

Prevalence of Bovine Babesiosis in and Around Sibru Sire District of East Wollega Zone, Oromia Regional State, Western Ethiopia

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Abstract: A cross-sectional study was carried out from November, 2017 to April, 2018 to assess the prevalence of bovine babesiosis infection in relation to age, sex, body condition, locality, tick infestation and anemic status in local zebu cattle randomly selected in and around the Sibru Sire district, East Wollega Zone, Oromia Region, Western Ethiopia. A total of 402 blood samples were collected from the marginal ear vein and jugular vein of cattle from seven kebeles. Blood smears were stained using Giemsa's technique and examined under oil immersion for the detection of *Babesia* as well as hematocrit centrifugation techniques were used to determine packed cell volume (PCV) for the detection of anemia. In this study microscopic examination of blood smears revealed an overall prevalence of 8.71% (35/402) for bovine babesiosis. From the identified *Babesia* species, 68.6% (24/35) of them were found to be *B. bovis* and the rest 31.4% (11/35) were *B. bigemina*. Study kebele's, sex and age categories didn't show significant difference in prevalence of babesiosis. The prevalence of bovine babesiosis had a significant association with anemic status (univariate: $p < 0.001$, $OR = 14.5$; multivariate: $p < 0.001$, $OR = 13.35$), tick infestation (univariate: $p < 0.001$, $OR = 5.12$; multivariate: $p = 0.04$, $OR = 3.13$) and body condition (univariate: $p < 0.001$, $OR = 2.64$; multivariate: $p = 0.02$, $OR = 2.2$) of the animals. The results of this study have indicated that bovine babesiosis was found to be prevalent in the study area and poses a threat to cattle production. It is important to design appropriate tick control and strategic prophylactic treatment in order to decrease the current challenges in the study area. There is a need for further epidemiological investigations using advanced serological and molecular techniques.

Key words: Babesiosis • Bovine • Associated Risk Factors • Sibru Sire District • Prevalence

INTRODUCTION

Ethiopia is one of the countries with the largest number of livestock in Africa. The estimated livestock population in Ethiopia is 59.5 million cattle, 30.70 million sheep, 30.20 million goats, 1.21 million camels, 2.16 million horses, 8.44 million donkeys, 0.41 million mules, 56.53 million poultry and 6,523,969 traditional, intermediate and modern bee hives [1,2]. In spite of having the largest livestock population in Africa, the contribution for the economic aspect of the country is still lowest and cattle productivity is low [3]. The little benefit from the enormous livestock resource of the country is attributable to a multitude of problems. This may be due to improper management, disease, nutritional deficiency, harsh environment and genetic factors [1].

Livestock disease is among the major factors that affect the production and productivity having negative

effects on the health of the livestock. Among these, tick-borne diseases are present throughout the world, but are most numerous and exert their greatest impact in the tropical and subtropical parts of the world including Ethiopia [4, 5]. Many of the tick-borne diseases are haemoparasitic [4,6]. Arthropod transmitted haemoparasitic disease of cattle is caused by *Trypanosoma*, *Babesia*, *Theileria* and *Anaplasma* species [7]. Their effects often depend on the species and immunity of the host and can vary from development of severe disease to a completely sub clinical infection without any signs of disease [8, 9].

Babesia species are intra erythrocytic protozoan parasites of domestic, wild and laboratory as well as human. They belong to protozoan parasites of genus *Babesia*, order Piroplasmida, phylum Apicomplexa and subclass piroplasmia and are commonly referred as 'piroplasmas' due to the pear like shaped merozoites

which live as small parasites inside the RBC of mammals. More than 100 known *Babesia* spp. have been identified which infect many types of mammalian host, out of these, 18 spp. cause disease in domestic animals notably in cattle, sheep, goats, horses, pigs, dogs and cats [10]. The principal species of *Babesia* that cause bovine babesiosis (BB) are: *Babesia bovis*, *Babesia bigemina* and *Babesia divergens*. Other Babesiathat can infect cattle includes *B. major*, *B. ovata*, *B. occultans* and *B. jakimovi* [11]. Two species, *B. bigemina* and *B. bovis*, have a considerable impact on cattle health and productivity in tropical and subtropical countries [10].

