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Study on Bovine Babesiosis in and Around Meki and Batu Towns, Oromia, Ethiopia

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Abstract: A cross-sectional study was conducted from November, 2015 to April, 2016 with the overall objectives of determining the prevalence and associated risk factors of bovine Babesiosis in and around Meki and Batu towns, Ethiopia. A total of 384 blood samples were collected from cattle of different age, sex and body condition for parasitological examination using thin blood smears followed by Giemsa staining method and Packed Cell Volume (PCV) determination. Accordingly, the overall prevalence of bovine Babesiosis was found to be 3.64%. In this study, *Babesia bigemina* (3.38%) and *Babesia bovis* (0.26%) were encountered. Out of the 3.64% positive animals, 13 were found to be anemic with PCV values less than 24%. Thus, there was a strong association between anemia and *Babesia* positive cases ($p=0.008$). The prevalence of tick infestation was 29.43%. Generic level tick identification revealed the occurrence of *Ambylomma* (36.13%), *Rhipicephalus* (23.16%) and *Hyalomma* (40.71%). The Prevalence of bovine babesiosis and tick infestation by respect to the host related risk factors were sex (3%, 28.6% male and 4.4%, 30.4% female), age (2.9%, 30.4% young, 3.3%, 28% adult and 7.5%, 37.5% old) and body condition (4.1%, 30.5% poor, 2%, 30.6% moderate and 3%, 24.2%, good) respectively. There was a statistically significant difference observed ($p<0.05$) between prevalence of Babesiosis with tick infestation and tick burden, tick infestation with season. In a nut shell, the findings of the present study disclosed that bovine babesiosis is one of the important hemoparasitic protozoan diseases of cattle that circulate in the study area. In order to tackle and manage the problems associated with bovine babesiosis and its tick vectors, it is essential to customize appropriate and integrated tick control measures and tactical treatment of overt bovine babesiosis clinical cases.

Key words: Batu · Babesiosis · Host Related Risk Factors · Meki · Prevalence · Tick Infestation

INTRODUCTION

Ethiopia is one of the countries with the largest number of livestock in Africa. Livestock production plays a major role in the development of Ethiopia's agriculture. Despite the importance of livestock to the economy at large, the sub-sector has remained untapped. The little benefit from the enormous livestock resource of the country is attributable to a multitude of problems. Livestock disease is among the major factors that affect the production and productivity having negative effects on the health of the livestock. The presence of diseases

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caused by haemoparasites is broadly related to the presence and distribution of their vectors. Arthropod transmitted haemoparasitic disease of cattle is caused by the *Trypanosome*, *Babesia*, *Theleria* and *Anaplasma* species. Bovine Babesiosis is a tick born hemoparasitic disease caused by intra erythrocytic protozoan parasite known as *Babesia*, causing significant morbidity and mortality in cattle and buffaloes [1].

Taxonomically, *Babesia* belongs to the phylum Apicomplexa, orders Piroplasmida and subclass Piroplasmia and are commonly referred to as 'piroplasmas' due to the pear-like shaped merozoites. More than 100 known *Babesia* species have been identified which infect many types of mammalian host, out of these, 18 cause disease in domestic animals notably in cattle, sheep, goats, horses, pigs, dogs and cats [2]. The major *Babesia* species that affect cattle include: *Babesia bigemina*, *Babesia bovis*, *Babesia divergens* and *Babesia major*. Two species, *B. bigemina* and *B. bovis*, have a considerable impact on cattle health and productivity in tropical and subtropical countries including Ethiopia [3].

Babesia bovis infections are characterized by high fever, ataxia, anorexia, general circulatory shock and sometimes also nervous signs as a result of sequestration of infected erythrocytes in cerebral capillaries. Anemia and haemoglobinurea may appear later in the course of the disease. In acute cases, the maximum parasitaemia (percentage of infected erythrocytes) in circulating blood is less than 1%. This is in contrast to *B. bigemina* infections, where the parasitaemia often exceeds 10% and may be as high as 30%. In *B. bigemina* infections, the major signs include fever, haemoglobinurea and anemia. Infected animals develop a life-long immunity against reinfection with the same species of *Babesia*. There is also evidence of a degree of cross-protection in *B. bigemina*-immune animals against subsequent *B. bovis* infections. Calves rarely show clinical signs of disease after infection regardless of the *Babesia* species involved or the immune status of the dams [4].

