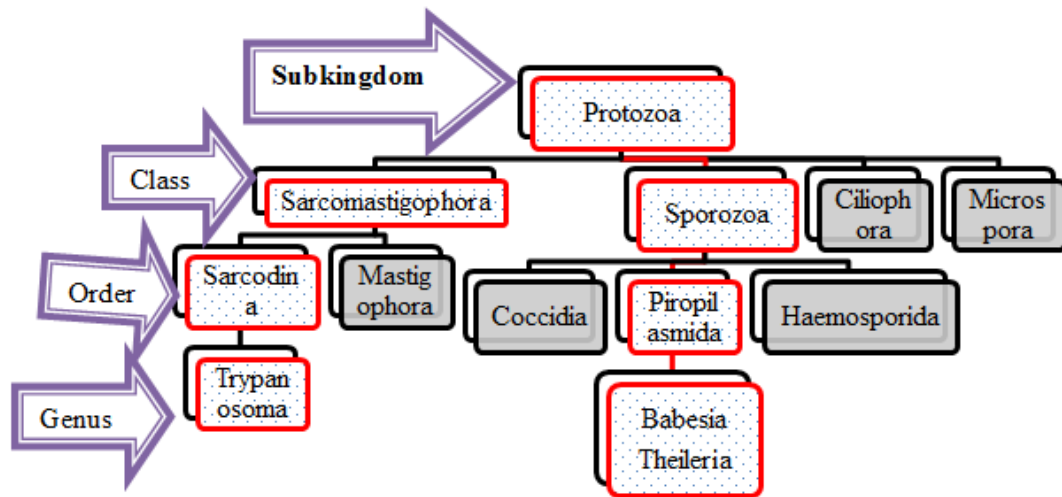


Figure 1. Schematic presentation of taxonomic classification of hemoprotozoa spp (Urquart, 1996)

2.1.2 Epidemiology

Hemoprotozoan diseases of cattle have a global distribution, stretching from the polar circle to the equator. This is due to the fact that their vectors, ticks and blood sucking flies have a global distribution (Uilenberg, 1995). Their prevalence depends upon geographical region and several other factors like tick and blood sucking flies density, climatic conditions, age, gender, management practices and immunity, either passive or active. The incidence rate is high during rainy season due to the warmth and humidity which favors ticks and blood sucking flies and subsequently parasite transmission (Vahora *et al.*, 2012). *Babesia* is the second most common parasite found in the blood of mammals after trypanosomes (Yabsley and Shock, 2013). *B. bovis* and *B. bigemina* have the same distribution, but in Africa *B. bigemina* is more widespread than *B.bovis* because of the ability of *Rhipicephalus (Boophilus) decoloratus* and *Rhipicephalus evertsi* act as vectors for this parasite. Both species are transmitted transovarially by *Rhipicephalus (boophilus)* ticks, but only tick larvae transmit *B. bovis*, whereas nymphs and adults transmit *B. bigemina* (Esmaeilnejad *et al.*, 2015). Economically important *Theileria* species that infect cattle and small ruminants are transmitted by iodide ticks of the genera *Rhipicephalus*, *Amblyomma*, *Hylomma* and

Haemaphysalis. Theileria sporozoites are transmitted to cattle in the saliva of the feeding tick (Mandal, 2012). *T. parva* occurs in 14 countries (Tarimo, 2013) in sub-Saharan Africa causing East Coast fever (ECF) and still ranks first among the tick-borne diseases of cattle in sub-Saharan Africa (Nene *et al.*, 2016), whilst *T. annulata* occurs in southern Europe as well as North Africa and Asia (OIE, 2014).

When we see the epidemiology of trypanosomosis it is highly dependent on the parasite, vector and host factors. The disease is distributed over approximately 10 million km² of Sub Saharan Africa between latitudes 14°N and 29°S which directly coincide with distributions of tsetse flies (Radostits *et al.*, 2007). Trypanosome species occur in a variety of genotypes with different strains, virulence, immunogenicity and response to chemotherapeutic agent. Since the parasite infects a wide range of animals including wild animals which constitute the reservoirs of the disease, the epidemiology of trypanosomosis is extremely complex. The degree of risk to which domestic animals are exposed to the trypanosomosis depends on the species and density of tsetse present, infection rate in tsetse, species and strain of trypanosomes, source of infection (wild or domestic animals) and feeding preference of the flies (MacLennan, 1980). Six species of *Glossina* (*Glossina morsitans submorsitans*, *G. pallidipes*, *G. tachinoides*, *G. f. fuscipes* and *G. longipennis*, *G. bravis*) have been reported in Ethiopia (Getachew, 2005). According to National tsetse and Trypanosomosis Investigation and Control Center (2002), tsetse transmitted animal trypanosomosis still remain as one of the largest causes of livestock production losses in Ethiopia.

2.1.3 Life cycle and transmission

Babesiosis: Ticks become infected by the ingestion of intraerythrocytic parasites. In the gut lumen of the tick, 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 hemolymph, which is the tick's 'blood'. Further development outside the intestine occurs in a variety of tissues, the salivary glands and ovaries being especially important for transmission. Once the adult female tick is infected it can transmit the infection for 32 generations (Taylor *et al.*, 2007).

The merozoites are motile and are able to swim. Some enter the oviduct and invade the developing eggs in the female tick. 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, sporozoites in tick salivary glands are injected into the mammalian host at the next blood meal and the *Babesia* is activated and development recommences. The infective forms of *B. bovis* are injected into cattle by larval ticks; those of *B. bigemina* are injected into cattle by nymphal and adult ticks. Sexual development occurs in the tick. *B. bovis* is transmitted transovarially but not transtadially (one tick stage to another stage) (Freeman *et al.*, 2010).

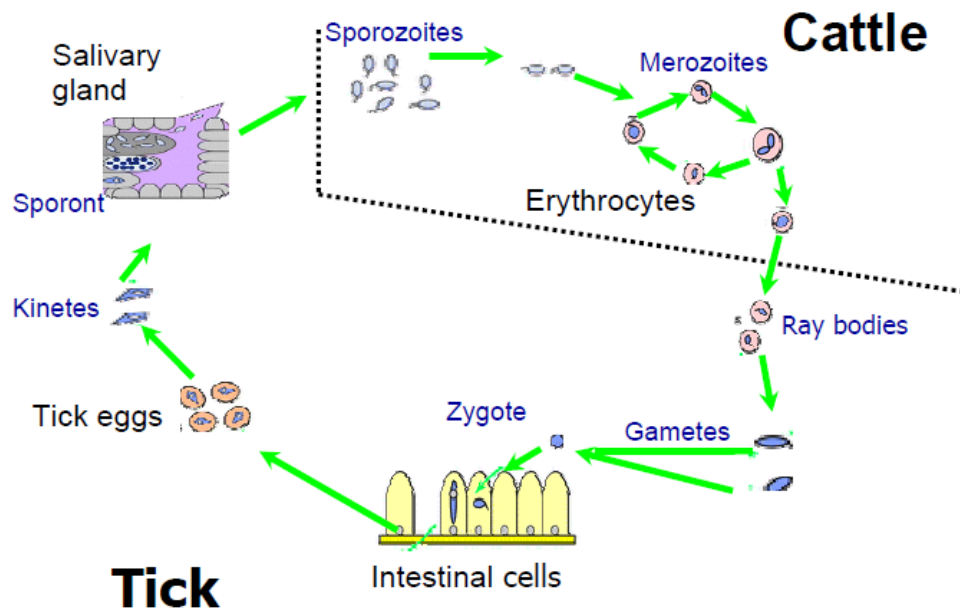


Figure 2. Life cycle of *Babesia* spp (Simuunza, 2009)

Theileriosis: The life cycle of *Theileria* spp is complex, involving morphologically distinct phases in two hosts. Sporogony and merogony take place in the cattle host while zygote and kinete are formed in ticks (Gul *et al.*, 2015). *Theileria* sporozoites enter their cattle host during tick feeding and they rapidly invade mononuclear leukocytes (lymphocytes and monocytes), where they mature into macroschizonts and induce proliferation in host cells (Shahnawaz *et al.*, 2011). Microschizonts gradually develop into macroschizonts and

ultimately into merozoites, which are released from leukocytes. These merozoites invade erythrocytes and develop into piroplasms (Khattak *et al.*, 2012). Theileriosis have a variety of tick vectors which cause infections ranged from clinically inapparent to rapidly fatal (Taylor *et al.*, 2007).