Bovine babesiosis is the most important arthropod borne disease of cattle worldwide that causes significant morbidity and mortality. It is the second most common blood borne parasitic disease of mammals after the trypanosome and it was classified as list B disease according to OIE [12] [9]. Babesiosis is a haemolytic disease and characterized by fever (40-42°C) which may be sudden in onset, anemia, icterus, hemoglobinuria, listless, anorexic, jaundice and death [13]. Disease signs vary in severity from silent infection to acute circulatory shock with anemia, depending on susceptibility, immunity and age of the host, and on *Babesia* species and parasite load [14]. The most prevalent species, *B. bovis* and *B. bigemina*, are found throughout most tropical and subtropical regions, particularly important in Asia, Africa,

Central and South America, parts of southern Europe, and Australia. The economic losses from these two organisms can be considerable, particularly in developing countries [12].

In Ethiopia, previous surveys have been conducted on distribution, abundance, and prevalence of hemoparasite species affecting livestock in different regions of the country. These investigations have showed wide spread existence haemoparasites like anaplasmosis, babesiosis, cowdriosis, trypanosomosis and theileriosis [15,16]. However, the detailed status of the hemoparasiticdiseases in ruminant's particularly bovine babesiosis is not thoroughly studied in and around Sibru Sire district, East Wollega Zone, Western Oromia Regional State. Thus, the present study was conducted to determine the prevalence of bovine babesiosis in and around Sibru Sire district, and to investigate the risk factors associated with the disease in the study area.

MATERIALS AND METHODS

Study Area: The study was conducted from November 2017 to April 2018 in seven (7) selected kebeles of Sibru Sire district, located in East Wollega Zone, Oromia Region, Western Ethiopia. It is one of the districts in East Wollega Zone and is located 281km in West from Addis

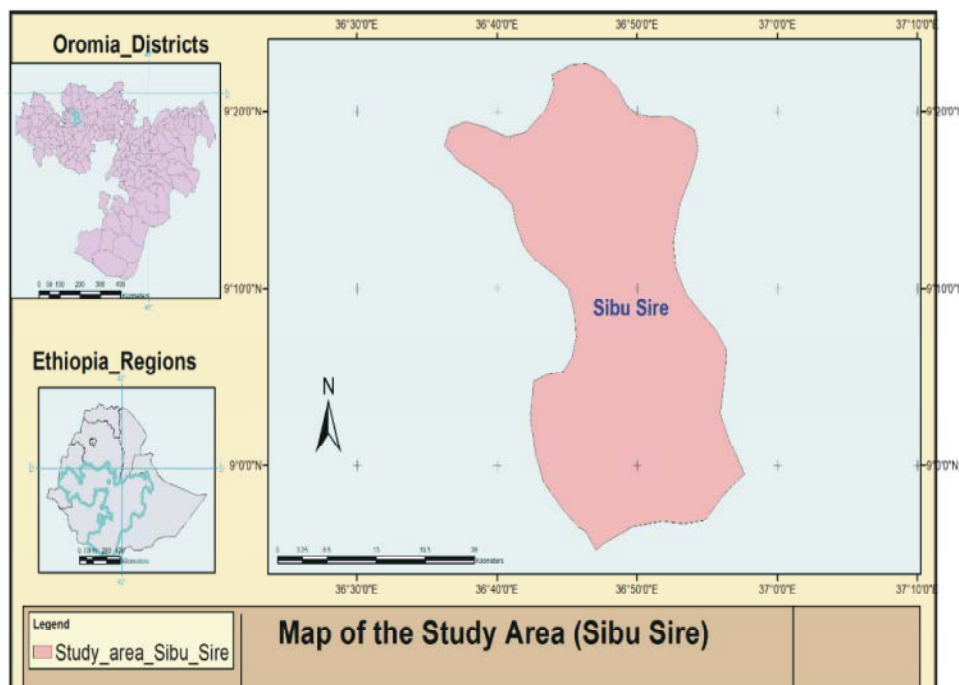


Fig. 1: Map of the Study area (Sibu Sire, East Wollega)