Ticks are among the most common ectoparasites and vectors of important animal diseases on global scale particularly, in tropical and sub-tropical parts of the world. As ectoparasites ticks are responsible for tick-borne disease, anemia, irritations, skin abrasions, tick toxicosis, tick worry, bite wound, wounds and myiasis and loss of udders, which provides portals of entry for secondary bacterial infection [5]. Numerous studies have been conducted on the ticks and tick-borne diseases of cattle in various parts of Ethiopia and several species of ticks belonging to genus *Amblyomma*, *Rhipicephalus (Boophilus)*, *Hyalomma* and *Haemaphysalis* have been reported [6]. More than 60 species of ticks are infesting both domestics and wild animals have been recorded and 33 of these are known to be important parasites of livestock [7].

In Ethiopia, various surveys have been carried out on distribution, abundance and prevalence of hemoparasite species of livestock in different regions of the country by various investigators including Trypanosomiasis of ruminants, Babesiosis in donkeys and canine [8]. Such investigations have been conducted in different regions of the country. However, the current status of bovine Babesiosis and its tick vectors are not studied in Meki and Batu and the surrounding areas. Hence, in light with the above back ground information and justifications, the present study was initiated and designed to address the status of bovine babesiosis and its tick vectors in and around Meki and Batu towns of Oromia regional state with the objectives of ;

- Determining the prevalence of bovine babesiosis and its tick vectors in and around Meki and Batu towns
- To identify the associated risk factors and
- Forward appropriate and practical intervention and control strategies that can combat the impact of bovine babesiosis and its tick vectors.

MATERIALS AND METHODS

Description of the study area: The study was conducted in the East Shewa Zone of Oromia Region State of Ethiopia from November, 2015 to April, 2016. The area has a latitude and longitude of 8°9'N 38°49'E with an elevation of 1636 meters above sea level. Meki is the administrative center of Dugda District. Batu is a town and separate districts in central Ethiopia. It is located on the road connecting Addis Ababa to Nairobi.

Study Population and Sample Size Determination: The study animals were cattle of different age and sex which were brought to veterinary clinics found in and around Meki and Batu towns. The age, sex, seasonal tick infestation and body condition scores were recorded. Taking an estimated prevalence of 50%, the minimum sample size at 95% confidence interval and at 5% precision or accuracy level the sample size were calculate to be 384 using the formula given by Thursfield [9] as follows:

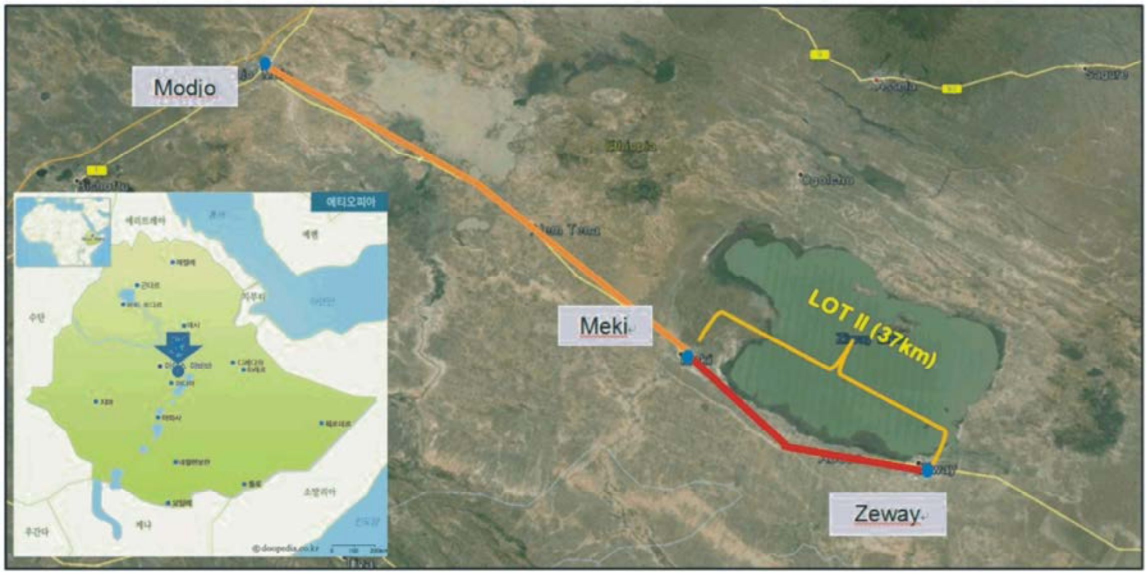

Fig. 1: Map of the study area

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

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

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

Study Design and Sampling Methodology: A cross sectional study was conducted from November, 2015 to April, 2016 to determine the prevalence of bovine Babesiosis and its risk factors associated with Babesiosis in and around Dugda (Meki) and Batu districts, East Shewa Zone of Oromia Regional State. Blood samples were collected from ear and jugular vein of randomly selected 384 bovines from selected different Veterinary Clinics of Dugda district and Batu town based on the cattle population density of the area, accessibility of roads and transportation systems and vegetation coverage of the focus area.

Sample Collection and Transportation: Blood samples were collected after proper restraining of the animals according to Urquhart *et al*. [10]. For the purpose of blood collection, jugular vein by using EDTA coated vacationer tubes and ear vein (marginal ear vein was prepared for disinfection with the help of methyl alcohol (70%) and a slight tearing of vein was made with lancet then the blood sample was taken with one end sealed microhaematocrit tube (capillary tube) were used. After labeling, there were transported to Meki Veterinary Clinic Laboratory with ice box as soon as possible when the samples were collected from field.

Preparation of Slides: Thin blood smear on clean and dry glass slides were prepared from the blood taken from marginal ear vein or jugular vein. These smears were air dried and fixed in methyl alcohol (99%) for 3 minutes and stained with working solution of Giemsa stain (1:9) ratio with phosphate buffer solution having pH 6.8 and wait for 30 minutes. The smears were washed with tap water to remove extra stain and air dried and slides were examined under the oil immersion lens of a light microscopic.

Blood Films Examination: Giemsa staining procedures and microscopic examination of slides were conducted according to OIE [11]. Thin blood films were prepared from blood samples, air dried, fixed with absolute methyl alcohol for 3 minutes and then stained by Giemsa stain 10% for 30 minutes then examined microscopically using oil immersion lens (100x) of a light microscope according to Zafar *et al*. [12]. The parasites were identified according to the characters described by Soulsby [13]. The smears were recorded as negative for *Babesia* if no parasites were detected in oil-immersion fields According to Moretti *et al*. [14].

Packed Cell Volume (PCV): Packed cell volume, which is a measure of the proportion of the volume of the whole blood that is occupied by red blood cells, was determined by the microhaematocrit centrifugation technique [15]. Blood in a sample vacutainer tube was mixed by gently inverting the tube. The blood was drawn three quarters of the microhaematocrit capillary tubes. Blood was wiped off the tip of the capillary tube and the ends of the capillary tubes were carefully plugged with plasticine. The capillary tubes were placed, with the closed end outwards, in a microhaematocrit centrifuge and spun at 12000 rpm for 5 minutes [16]. The capillary tube was removed from the centrifuge, placed on a haematocrit reader and the PCV was recorded.

Tick Collection and Identification: Ticks were collected by forceps and gloved hand picking by a simple random sampling method from different body parts of the cattle without damaging their mouth parts. The collected ticks were preserved in 70% ethyl alcohol in clean, well-stopped glass vials and labeled properly. Morphological characterization of ticks was carried out using stereoscopic microscope [17]. Prevalence for each tick species was calculated as P=d/n x 100, where p = the prevalence, d = the number of animals that tested positive for a particular tick genera and n the total number of animals sampled [18].

Body Condition Scoring and Age Estimation: On subjective basis, body condition scores of animals were evaluated during sample collection. They were classified as emaciated (poor), moderate (medium) and good based on anatomical parts and the flesh and fat cover at different body parts [19]. Animals were conveniently classified as young (<3 years), adult (4-6 years) and old (>7 years) age categories as described by De- lahunta and Habel [20].