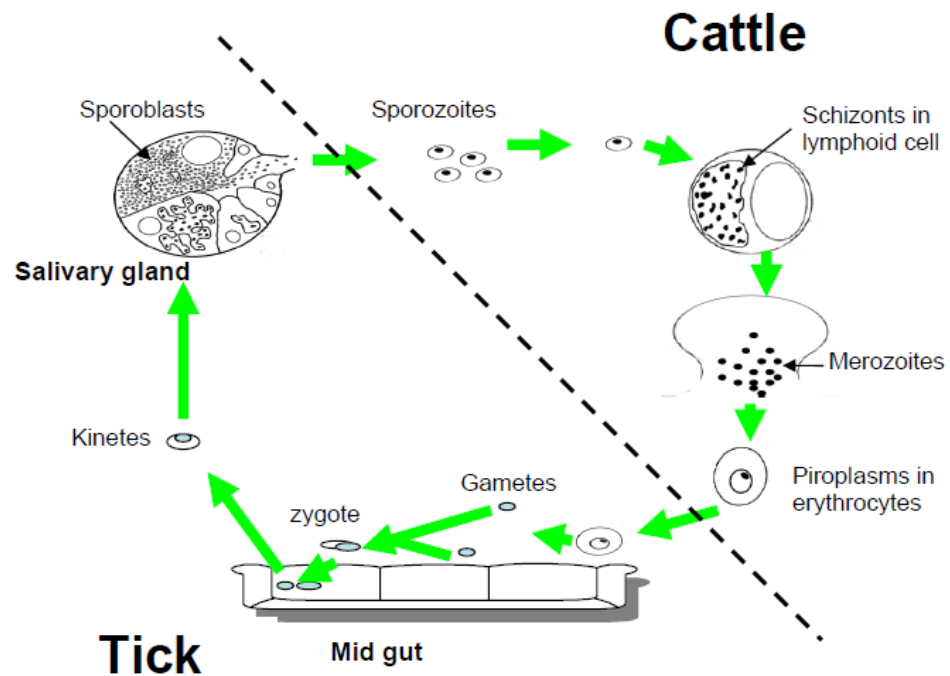


Figure 3.Life cycle of *Theileria* using *T. parva* as an example (Simuunza, 2009).

Trypanosomosis: transmitted through bites by different species of *Glossina* (Tsetse fly) and mechanically by a number of biting flies such as *Tabanus* and *Stomoxys* spp (Mulligan, 2006). Most tsetse-transmission is cyclical and begins when blood from a trypanosome infected animal is ingested by the tsetse fly. The trypanosome loses its surface coat, multiplies in the fly, then reacquires a surface coat and becomes infective. *Trypanosoma brucei* species migrate from the gut to the proventriculus to the pharynx and eventually to the salivary glands; the cycle for *T. congolense* stops at the hypopharynx and the salivary glands are not invaded; the entire cycle for *T.vivax* occurs in the proboscis. The animal-infective form in the tsetse salivary gland is referred to as the metacyclic form. The life

cycle in the tsetse may be as short as 1 week with *T. vivax* or extend to a few weeks for *T. brucei* species (OIE, 1982).

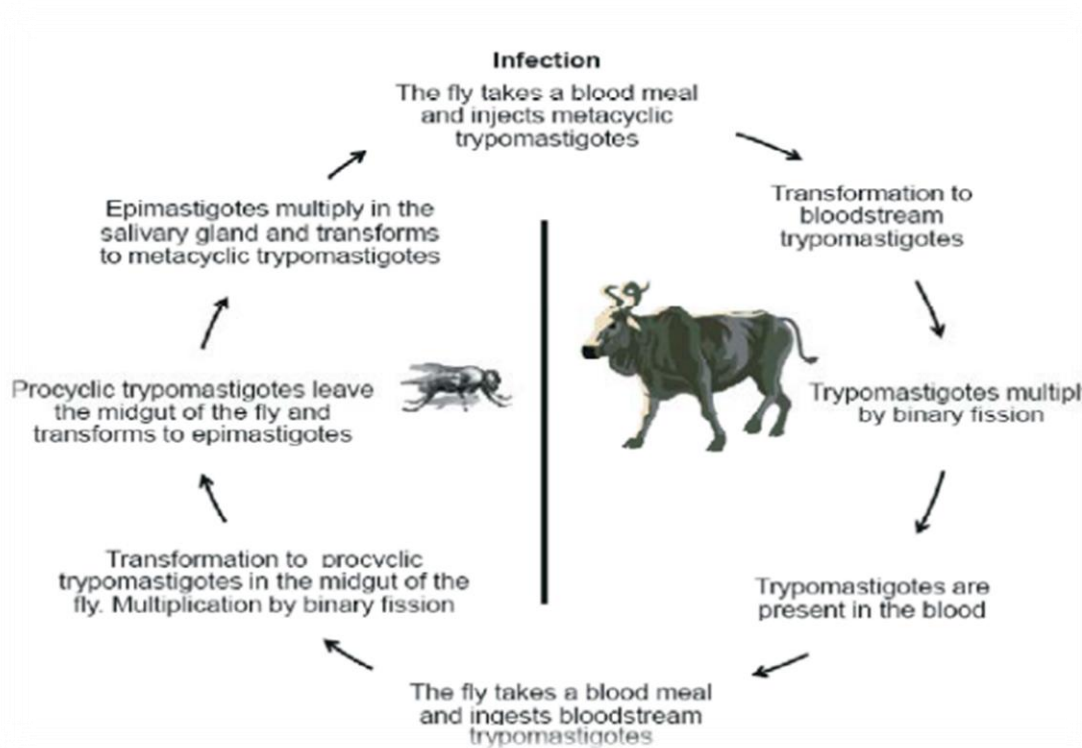


Figure 4. Life cycle of the trypanosome (Stein, 2011)

2.1.4 Pathogenesis and clinical signs

Babesiosis: *Babesia* produces acute disease by two mechanisms; hemolysis and circulatory disturbance. The rapidly dividing parasites in the red blood cells produce rapid destruction of the erythrocytes with accompanying haemoglobinaemia, haemoglobinuria and fever. This may be so acute as to cause death within a few days, during which the packed cell volume falls below 20% which will lead to anemia (Demissie and Derso, 2015). Despite, being closely related and transmitted by the same *Boophilus* ticks, *B. bovis* and *B. bigemina* cause remarkably different diseases in cattle. In *B. bovis* infections, the disease pathology can be both due to over-production of pro-inflammatory cytokines and the direct effect of red blood cell destruction by the parasite. During an acute infection, macrophages activated by the

parasite produce pro-inflammatory cytokines and parasitocidal molecule (Simuunza, 2009). The outcome of infection is related to the timing and quantity of production of these substances. Over-production of inflammatory cytokines results in severe pathology leading to vasodilatation, hypotension, increased capillary permeability, edema, vascular collapse, coagulation disorders, endothelial damage and circulatory stasis (Ahmed, 2002). Although stasis is induced in the microcirculation by aggregation of infected erythrocytes in capillary beds, probably, the most deleterious pathophysiological lesions occur from the sequestration of parasitized erythrocytes in microcapillaries of the lungs and brain. These results in cerebral babesiosis and a respiratory distress syndrome associated with infiltration of neutrophils, vascular permeability and edema. Coagulation disorders, cyto-adherence and the hypotensive state seen in *B. bovis* are not features of *B. bigemina* infections (Bock *et al.* 2004).