Ababa and 50km East from Nekemte, the administration town of East Wollega Zone. The district lies between 8°56'- 9°23'N latitudes and 36°35'- 36°56' E longitudes. The altitude of the district ranges between 1360 and 2500 meters above sea level. It covers an estimated area of 1,132.51 km². This district is bordered in the East by GobuSeyo, in the West by WayuTuka, in South by WamaHagalo and BilloBoshe and in the North by GudeyaBila and Guto Gida (Fig. 1).

There are three agro-ecological zones represented in this district. The majority (74.3%) of the district is classified as mid-land with lowland (18.27%) and only 7.53% is considered as highland. The minimum, maximum and mean temperature of this area was 14.09°C, 27.30°C, and 22.55°C, respectively. The highest temperature occurs in February and March. The lowest temperature occurs in July and August. The area has typically two rainy seasons: a long rainy season from June to September, with the peak rainfall in July and August, and a short rainy season from April to May. Averagely, the annual rainfall of the district is 1295 mm [17].

This district has 23 kebeles from these 19 are PAs and 4 municipals. According to data collected by district animal statistics agency since 2017, the animal population of this district accounts: 192575 cattle, 39605 sheep, 38895 goats, 13145 horses, 1545 mules, 17506 donkeys, 78669 poultry, and 242 swine. For this study, seven (7) kebeles are selected based on the cattle population density. Accordingly, Baro-Titita, Bujura, Burka Talo, Bako Jima, Dicho, Lalisa and Chari which have total cattle population of 12985, 10578, 9890, 13204, 14892, 8535, and 13763, respectively.

Study Animal: The animals used for this study were local zebu cattle (*Bos indicus*), which are usually kept under an extensive husbandry system. The studied cattle were herded together during the day time and returned to their individual owner's farmstead each evening. Their age was determined based on owners' information and as described by De-lahunta and Habel [18]. Age was categorized into three groups (< 3 years, 4-6 years, and >7years) of age as young, adult and old, respectively based on dentition and horn rings as well as information provided by the owners. The body condition score was grouped into poor, medium, and good conditioned animals based on the appearance of ribs and dorsal spines applied for zebu cattle [19].

Study Design: A cross-sectional study design was conducted from November 2017 to April 2018 in the Sib

Sire district of East Wollega zone to estimate the prevalence of bovine babesiosis and to investigate the risk factors associated with the disease from local zebu cattle with consideration of their age, sex breed, body condition, locality and tick infestation.

Sampling Method and Sample Size Determination: The study animals were sampled randomly involving cattle of both sex, all age groups, and all types of body conditions. The desired sampling size was calculated according to the formula given by Thrustfield [20]. As there have been no published studies reported in this area, the sample size was determined based on the expected prevalence of 50%, confidence level of 95%, and 5% desired absolute precision. Accordingly, a total of 402 samples were collected from seven PAs of the area (villages) in the district. Of which, 55 animals from Baro Titita, 49 from Bujura, 40 from Burka Talo, 60 from Bako Jima, 87 from Dicho, 43 from Lalisa and 68 from Chari.

$$n = \frac{1.962 p \exp(1 - p \exp)}{d^2}$$

where, n is the required sample size (n), Pexp, is the expected prevalence and d is the desired absolute precision. To increase the precision, additional samples were collected. Thus, a total of 402 blood samples were collected from the selected kebeles.

Sample Collection and Transportation: Blood samples were collected from 402 animals during the period of November 2017 to April 2018 from 7 PAs of the Sib Sire district. The cattle were selected randomly and restrained by farmers for blood sampling. Sample collection procedures were done according to Urquhart *et al.* [21]. Before blood was collected, the area of puncture was cleaned, hair removed and disinfected with 70% alcohol. First, blood was collected from jugular vein using EDTA coated vacutainer tubes and labeled with all necessary information's, for PCV determination. And again for thin smear, blood sample was collected with heparinized microhaematocrit tube (capillary tube) after proper disinfection and preparation of marginal ear vein with alcohol. Thin smear was prepared by applying the slide with blood onto a clean slide at an angle of 45° and then gently moving forward. The slides were labeled with pencil where information like sex, age, body condition, and PAs were recorded. The thin smeared slide was air dried and fixed for 5 minutes in methyl alcohol (absolute methanol).