Data Analysis: The data collected during the study period were stored in Microsoft Excel spread sheet and analyzed using SPSS version 20 for Windows. The prevalence was calculated by dividing the number of cattle found to be positive for *Babesia* and tick infested by the total number of cattle examined for *Babesia* and tick infestation. The association of risk factors like age, sex, body condition and seasons of study period for Babesiosis were assessed using Chi-square test. In all the analyses, confidence interval (CI) was held at 95% and P<0.05 was set for statistical significance.

RESULTS

A total of 384 blood samples were collected, examined and tested by using thin blood smear and an overall prevalence of 3.64% (14/384) *B. bovis* and *B. bigemina* were recorded in the study areas. In this study, highest prevalence of Babesiosis was noted among old age (7.5%) followed by adult (3.3%) and young age cattle (2.9%). Origin, age, body condition of the animals and sex were not significant (p>0.05) with positivity for Babesiosis (Table 1). Two species of *Babesia* were identified with the prevalence of 0.26% (1/384) and 3.38%(13/384) for *B. bovis* and *B. Bigemina,* respectively (Table 2).

Sex and Age Wise Prevalence: As indicated from the above table, both sexes of animals were compared and a prevalence rate of 4.4% (8/181) for females and 3% (6/203) for males were found. Highest infection rates was observed in old cattle (7.5%) followed by adult (3.3%) and young (2.9%).

Body Conditions and Season Wise Prevalence: As shown from the above table, the external factor of both seasons were compared and a prevalence rate of (3.8%) for autumn and (3.3%) for winter were found. The body condition wise prevalence of Babesiosis was highest in poor (4.1%) followed by good (3%) and medium (2.9).

Tick Infestation Wise Prevalence: Out of the total animal which was infested with ticks, (11.5%) was prevalence of infection. On the other hand, (0.4%) prevalence was recorded in the animals of free tick infestation. Nevertheless, a significant difference was observed (x^2=28.149, p= 0.000) (Table 2).

The prevalence of babesiosis in cattle on the basis of tick genera (*Ambylomma*, *Rhiphicephalus* and *Hyalomma*). The three genera (*Ambylomma*, *Hyalomma* and *Rhiphicephalus*) did not show any statistical significant associations (P>0.05) with the occurrence of the disease while *Rhiphicephalus* formerly *Boophilus* has shown high statistical significance (p=0.000) with the occurrence of the disease (Table 2).

Mean PCV in Parasitemic and Aparasitemic Cattle: The proportion of Babesia infection with species level indicated that 0.26% and 3.38% cattle were found to be infected B. *bovis* and *B. bigemina* (Table 3). PCV of individual animals was measured for the assessment of

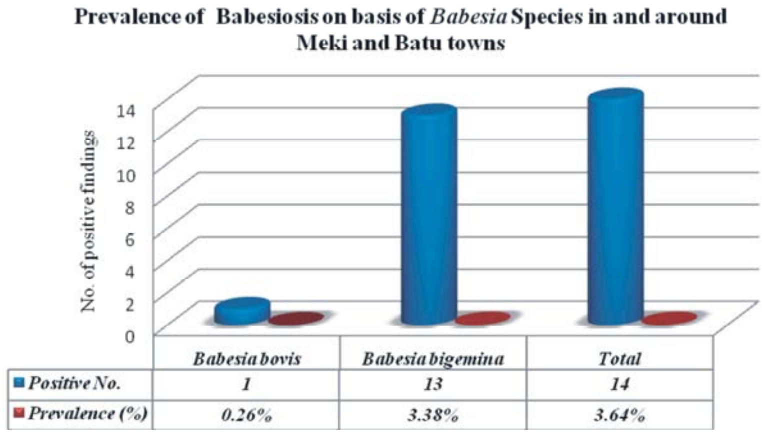

Fig. 2: Prevalence of Babesiosis on basis of Babesia Species

Table 1: Prevalence of bovine babesiosis in and around Meki and Batu towns

Risk factor		No. of tested animals	Positive	Prevalence (%)	Chi-square	P-Value
Age	Young	69	2	2.9		
	Adult	275	9	3.3	1.910	0.289
	Old	40	3	7.5		
Sex	Male	203	6	3	0.82	0.445
	Female	181	8	4.4		
BCS	Good	66	2	3		
	Moderate	49	1	2	0.581	0.748
	Poor	269	11	4.1		
Season	Winter	150	5	3.3	0.068	1.0000
	Autumn	234	9	3.8		
Tick infestation	Present	113	13	11.5	28.149	<0.001
	Absent	271	1	0.4		
Tick burden	None	270	1	0.4		
	Few	112	12	10.4	36.409	<0.001
	Moderate	2	1	1		