Theileriosis: The pathological damage is induced in cattle by schizont stage of *T. annulata* and *T. parva* (Bishop *et al.*, 2004). *Theileria* spp is classified into 2 groups. In first group (*T. parva* and *T. annulata*), proliferate is seen in lymphocytes but in the second group (*T. orientalis*) it is seen in erythrocytes that causes hemolytic anemia (Magona *et al.*, 2010). The cells infected by schizonts induce massive and uncontrolled proliferation of both specific and nonspecific T-lymphocyte resulting in enlarged lymph nodes (Schneider *et al.*, 2007). Affected lymph nodes show reactive follicular hyperplasia, reticulo-endothelial hyperplasia, enlarged germinal centers and slight increase of inter-follicular lymphoid tissue within the paracortical and cortical regions (Hassan *et al.*, 2012).

Due to schizogony in the lymphoid tissue hyperplasia occurs causing swelling of lymph nodes. Due to sudden release of toxins of macroschizonts high rise in body temperature and formation of the ulcers on the mucosal layers of abomasum. In acute cases there is dispoena and death due to anemia. Pulmonary congestion, edema, hemorrhage and emphysema of variable extents are also observed in clinically infected cattle. These lesions are characterized by the occurrence of proteinacious fluid in alveolar spaces, enlargement of pulmonary blood vessels with erythrocytes, presence of emphysematous areas (interstitial and alveolar emphysema) and infiltration of inflammatory cells within the lung's interstitial tissue (Hassan *et al.*, 2012). Tropical theileriosis is characterized by hemolytic anemia.

Hemolytic anemia is caused by immune mediated hemolysis (Omer *et al.*, 2002). The infected erythrocytes show morphological disorders which may be attributed to the presence of *Theileria* schizonts, immune-mediated processes and intravascular thrombi (Singh *et al.*, 2001).

Trypanosomosis: The pathogenesis of trypanosomosis depends on the pathogenicity of the strain, the hosts breed, genotype, age, sex, skin type and method by which the infection is induced i.e. natural or artificial (Leak *et al.*, 1987). The trypanosomes affect firstly the bite site or in other words the inoculation site in the animal skin causing a swelling and a chancre. The fly deposits during the blood sucking process the metacyclic proliferating trypanosomes in a limited number of metacyclic variant antigenic types. This stimulates the immune response causing the chancre. The chancre not only forms a site for the establishment of the infection but also it is a focus for multiplication and persistence of trypanosomes before their dissemination into blood stream (Elnasri, 2005).

The primary clinical signs are intermittent fever, anemia and weight loss. Cattle usually have a chronic course with high mortality, especially if there is poor nutrition or other stress factors (Riviere and Popich, 2009).

2.1.5 Diagnosis

Babesiosis: Traditionally, the microscopic detection of *Babesia* parasites has always been considered as the gold standard for the diagnosis of acute babesiosis (OIE, 2010). However, the low sensitivity of the technique is the major drawback which makes it difficult to detect low parasitaemia in the chronic stage of infection as well as in the carrier animals (Almeria *et al.*, 2001). These parasites are found within RBCs and all divisional stages ring (annular) stages, pear shaped (pyriform) trophozoites either singly or in pairs and filamentous or amorphous shapes can be found simultaneously. Filamentous or amorphous forms are usually seen in animals with very high levels of parasitaemia. *B.bovis* trophozoites are small (usually 1–1.5 μm x 0.5–1.0 μm), often paired and usually centrally located in RBCs. *B. divergens* resembles *B. bovis*, but the pairs are often found at the edge of the RBC. *B. bigemina* is large and can fill the RBC. Babesiosis should be suspected in cattle with fever, anemia, jaundice and hemoglobinuria (Spickler *et al.*, 2010).

Serological test-like IFAT, due to their better sensitivity, are considered as a suitable protocol for diagnosis of infection (Mtshali and Mtshali, 2013). But cross reactivity among species and also in genus level is really a major drawback for species-specific diagnosis. Nucleic acids-based detection methods developed in recent past with increased specificity and sensitivity. PCR-based assays have been widely used for the detection of *Babesia* parasites owing to their high specificity and sensitivity (AbouLaila *et al.*, 2010).

Theileriosis: Giemsa staining technique is the traditional method that involves microscopic examination of piroplasms in blood smear as well as in lymph node smears and is differentiated from other parasites by morphological properties (Aktas *et al.*, 2006). This method is frequently used for detection as it is comparatively inexpensive. However, this method is insensitive and not suitable for carrier animals because the pathogen level is usually low in the blood stream making it an unreliable technique for accurate results. Morphological differentiation of *T. annulata* and *T. parva* is also difficult, but both species are geographically separated (Hoghooghi-Rad *et al.*, 2011).

Sub-clinical infections can be diagnosed using serological tests such as coagulation, IFAT (immunofluorescent antibody test), CA (capillary tube agglutination), IHA (indirect hemagglutination assay) and ELISA (enzyme-linked immunosorbent assay) in epidemiological studies (Molad *et al.*, 2006). Molecular diagnosis (PCR) is highly sensitive tools employed for diagnosis of pathogens in carrier animals as compared to conventional techniques. However, contamination can lead to false positive results. Mixed infections are also not always detected by PCR (Yusufmia *et al.*, 2010).

Trypanosomosis: The standard laboratory method for diagnosis of trypanosomiasis is to demonstrate and identify trypanosomes in the blood of the infected animal.

Blood Smear Technique: There are several techniques for parasite detection, which include direct microscopy by wet, thick, and thin smears (Woo, 2000). Final confirmation of the species is made by the examination of the stained preparation. Usually, both a thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity, while thin smears allow trypanosome species identification. Thick blood smear method is simple and relatively inexpensive, but

results are delayed because of the staining process. Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species (Taylor, 1998).

Trypanosoma congolense, *T. vivax* and *T. b. brucei* are the predominant trypanosome species in Ethiopia. *T. congolense* resides in the subgenus Nannomonas, a group of small trypanosomes with medium-sized marginal kinetoplast, and no free flagella and poorly developed undulating membranes. *T. vivax* is a member of the subgenus Duttonella, a group of trypanosomes with large terminal kinetoplast, distinct free flagella and inconspicuous undulating membrane, although this organism is considered to be less pathogenic for cattle than *T. congolense*. This trypanosome readily persists in areas free of tsetse, where it is transmitted mechanically by biting flies or contaminated needles, syringes and surgical instruments. *T. b. brucei* resides in the subgenus Trypanozoon. *T. b. brucei* is an extremely polymorphic trypanosome occurring as short, stumpy organisms without free flagella, long slender organisms with distinct flagella and intermediate forms that are usually flagellated (Getechew, 2005).

Indirect diagnosis: Blood smear techniques are not sufficiently sensitive. Although significant improvements have been made in diagnosis, a high proportion of infections still remain undetected as the chronic, more common form of the disease is often aparasitaemic. In the face of these constraints, alternative methods of diagnosis have been developed, most of which are for the detection of antibody responses to the antigens of the infecting trypanosomes. The most useful of these tests in view of their sensitivity and specificity are the indirect immunofluorescent antibody test, enzyme Linked immune-sorbent assay (ELISA) and Polymerase Chain reaction (PCR) (Kukla *et al.*, 1999).