Soon after the slides were fixed and air dried it was put into slide box and blood samples collected in EDTA coated vacutainers were transferred to ice box and transported to Wollega University School of Veterinary Medicine, Veterinary Parasitology and Pathology Laboratory for examinations of parasites without delay.

Study Methodology

Blood Film Preparation and Examination: Giemsa staining procedures and microscopic examination of slides were conducted according to OIE [22]. The slides were immersed in Giemsa stain (1:10 solution i.e. 10 ml of Giemsa stock solution and 90 ml of distilled water) in staining rack for 30 minutes. Then the slides are washed with tap water to remove excess stain and were air dried. After a drop of oil immersion was added on the slide and it was finally examined under high power (100x) adjusted objective microscope for appreciation and identification of different *Babesia* species according to their morphological characteristics [23, 24].

Packed Cell Volume (PCV) Determination: Blood samples obtained from jugular veins were used for PCV determinations. Capillary microhaematocrit tubes were filled approximately $\frac{3}{4}$ of its length, then the tubes were sealed at one end and placed in microhaematocrit centrifuge with sealed end lying at the outer most. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 minutes. After centrifugation, the capillary tubes were placed in a haematocrit reader and the length of the red cells column was expressed as a percentage of the total volume of blood [25]. Animals with PCV less than 25% were considered to be anemic [24].

Data Analysis: The raw data collected during the study period were coded and stored in Microsoft Excel spread sheet 2007 data base system and analyzed using STATA version 15 statistical software for Windows (Stata Corp. College Station, USA). The Prevalence of bovine babesiosis was analyzed by determining total positive cases for *Babesia* out of the total number of cattle examined. Univariate and multivariate analyses were performed to assess the association of potential risk factors (age, sex, body condition, anemic status, kebele and tick infestation) with the prevalence of bovine babesiosis. The Pearson's chi-square test (χ^2) was used to measure the association between prevalence of the parasite with those mentioned potential risk factors and odds ratio was calculated to determine the level of

association. In all the analyses, confidence interval (CI) was held at 95% and $p < 0.05$ was set for statistical significance.

RESULTS

Hematological Findings: PCV of individual animals was measured for the assessment of degree of anemia. The overall anemia prevalence in the studied district was 39.6% (159/402). The mean PCV value was 26.62%. The most frequently recorded PCV value was 22% and was recorded in 58 cattle from the overall studied animals in the district. A prevalence of bovine babesiosis was 19.45% (31/159) for anemic animals and 1.65% (4/243) for normal animals. The difference was statistically significant ($p < 0.001$) (Table 1). The frequency distribution of PCV % for the overall studied 402 cattle is indicated in Fig. 2.

PCV According to Age Group of Animals: The mean PCV value was 27.35%, 26.61% and 26.11% in young, adult and old age groups, respectively. In young and adult animals, the minimum PCV value was 18% and the maximum was 44%. But in older group animals minimum value was 18% and maximum was 37% (Fig. 3).

PCV According to Sex: The mean PCV was 26.4% and 26.77% in male and female animals, respectively. In both sex the minimum and maximum PCV recorded was 18% and 44%, respectively (Fig. 4).

PCV According to Body Condition Score of Animals: The minimum PCV scored in all body condition groups was 18% and the maximum was 44%, which were registered in good and medium body conditions while in poor body condition of the animal the maximum PCV was lower (42%) (Fig. 5).

PCV of Animal According to Tick Infestation: The mean PCV value of animals with no tick infestation (27.36%) was higher than that of tick infested animals (26.06%). The maximum PCV recorded in tick infected animals was 43%, while that of animals with no tick infection was slightly higher (44%) (Fig. 6).