2.1.6 Treatment and control

Babesiosis: Imidocarbs are the drug of choice for cattle babesiosis, which can prevent clinical infection up to 2 months (Saad, *et al.*, 2015). Sick animals should be treated as soon as possible with an antiparasitic drug. Midocarb (Imizol) and the allied drug amicarbalide are effective babesiocides for cattle at the dose rate of 1-3 mg/kg and 5-10 mg/kg body weight respectively (Beckley, 2013). Vitamin E also acts as supportive therapy as vitamin E

ameliorates the oxidative effect of *Babesia* by increase antioxidant effect (Abdel Hamid, 2014).

Theileriosis: Buparvaquone is the most effective drug and the recommended dose in cattle is 2.5mg/kg BW (Taylor *et al.*, 2007). In control of the disease use of genetically resistant breed, a judicious and selective application of acaricides at 3- week intervals and the use of vaccines are recommended (Radostits *et al.*, 2008).

The control of the piroplasmosis depends on effective quarantine to prevent the introduction of the vector tick by dipping or spraying animals at risk with recommended acaricide. In routine surgery, care should be taken to prevent accidental transfer of blood from one animal to another (e.g. castration, dehorning). Widespread use of tick vaccines may also have a significant influence on the incidence of infection in cattle (Taylor *et al.*, 2007).

Trypanosomosis: If detected early Trypanosomosis can be treated with trypanocidal drug for therapeutic and prophylactic purpose. Therapeutic drugs include diminazene aceturate (3.5-7mg/kg), homidium bromide and chloride (1mg/kg). Prophylactic drugs for cattle include homidium bromide, homidium chloride and isometamidium(0.25-1mg/kg) (Achenef and Bekle, 2013).

The control of trypanosomosis in enzootic countries involves control of tsetse fly population, prophylactic treatment and good husbandry of animals at risk and use of trypano-tolerant animals. Control of tsetse has been successfully attempted, but reinvasion is frequent if the land is not properly utilized. The earliest methods involved bush clearing and elimination of game animals on which tsetse feed. More recent methods involved the use of insecticides applied strategically in the form of ground and aerial spraying over large expanses of land (Getachew and Eley, 1993).

2.2 Distribution of major hemoprotozoan parasites of cattle in Ethiopia

In Ethiopia, quite a number of epidemiological studies have been conducted including babesiosis, trypanosomosis and theileriosis. These epidemiological studies were carried out

using conventional parasitological techniques such as dark phase buffy coat, thin and thick smear and different serological and molecular techniques.

The prevalence of hemoprotozoan parasites of cattle varies from place to place. In 2015 62.2% of *B. bigemina* prevalence was reported in Jimma, western Ethiopia by Lemma *et al* (2015). In the same year Hamsho *et al.*, (2015) revealed that the prevalence of *B. bigemina* and *B.bovis* was 9.9% and 7% respectively in Borana, Southern Ethiopia. In the year of 2015 the prevalence of *T. congolence* 2.6%, 7.04% and 13.35% as reported by Dawit *et al.*, (2015), Keffale *et al.*, (2015) and Kassaye *et al.*, (2015) in Abaya(southern), Dara (Southern) and Soyonole (western) respectively. When we see the distribution of *Theileria* spp in the recent study by Gebrekidan *et al.* (2014) four species of *Theileria* in cattle: *T. velifera*, *T. mutans*, *T. orientalis* complex and *T. annulata* were reported from northern Ethiopia (Addis Zemen, Humera and Sheraro) with infection rates of 66, 8, 4, and 2%, respectively.

3. MATERIALS AND METHOD

3.1 Description of the Study Area

The study was conducted in Ambo and Toke Kutaye districts, West Shewa zone of Oromia Regional State from October, 2016 to May, 2017 on the epidemiology of hemoprotozoal parasites of zebu cattle. Ambo is the administrative center of the zone and Ambo district located at a latitude and longitude of 8°59'N, 37° 51'E, respectively and an elevation of 2101 m and 114 km West of Addis Ababa. The livestock population of the Ambo district includes 14,5371 cattle, 50,152 sheep, 27,026 goats, 9,088 horses, 2,914 donkeys and 256 mules (AARDB, 2016). Guder is the administrative town of Toke Kutaye district and geographically located at 8°58'N, 37°46'E and altitude of 1946 m above sea level. Toke Kutaye has a latitude and longitude of 9°00'N-9°27'N and 37°51'E-38°20'E, respectively. It is located at 137 km west of Addis Ababa (Fig. 5).

The rainfall is bi-modal for both districts with the short rainy season from February to May and long rainy season from June to September (AARDB, 2016). The area receives a mean annual rainfall of 900 mm (800 to 1000 mm) and annual temperature ranging from 15 to 29°C with average temperature of 22°C. West Shewa zone has generally a highland topography, which gave the area a characteristic climate that is conducive for the cattle husbandry. The livestock market of Guder is one of the largest livestock market in Oromia regional state and the animals were transported from 14 different places in Ethiopia to Guder livestock market (Jerlstrom, 2013).

Mixed agricultural practices (crop production and livestock rearing) are the major means of livelihood of the community of the study areas. The livestock sub-sector plays an important role in the livelihood of the rural people in terms of providing alternative income sources, as a strategy in building emergence to stress and also in contributing to their food security. Both local and cross breeds cattle are raised in the areas. While all local breeds and few cross breed of dairy cows are mainly managed under semi-intensive farming system (TKADB, 2016).

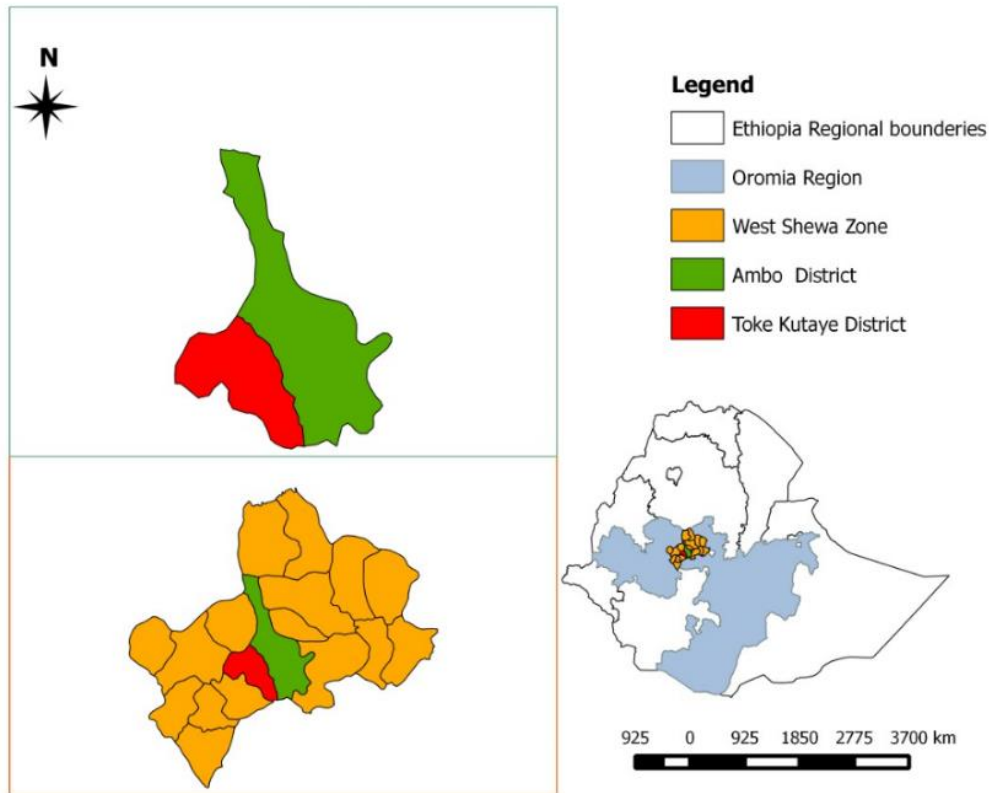


Figure 5. A map showing study area

3.2 Study Design and Sample Size Determination

A cross sectional study was conducted to know the prevalence of hemoprotozoal parasites of cattle in the study area. Systematic random sampling technique was used to recruit cattle for the study. The sample size was calculated according to Thrusfield (2005) by considering 50% expected prevalence with 95% confidence interval and at 5% desired absolute precision ($d=0.05$) as follow:

$$n = \frac{[1.96P_{exp}(1 - P_{exp})]}{d^2}$$

$$n = \frac{[1.96 * 0.5(1 - 0.5)]}{0.05 * 0.05} = 384$$

Where: n = sample size

Pexp = expected prevalence = 50%

1.96 = the value of Z at 95% confidence interval

d = Desired accuracy level at 95% confidence interval

3.3 Study Animal

The study was conducted on local breed (zebu) cattle of different age, sex and body score condition (BSC) reared under extensive farming system. Body condition scores of each cattle was evaluated during sample collection and the cattle were classified as emaciated (poor), moderate (medium) and good based on anatomical parts and the flesh and fat cover at different body parts (Nicholson and Butterworth, 1986) (Annex 6). Animals were conveniently classified as young (<3 years), adult (4-6 years) and old (>6 years) age categories as described by Delahunta and Habel (1986).

3.4 Sample Collection and Examination

3.4.1 Collection and transportation of blood samples

A total of 384 blood samples were collected from ear vein of randomly selected cattle from Ambo and Toke Kutaye districts following the standard protocol described by Urquhart *et al.* (1996). Briefly, after proper restraining of the animal, marginal ear vein was disinfected with alcohol (5%) and the hair around the intended area was shaved with scalpel blade followed by a slight tearing of the vein with lancet. Then the blood was collected with heparinized capillary (microhaematocrit) tube and sealed. After labeling, it was kept in cold chain and transported to Ambo University, College of Agriculture and Veterinary Science, Department of Veterinary Laboratory Technology Laboratory for laboratory examination.

3.4.2 Blood sample examination

Packed cell volume and buffy coat study: Capillary tube containing blood was centrifuged for 5 minutes at 12,000 revolutions per minute. The result was recorded for each sample. The readings were expressed as a percentage of packed red blood cells to the total volume of whole blood. Animals with packed cell volume < 25% were considered to be anemic (Murray *et al.*, 1977). To detect the presence of trypanosomes the centrifuged capillary tubes were cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most

layers of the red blood cells and 3 mm above to include the plasma. The content of the buffy coat was placed on glass slide, homogenized, covered with cover slip and examined under microscope for the movement of the parasite (Paris *et al.*, 1982).

Thin blood smear: A thin blood smear was prepared by taking drops of blood from a microhaematocrit capillary tube to two slides and spread by using other two clean slides at an angle of 45°, air dried and fixed with methyl alcohol for 2 min. Giemsa staining procedures and microscopic examination of the slides were conducted according to OIE (2010). The slides were immersed in Giemsa stain (1:10 solution) for 30 minutes according to Zafar *et al.* (2006). The excess Giemsa solution was drained and washed using distilled water, allowed to dry by standing up right on the rack and examined under the microscope with oil immersion objective lens (Hendrix and Robinson, 2006). Fifty fields from each stained slides were examined for identification of blood protozoa at genus and species level. (Urquart *et al.*, 1996).

3.5 Collection and Examination of Arthropod Vectors

3.5.1 Fly vector collection and examination

To catch biting flies, a total of 43 traps with a monoconical shape were placed approximately 250m apart and left in position for three consecutive days (Marquardt *et al.* 2000). The traps poles were smeared with grease to avoid ants climb. After 3 days of deployment, the catchments of each trap were sorted by fly species and then counted, identified and analyzed (Murray *et al.*, 1983) using their morphological structures such as size, color, wing venation and proboscis at the genus level (Wale and Shearer, 1997). The apparent densities of biting flies were determined based on the mean fly catches in traps baited with acetone and cow's urine (Challier and Laveissor, 1973).

3.5.2 Tick vector collection and examination

Ticks were collected by gentle rotation of ticks (replete ticks or ticks starting to engorge) from their attachment sites using thumb forceps and fingers. Adult and nymphal ticks were collected from the restrained animals and stored in universal bottles and plastic containers, filled with 70% ethyl alcohol (Baker and Ducasse, 1967).

The ticks in the containers were transferred to a Petridish, spread onto filter paper to absorb excess preservatives and examined under stereomicroscope for identification using standard identification keys described by Walker *et al.* (2003).

3.6 Data Analysis and Management

The collected data were coded and entered into Microsoft Excel and summarized using descriptive statistic. The point prevalence was calculated for all data by dividing positive samples by total number of examined samples and multiplied by hundred. All statistical analyses were done using STATA statistical software version SE 13 (Stata Corp., 2013). Logistic regression was used to assess if there was a statistically significant difference between the occurrence of hemoprotozoal parasites and potential risk factors. Statistical significance was considered to exist if p-value less than 0.05.

4. RESULTS

4.1. Overall prevalence of hemoprotozoan parasites

Of 384 blood samples collected from cattle and examined using Giemsa staining technique, 36 (9.4%) of them were infected with *Babesia bovis*, *B. bigemina* and *Trypanosoma vivax*. Out of the total animals exposed to hemoprotozoan parasite 18 (4.6%) and 18 (4.6%) were from Ambo and Toke Kutaye districts, respectively. Out of the total positive cattle, 4 (1%), 29(7.6%) and 3(0.8%) animals were exposed to *B. bigemina*, *B. bovis* and *T. vivax*, respectively.

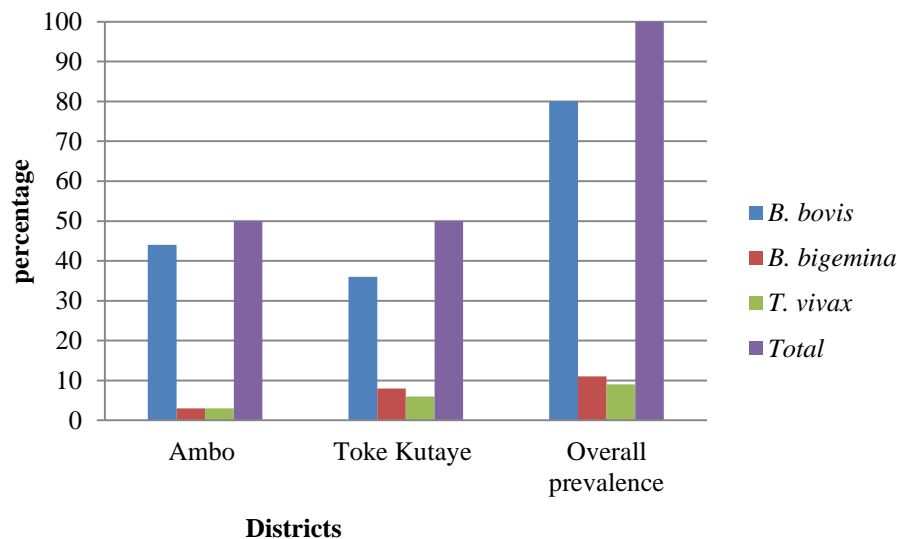


Figure 6. Prevalence of hemoprotozoan parasites in Ambo and Toke Kutaye districts

4.2 Prevalence of Hemoprotozoal Parasite Based on Packed Cell Volume (PCV) Values of Animals

Out of the total cattle affected with hemoprotozoan parasite, 7.2 % (26/384) and 2.8% (7/384) of them were found to be anemic (PCV<25%) and non-anemic (PCV \geq 25%), respectively with statistically significant variation (pvalue = 0.000).

4.1. Prevalence of Hemoprotozoan parasites based on risk factors

The overall prevalence of *T. vivax* in the study area was 0.8% (3/384). There was no statistical significance difference between category of districts, age, BSC, PCV and sex of cattle with prevalence of trypanosomosis. As indicated in Table 1, there was no statistical significance difference in the presence *B. bigemina* between category of districts, sex, age, PCV and body score condition (BSC) of cattle in the study areas .The prevalence of *B. bovis* is higher in Ambo than Toke Kutaye district, in old cattle than young and adult, in female than male cattle, in poor than medium and good BSC and in non-anemic than anemic cattle. Except body condition, sex and PCV of cattle, there was no statistically significant difference between the overall prevalence of hemoprotozoal parasites and the categories of variables.

Table 1. Prevalence of hemoprotozoan parasites by risk factors

Variables	Categories	N examined	<i>T. vivax</i>			Babesia			Overall prevalence		
			N (%)	OR	P value	N (%)	OR	P value	N (%)	OR	P value
District	Ambo	198	1 (0.26)	2.141	0.62	17(4.42)	1.002	0.995	18(4.7)	1.071	0.480
	T.Kutaye	186	2 (0.52)			16(4.16)			18(4.7)		
Age	Young	139	0 (0)	0.5543	0.568	7(1.82)	1.299	0.171	7(1.8)	1.13	0.082
	Adult	155	2 (0.52)			15(3.9)			17(4.4)		
	Old	90	1 (0.26)			11(2.86)			12(3.1)		
Sex	Male	191	1(0.26)	0.492	0.219	12(3.12)	1.821	0.112	13(3.4)	1.626	0.038 ^a
	Female	193	2 (0.52)			21(5.46)			23(6)		
PCV	<25%	252	3 (0.26)	0.822	0.874	26(6.77)	7.241	0.000 ^a	29(7.6)	5.847	0.000 ^a
	>=25%	132	0(0.52)			7(1.82)			7(1.8)		
BSC	Poor	171	3 (0.52)	2.427	0.945	25(6.51)	2.95	0.001 ^a	28(7.3)	10.15	0.00 ^a
	Medium	149	0(0)			7(1.82)			7(1.8)		
	Good	64	0 (0)			1(0.26)			1(0.3)		
Tick infestation	+ve	197	2(0.52)	1.907	0.599	24(6.25)	2.743	0.013 ^a	26(6.8)	2.691	0.004 ^a
	-ve	187	1(0.26)			9(2.34)			10(2.6)		

Keys: N= No. of animals, +ve= positive,-ve = negative %=Percentage, T.Kutaye= Toke Kutaye

^a=statistically significant (P<0.05), OR= Odd Ratio

4.3 Findings of Vector study

4.3.1 Tick vector distribution in the study area

Out of 384 cattle examined, 211 of them were exposed to different species of ticks including *Amblyomma* spp, *Rhipicephalus (Boophilus)* spp, *Rhipicephalus* spp and *Hylomma* spp. *Rhipicephalus (Boophilus)* spp, which is the vector of *Babesia* spp, was the most predominating species of tick (Fig. 9).

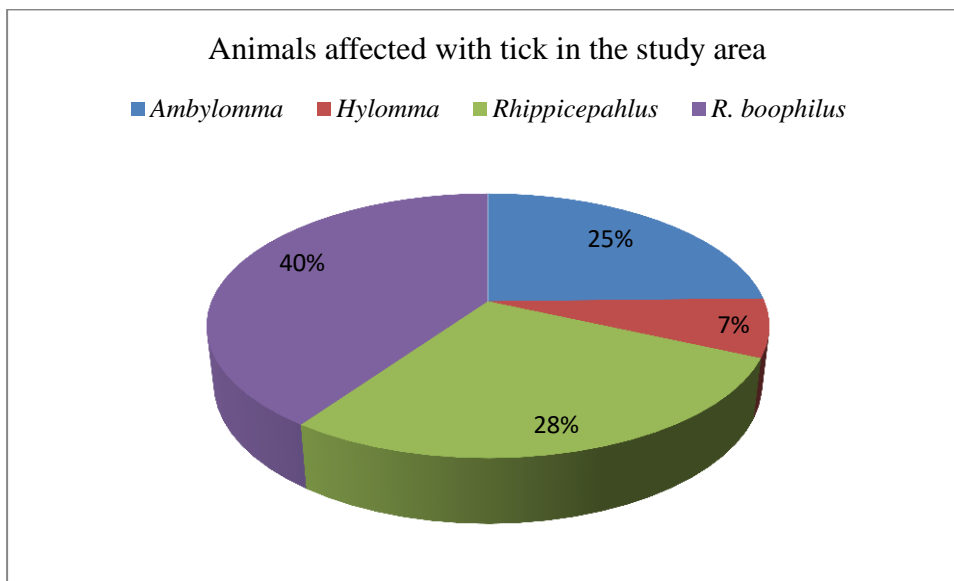


Figure 7. Number of animals infested with tick in the study area

4.3.2 Fly vector surveyed in the study areas

A total of 43 monoconical traps were deployed in the study areas. A total of 1,052 biting flies were caught during the study period. Out of the caught biting flies 512, 430 and 110 belong to family *Stomoxys* (stable fly), *Tabanus* (horse fly) and *chrysops* (deer fly) (Fig. 10). Tsetse fly was not caught in the study area. The overall apparent density of biting flies in the study area was 0.81flies/trap/day.

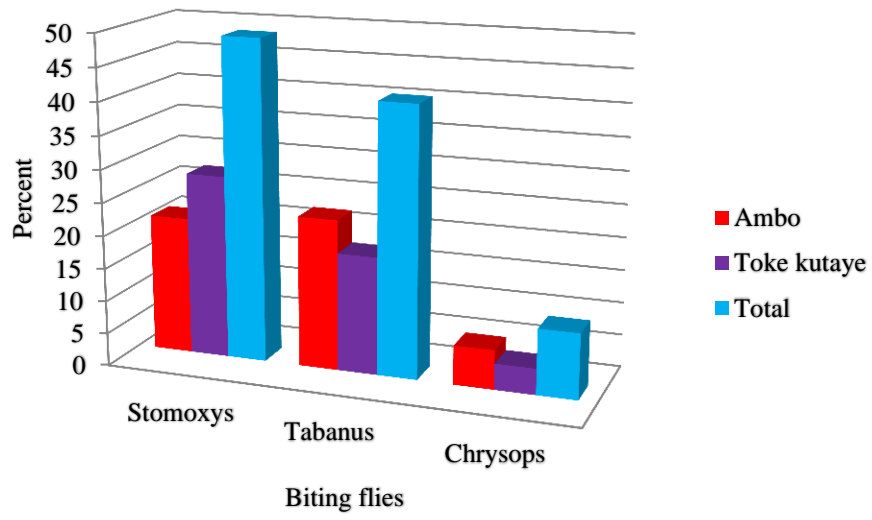


Figure 8. Distribution of biting flies in the study area

5. DISCUSSION

Out of 384 cattle randomly selected for investigation of hemoprotozoan parasites, *Babesia bigemina* and *B.bovis* and *Trypanosoma vivax* were identified in the study area. The overall prevalence of hemoprotozoan parasites of cattle in this study was 9.4% (36/384). This result is relatively similar with that of Alim *et al.*, (2011) who reported 12.02% of hemoprotozoal parasite in Chittagong Division, Bangladesh. This prevalence is lower than that of Ananda *et al.* (2009), who reported 43.18% in Bangalore. The lower prevalence reported in this study area may be due to random sampling rather than selection of clinically susceptible cattle. However, variation in geo-climatic condition, breed and exposure of vectors and age of the animals might contribute to variable prevalence of hemoprotozoan diseases in the study area (Muhanguzi *et al.*, 2010). Constant exposure of infections and development of immunity against such infections might also be responsible for lower prevalence in cattle (Siddiki *et al.*, 2010). Other cause of variation may be due to different geographical conditions and or due to different breeds of cattle studied (Nasir *et al.*, 2000).