Parasitological Findings: From a total of 402 cattle examined the overall prevalence rate of bovine babesiosis in this study area was 8.71% (35/402). Out of this prevalence, 68.6% (24/35) of the species were found to be *B. bovis* and the remaining 31.4% (11/35) were *B. bigemina*.

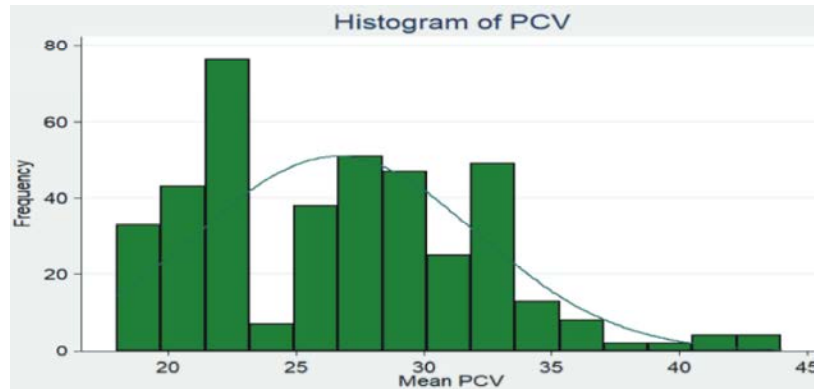


Fig. 2: PCV distribution in cattle population of Sibiu Sire district

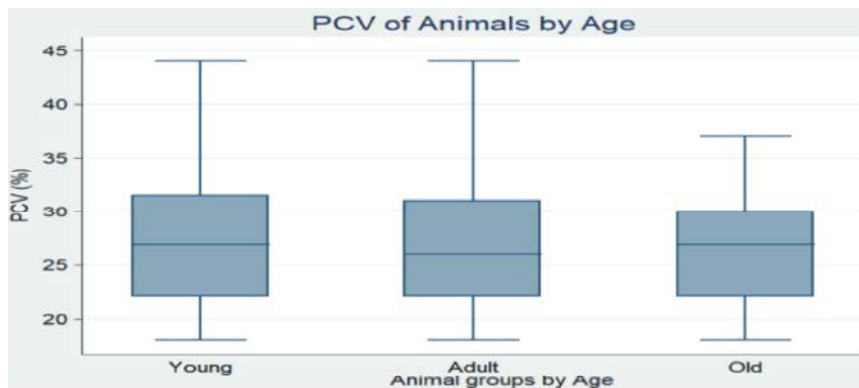


Fig. 3: PCV distribution of animal by age

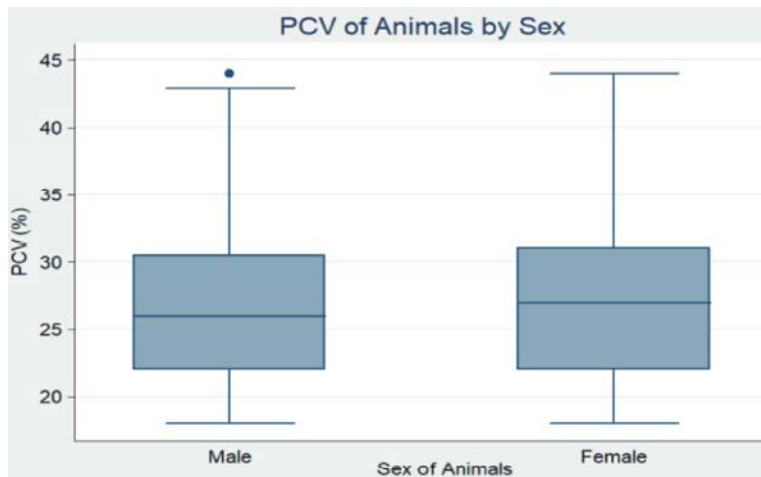


Fig. 4: PCV distribution of animal by sex

Association of Risk Factors with Prevalence of Bovine Babesiosis

Univariate Analysis: All the seven investigated kebeles in Sibiu Sire district belong to rural areas. Of the total 402 samples collected, the lowest prevalence (2.5%) was recorded in Burka Talo while the highest (13.95%) was recorded in Lalisa (PAs) (Table 1). But there was no

statistical significant difference observed ($OR=1.12$, $CI=0.933-1.336$, $p>0.05$) between the different PAs in the study area. The prevalence of babesiosis was slightly higher in males (8.75%) as compared to female animals (8.68%) (Table 1). However, the difference was not statistically significant ($OR= 0.99$, $CI= 0.488-2.011$, $p=0.22$). The highest prevalence was observed in the

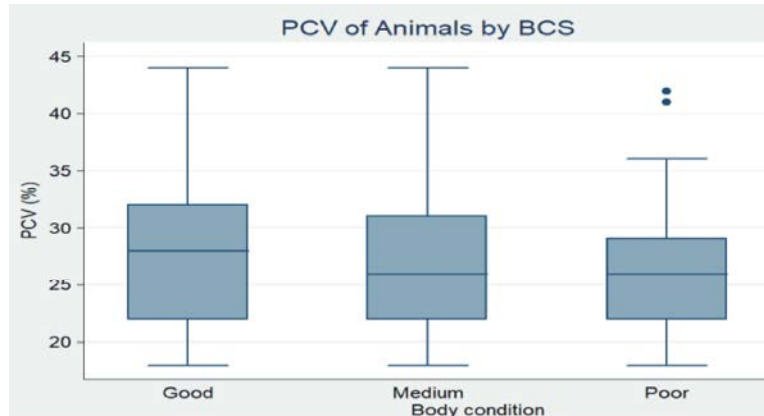


Fig. 5: PCV distribution according to body condition of animals

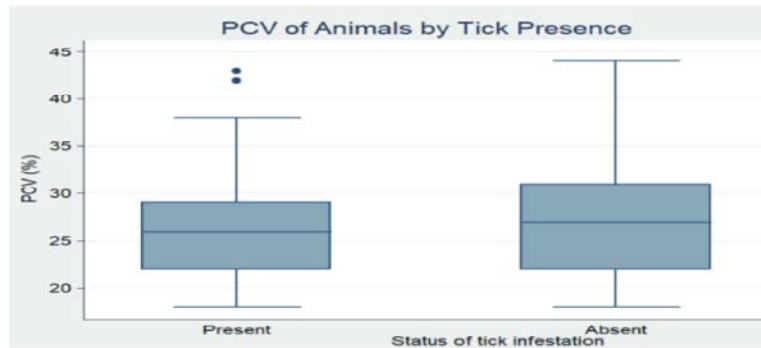


Fig. 6: PCV distribution of animal by tick infestation

Table 1: Univariate and multivariate analysis of bovine babesiosis with potential risk factors

Risk factors	Groups	No. examined	No positive (%)	Univariate analysis				Multivariate analysis		
				OR	95% CI	χ^2	P-value	OR	95% CI	P-value
PA	BaroTitita	55	6 (10.91)	1.12	0.933-1.336	1.48	0.22	1.04	0.855-1.254	0.72
	Bujura	49	3 (6.12)							
	Burka Talo	40	1 (2.5)							
	Bako Jima	60	2 (3.33)							
	Dicho	87	9 (10.34)							
	Lalisa	43	6 (13.95)							
	Chari	68	8 (11.76)							
Sex	Male	160	14 (8.75)	0.99	0.488-2.011	0.98	0.98	0.99	0.458-2.167	0.99
	Female	242	21 (8.68)							
Age	Young	96	3 (3.13)	1.48	0.913-2.395	2.61	0.11	1.11	0.605-2.039	0.74
	Adult	173	19 (10.98)							
	Old	133	13 (9.77)							
Body condition	Good	93	2 (2.15)	2.64	1.504-4.613	13.80	<0.001	2.20	1.150-4.219	0.02
	Medium	162	11 (6.79)							
	Poor	147	22 (14.97)							
Anemic status	Normal	243	4 (1.65)	14.47	4.998-41.901	40.07	<0.001	13.35	4.541-39.251	<0.001
	Anemic	159	31 (19.45)							
Tick infestation	Absent	174	5 (0.29)	5.12	1.944-13.493	14.83	<0.001	3.13	1.039-9.432	0.04
	Present	228	30 (13.16)							