This study revealed a higher prevalence of hemoprotozoal parasites in adult age (4.4% (17/384)) than young (1.8% (7/384)) and old (3.1% (12/384)) although, not statistically significant ($p=0.08$). This finding agrees with that of Abdul *et al.* (2011) who reported high prevalence of hemoprotozoal diseases in adult animals. The lower prevalence in the young animals compared to adult animals may be due to restricted grazing of young animals which tends to reduce their chance of contact with the vectors of hemoprotozoal parasites. Adult animals were also transported long distance for grazing and draught activities and exposed to vectors of hemoprotozoal parasites. The other reason may be due to innate resistance in young animals which usually protects and limits mortality to a low level in young animals (Urquhart *et al.*, 1996).

When comparing the prevalence of hemoprotozoal parasites in the study depending on the sex of animals, higher prevalence was seen in female (6% (23/384)) than in male (3.4% (13/384)) cattle, which is statistically significant ($p=0.00$). This agrees with the finding of Kamani *et al.* (2010) who reported higher prevalence in female than male animals. The higher prevalence of hemoprotozoal diseases in female animals may be due to the fact that

female animals are kept longer for breeding and milk production purposes (Kamani *et al.*, 2010).

Moreover, higher prevalence of hemoprotozoal parasite in female animals might be due to hormonal disturbances as they are used in milk production and breeding system and also supplied insufficient feed against their high demand which lowers the immune system of the animals (Kamani *et al.*, 2010) or variation in sample size.

During the study period, animals with PCV value $\leq 24\%$ were considered to be anemic (Van den Bossche and Rawlands, 2001). High prevalence of hemoprotozoal parasite was found to be in anemic (PCV $\leq 24\%$) (7.6% (29/384)) than non-anemic (PCV $\geq 25\%$) (1.8% (7/384)) and it is statistically significant ($p=0.00$). The variation between anemic and non-anemic animals may be due to replication of all hemoprotozoal parasites inside or between erythrocytes leading to hemolysis and anemia. All the hemoprotozoal parasites mentioned in combination led to significant ($p<0.05$) reduction in mean packed cell volume in infected animals.

The prevalence of hemoprotozoal disease based on the body condition of the animals was 0.26% (1/384), 1.8% (7/384) and 7.3% (28/384) for good, medium and poor scoring, respectively with significant statistical association ($P<0.05$). This could be due to the fact that animals with poor body condition have lower immunity which encourages infection of animal by different hemoprotozoal parasites. In addition, during this survey it was very common to see high burden of ectoparasite (ticks) on animal with poor body condition and this can increase rate of infection from *Babesia*.

Trypanosoma vivax is the only *Trypanosome* species identified in the study area. This result agrees with the finding of Sitotaw *et al.* (2015) who found only *T. vivax* of *Trypanosoma* species from Bishoftu, central Ethiopia. This may be due to *T. vivax* being spread beyond the tsetse infested area of north west and south west of Ethiopia by transmission through mechanical vectors (CFSPH, 2009). The overall prevalence of *T. vivax* in the study area is 0.8% (3/384). This result is lower than the report of Tadesse and Getaneh (2015) who reported 2.34% prevalence of trypanosomosis in Womberma and Bure districts of west Gojjam Zone, Ethiopia.

There was no statistical significant difference in the prevalence of trypanosomosis between the age categories with high prevalence in adult animals. The higher prevalence of *T. vivax* in the current study agrees with the finding of Kitila *et al.*, (2016) in Yayo district of Ilu Ababora zone, western Ethiopia and Nabulime *et al.*(2014) in Mulanda, Tororo District of Eastern Uganda who reported the high prevalence of trypanosomosis in adult animals. This lower prevalence reported in young animals in this research may be due to the fact that young animals naturally protected to some extent by maternal antibody (Fimmen *et al.*, 1992). The old animals also stayed in the area for a long period of time experienced to many challenges with a disease which make their immunity so strong than adult animals.

There is higher prevalence of *T. vivax* in anemic 0.8 % (3/384) than non-anemic 0% (0/384) animals. Similar result was reported in finding of Konta Special district, Southern Ethiopia by Abera *et al.*(2015) who reported the high prevalence of trypanosomosis in anemic animals than non-anemic animals. This could be due to anemic nature of trypanosomosis and malnutrition. Therefore, trypanosomosis may be involved in adversely lowering the PCV value of infected animals.

All the positive samples for trypanosomosis (3/384) were from poor body conditioned cattle. The presence of trypanosomosis only in poor body condition cattle in this study was not statistically significant. Likewise, the same result reported by Gebreyohannes and Bekele (2014) in Woliso, South west Shewa, Ethiopia. The result revealed the marked effect of trypanosomosis on the body condition of cattle. Cattle with poor body condition were more associated with the disease as compared to those with good body condition. Weight loss (cachexia) and emaciation is the characteristic sign of trypanosomosis (Urquhart *et al.*, 1996) and/or poor body condition cattle are less immune to trypanosomosis than medium and good body condition.

The overall prevalence of babesiosis in the study area was 8.6 % (33/384). This is accordance with that of Seyyed *et al.*, (2011) reported 9.76%. But it is lower than that of Kamani *et al.*, (2010) and Lemma *et al.*, (2015) who reported 16% and 23% prevalence of babesiosis in cattle in north central Nigeria and Jimma, Ethiopia, respectively. The discrepancy in the prevalence of bovine babesiosis might be due to different factors like

management condition of the focus area, use of acaricides during tick infestation, farming system and proper use of antiparasitic drugs, fluctuations of parasites during chronic course of the disease and in carrier cattle, sensitivity of test used in this study, and distribution of infected vector in the study area. Other cause of variation may be due to different geographical conditions and or due to different breeds of cattle studied.

In the current study, 1.8%, 3.9% and 2.9% prevalence of *Babesia* was registered in young, adult and old cattle, respectively. The lower prevalence of babesiosis in young cattle in this investigation may be due to an inverse age resistance of the disease where adult showed more susceptibility than calves (Urquhart *et al.*, 1996). This might be due to rapid immune responses to primary infection by the calves through a complex immune mechanism (Annetta *et al.*, 2005).

Based on the sex of cattle, a higher prevalence of *Babesia* was reported in female (5.46% (21/384)) than male (3.12% (12/384)) cattle. The variation of prevalence of babesiosis between each sex is not statistically significant. This agrees with the report of Hamsho *et al.* (2015) who found 17.48% female as compared to male cattle 16.29%.

Cattle with PCV value <25% are significantly more affected (6.8%) with babesiosis when compared to those whose PCV value \geq 25% (1.8%). This result agrees with that of Mahmmod (2014) who reported more prevalence of babesiosis in anemic animals. Anemia develops as a result of blood hemolysis and hemolysis occurs due to mechanical damage by trophozoites to RBC when multiplied by binary fission, phagocytosis of infected RBC by host immune system and toxic substances secreted by the parasites (Ibrahim, 2011, CFPH, 2008).

Ticks were found on 54.9 % (211/384) of sampled cattle. There is significant correlation between incidence of babesiosis and tick infestation as indicated in the table 1. This indicates that ticks may act as a vector for transmission of *Babesia* spp in the study area but parasite detection was not carried out in ticks found on the cattle under the study because constraints of laboratory facility and time. A similar trend of tick infestation and babesiosis was previously reported by Iqbal *et al.*, (2011).