adult animals and the variation in prevalence between the different age groups was also not statistically significant ($OR=1.48$, $CI=0.913-2.395$, $p=0.98$). The prevalence of babesiosis between body condition scores was 14.97% in poor, 6.79% in medium, and 2.15% in good body conditioned animals and it was statistically significant ($OR=2.64$, $CI=1.504-4.613$, $p<0.001$). Animals with poor body condition were exposed 2.64 times to babesiosis than animals with good body condition.

Animals with tick infestation showed a higher prevalence (13.16%, 30/228) compared to animals with no tick infestation (0.29%, 5/174) and this was found to be statistically significant ($OR=5.12$, $CI=1.944-13.493$, $p<0.001$). Tick infected animals were more exposed (5.12 times) to babesiosis than animals with no tick infestation. A prevalence of bovine babesiosis was 19.45% (31/159) for anemic animals and 1.65% (4/243) for normal animals. The difference was statistically significant ($OR=14.47$, $CI=4.998-41.901$, $p<0.001$) and anemic animals were found to be 14.47 times more susceptible to babesiosis compared to normal animals (Table 1).

Multivariate Analysis: Among the different risk factors there was significant association between the prevalence of bovine babesiosis and body condition ($OR=2.64$, $CI=1.504-4.613$, $p=0.02$), tick infestation ($OR=3.13$, $CI=1.039-9.432$, $p=0.04$) and anemic status of animal ($OR=13.35$, $CI=4.541-39.251$, $p<0.001$) (Table 1).

DISCUSSION

The overall prevalence rate of bovine babesiosis in the study area was found to be 8.71%. The finding of the current study is higher than a range of studies conducted previously in Ethiopia and other countries. Hika *et al.* [26] studied prevalence of bovine babesiosis in and around Bishoftu town using Giemsa stained microscopic examination technique and found prevalence rate of 2.6%; Bihonegn *et al.* [27] reported 1.5% prevalence in Assosa Woreda, Benishangul Gumuz Regional State, Western Ethiopia; and Salm *et al.* [28] reported 7.69% in Egypt.

In contrary to this study, some researchers also reported higher prevalence rate of babesiosis from different world countries. Alemayehu and Nuraddin [29] and Fethu *et al.* [30] in Jimma town South Western Ethiopia reported the prevalence of 12.8% and 23%, respectively. Hamsho *et al.* [9] and Patcharathorn *et al.* [31] reported a prevalence of 16.9% in Teltele District,

Borena Zone, Southern Ethiopia and 26.6% forest in Salakpra Wildlife Sanctuary in Kanchanaburi province, respectively.

This disparity might be due to different factors like management systems, proper use of antiparasitic drugs or acaricides, sensitivity of the test used, distribution of infected vector, accessibility of animals to wildlife sanctuary and forest area harboring the Babesia vectors geographical locations, breeds of cattle and sample size [32, 33].

Out of the 8.71% overall prevalence of Babesia infection, 68.6% were due to *B. bovis*, and 31.4% were due to *B. bigemina*. The higher proportion of *B. bovis* in this study was in agreement with the previous results of Fethu *et al.* [30] where they reported 60.8% of the infection to be caused by *B. bovis*. Moreover, the results of Hamsho *et al.* [9] at Teltele District, Borena Zone, Southern Ethiopia (58.46%) and Bihonegn *et al.* [27] in and around Assosa Woreda, Benishangul Gumuz Regional State, Western Ethiopia (83.33%), had also shown higher results of *B. bovis* infections. A slight higher rate of *B. bovis* might be due to higher concentration of the *B. bovis* parasite in the capillary and veins than the *B. bigemina* parasite which evenly distributed in the whole blood vasculature. Previous studies have also indicated that cattle infected with *B. bovis* remain carriers for long periods, while those infected with *B. bigemina* remain carriers for only a few months [34].

The prevalence of bovine babesiosis was studied in different age groups of cattle and the highest prevalence of babesiosis was noted among adult age (10.98%) followed by old (9.77%) and young age cattle (3.13%). However, there was no statistically significant variation between the different age groups in the prevalence of babesiosis. This finding was in agreement with the result of Alemayehu and Nuraddin [29] from Jimma town. However, the results of this study disagree with Amorim *et al.* [35] who reported that calves were more susceptible to *Babesia* infection compared to adult cattle. The reason for the present result could be due to the fact that young animals, particularly calves under six months of age have maternal immunity acquired from colostrum feeding so that they are almost slightly resistant to infection as compared to adult animals. On the other hand, lower prevalence in young animals attributed due to restricted grazing of young animals which likely to reduce their chance of contact the vectors of the disease [36].

In the present study, a slightly higher infection rate was recorded in male (8.75%) than female animals (8.68%). Though this difference was not statistically significant, these findings are in agreement with the results of Hika *et al.* [26] and Bihonegn *et al.* [27]. In contrary to this finding Fethu *et al.* [30] from Jimma town reported a higher prevalence of babesiosis in female compared to male cattle. Moreover, the higher prevalence of tick borne diseases in male animals may be due to the fact that male animals are subjected to trek long journey for drought purposes and stressful work that suppress the immune system of the animals [37].

Furthermore, the present result stated that Babesiosis was higher in poor body condition (14.97%) followed by medium (6.79%) and good (2.15%) body condition. A body condition of the animal confers significant association for the occurrence of the disease. This could be due to the fact that animals with poor body condition have lower immunity which encourages infection of animal by different organisms like Babesia. In addition, during this survey it was very common to see high burden of ectoparasite (ticks) in animal with poor body condition unlike those animals with good body conditions and this can increase rate of infection from Babesia. This result was similar with the report of Bihonegn *et al.* [27] in and around Assosa district, and Alemayehu and Nuraddin [29] from Jimma.

The prevalence of babesiosis was higher in tick infested cattle (13.16%) than non-tick infested cattle (0.29%) and this was statistically significant ($p < 0.05$). This shows strong association between Babesia infection and tick vector. This finding was in agreement with the result of Fethu *et al.* [30] who found higher prevalence of babesiosis in those cattle having tick infestation compared to those that doesn't have tick infestation. The presence of diseases caused by hemoparasites is broadly related to the presence and distribution of the vectors [7].

Comparison of the anemic status of animal between those infected with Babesiaspp. and those not infected gave significant difference ($p < 0.05$). This may explain the low PCV values recorded in animals infected with Babesia spp. This finding is also supported by the findings of Olayemi and Oyewale [38] who indicated that the presence of blood parasites renders the erythrocytes to be more susceptible to osmotic lysis resulting in lowered PCV values. This agrees with the previous report of Mohammed [39] and Sitotaw *et al.* [16]. Anemia develops as a result of blood hemolysis and hemolysis occurs due to mechanical damage by trophozoite to RBC when multiplied by binary

fission, phagocytosis of infected RBC by host immune system and toxic substances secreted by the parasites [40].

CONCLUSION

In conclusion, this study indicated that bovine babesiosis is an important disease and a potential threat that affects the health and productivity of cattle in Sibru Sire district. The study revealed that the overall prevalence of the disease was 8.75%. *B. bovis* and *B. bigemina* were identified as the species responsible for bovine babesiosis with greater prevalence of *B. bovis*. Higher prevalence of babesiosis infection was observed in animals with poor body condition scores, tick infestation, and anemic condition. Further studies using advanced serological and molecular techniques should be conducted in order to monitor the herds for purpose of identifying factors that may pose risks to the current epidemiological status of the region. In order to alleviate the existing problem and to promote the status of livestock production more feasible in this district, the use of appropriate preventive measures is needed, especially with regard to the effective control of ticks in order to the control of *Babesia* parasite. Regular survey of cattle for tick is recommended for inclusion into routine management of cattle in the region.

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