During the study period, tsetse flies were not caught in the trap cages. The only flies found in the study area include *Tabanus* spp *Stomoxys* spp and *Chrysops*. Out of 1,052 caught biting flies 512, 430 and 110 belong to family *Stomoxys* (stable fly), *Tabanus* (horse fly) and *chrysops* (deer fly) (Fig. 8). The absence of *tsetse* fly in the study area may be due to the geo-climatic condition of the study area.

6. CONCLUSION AND RECOMMENDATION

This work was the first epidemiological study of hemoprotozoal parasite in the study area with the objective to determine prevalence, identify the genera and species of hemoprotozoal parasites and their associated risk factors. The findings revealed that *babesia* spp and *trypanosoma* spp were hemoprotozoal parasites of cattle identified in the study area. Body score condition of cattle, packed cell volume value and tick infestation of cattle were found to be statistically significant with prevalence of hemoprotozoal parasite. Ticks (*Amblyomma* spp, *Rhipicephalus* spp, *Rhipicephalus* (sub genus: *Boophilus*) spp and *Hylomma* spp) and biting flies (*Stomoxys* spp, *Tabanus* spp and *Chrysops* spp) were vector of hemoprotozoal parasite identified in the study area. In conclusion, hemoprotozoal disease is one of the most important cattle diseases in the study area. Therefore, the following points were recommended

- ☞ Integrated approach which is a combination of chemotherapy and vector control of hemoprotozoal parasite should be considered.
- ☞ Advanced and detailed studies should be conducted on the epidemiology of the disease in order to expand and implement disease investigation and control strategy.
- ☞ Awareness should be given to livestock owners in relation to vector control as one option of controlling the disease
- ☞ Veterinary service should be expanded through remote PAs to control the disease.
- ☞ The laboratories facility should be fulfilled to diagnose hemoprotozoal properly at district level
- ☞ The animals health worker should be trained how to diagnose and treat hemoprotozoal disease and how to control their vector.

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8. ANNEXES

Annex 1: Blood sample collection and result registration table

Date _____

District's name _____

SN	Owner's name	PAs/	Animal's ID	Breed	Sex	Age	BSC	PCV (%)	Tick identified on animal's body					Blood Parasite identified			
									Rhp	Hya	Amby.	Haemo phys	Other tick	<i>Babesia</i>	<i>Theile ria</i>	Tryps	
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	

Keys: BSC (Body score condition), PCV (Packed Cell Volume), Rhp (Rhipicephalus), Amby (Ambylomma), Haemophys (Haemophysalis), Tryps (*Trypanosoma*).

Annex 2: Fly vector registration sheet

SN	Date	Site/district of collection	Fly spp	Number of flies trapped(f/t/d)
1				
2				
3				

Annex 3: Buffy coat procedure (Bayou, 2005)

- A. Fill heparinized capillary tubes with blood from the animal to be examined
- B. Centrifuge the sample using hematocrit centrifuge
- C. Transfer the capillary tube on to a slide
- D. Use a small adhesive tape to attach the tube on the slide
- E. Examine the Buffy coat in the capillary tube under the microscope. (the Buffy coat is the grayish narrow space found between the plasma and the red blood cells in the capillary tube). The motile organisms such as the trypanosomes are seen flickering at this junction
- F. Cut the capillary tube at the junction between the Buffy coat and the red blood cells.(more toward the red blood cells)
- G. Blow the capillary tube containing the plasma, the Buffy coat and some red blood cells on clean slide
- H. Make a smear of this content and stain with Giemsa to identify(**Annex 4**)

Annex 4: Giemsa staining procedure (Bayou, 2005)

- A. Fix smear in methanol for two minutes
- B. Stain in 10% Giemsa in buffered pH 7.2 solution for 30 minutes in a coplin jar or upside down on staining plate

- C. Wash off stain with a stream of buffer.(phosphate buffer pH 7.2)
- D. Flood with buffer and leave until differentiation is complete (1-2 minutes)
- E. Dry by standing upright on the bench

Annex 5: Body score condition evaluation of zebu cattle

Score	Condition	Features
1	L-	Marked emaciation (animal would be condemned at ante mortem examination)
2	L	Transverse process project prominently, neural spines appear sharply
3	L+	Individual dorsal spines are pointed in the touch, hips, pins, tail-head, and ribs are prominent. Transverse process visible usually individually
4	M-	Ribs, hips and pins clearly visible. muscle mass between hooks and pins slightly concave, slightly more flesh above the transverse process than L+
5	M	Ribs usually visible, little fat cover, dorsal spine barely visible
6	M+	Animal smooth and well covered, dorsal spines cannot be seen, but are not easily felt
7	F-	Animal smooth and well covered, but fat deposit are not marked, Dorsal spines can be felt with firm pressure but fill rounded rather than sharp.
8	F	Fat covered in critical areas can be seen and felt; transverse process cannot be seen or felt.
9	F+	Heavy deposit of fat clearly seen on tail head, brisket and cod; dorsal spines, ribs, hooks and pins fully covered and cannot be felt even with firm pressure.

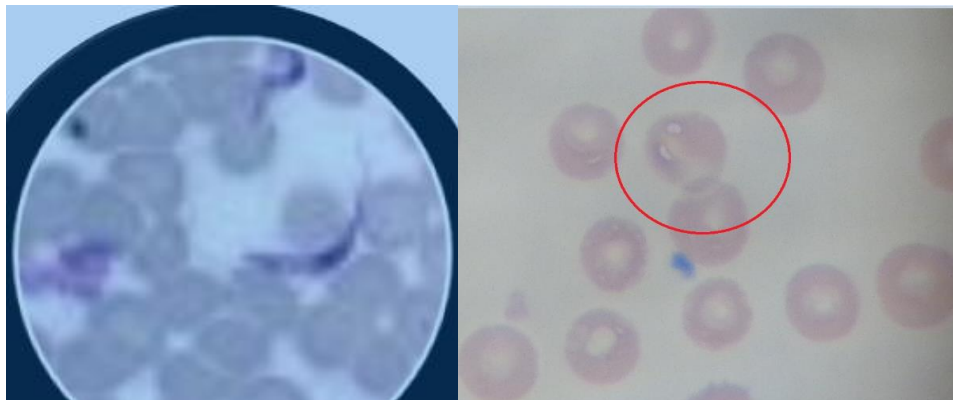
(Nicholson and Butterworth, 1986)

Annex 7: Pictures taken at field during study period

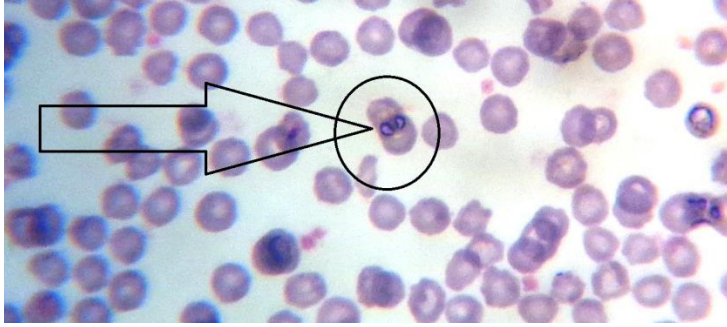


A. Monoconical trap used during study time. B. Flies caught in the cage of trap C. bottles filled with odor attractants (Top: cow's urine, Left: octenol, Right: Ethanol)

Annex 6: Blood smear pictures taken at Laboratory during study time



Blood smear of *T. vivax* positive Blood smear positive with *B. bovis* (encircled)



Blood smear containing *B. bovis* showed by arrow