Table 2: Prevalence of bovine babesiosis with tick genera in and around Meki and Batu towns

Tick genera	Status	No. tested animal	Positive	Prevalence (%)	Chi-square	P-Value
Ambyloma	Yes	41	0	0	1.737	0.379
	No	343	14	4.1		
Rhiphicephalus	Yes	111	12	10.8	18.517	<0.001
	No	273	16	5.8		
Hyalomma	Yes	46	3	6.3	1.230	0.267
	No	338	11	3.3		

Table 3: Mean PCV of infected and non-infected animals found in and around Meki and Batu towns

Condition	No. examined	Mean PCV (%)	Chi-square	P-Value
Infected	14	19.29	34.073	0.008
Non-Infected	370	25.45		

degree of anemia. A mean PCV of 19.29% and 25.45% were found for infected animals and non-infected animals respectively (Table 3). The difference was statistically significant ($p = 0.008$).

In the study, the overall prevalence of tick infestation was 29.43% (113/384). As indicated from the above Figure 2, old age, medium body condition and female animals had the highest prevalence of 37.5(15/40), 30.6(15/49) and 30.4% (55/181) respectively.

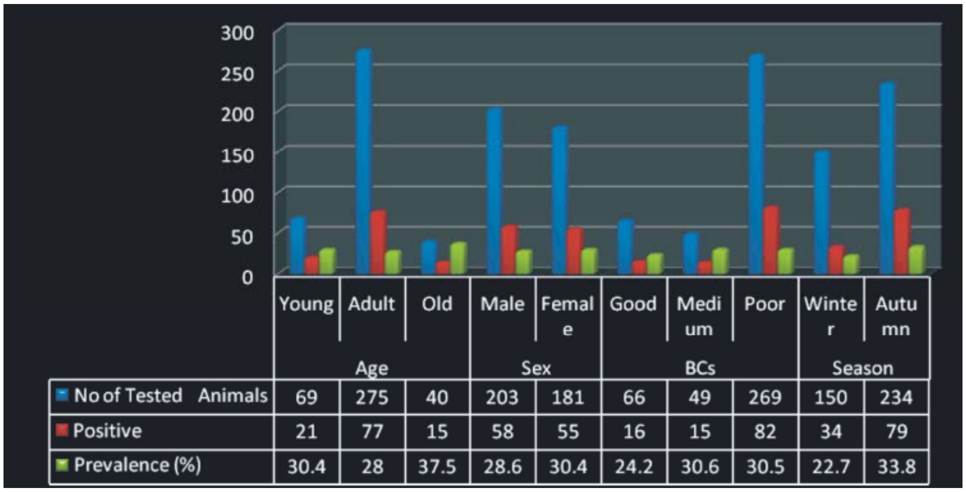

Fig. 2: Prevalence of tick infestation with different risk factors in and around Meki and Batu towns.

DISCUSSION

In this study, the overall prevalence rate of bovine babesiosis was found to be 3.64% out of these two species of *Babesia* comprising of *B. bovis* (0.26%) and *B. bigemina* (3.38%) were identified using Giemsa stained microscopic examination. This finding was lower than the earlier 42% prevalence reported from Malaysia [21], 40% from Benha, Qalubia Governorate, North Eastern Egypt [22], 26.6% from Salakpra Wildlife Sanctuary, Kanchanaburi province [23], 23% from Jimma Town, South Western Ethiopia [24], 16.9% from Teltele District, Borena Zone, Southern Ethiopia [25], 9.9% from District Kohat and Karak, Khyber Pakhtunkhwa Pakistan [26] and 6.6% from Lahore, North East Pakistan [27]. However, this current study finding was slightly higher than the reported prevalence 1.5% from Around Assosa district, Benishangul Gumuz Regional State, Ethiopia [19] and from Debre-Zeit, Central Ethiopia [1].

The discrepancy in the prevalence of bovine babesiosis might be due to different factors like management systems of cattle on the focus area, use of acaricides during tick infestation, sort of farming system and proper use of antiparasitic drugs, fluctuations of parasites during chronic course of the disease and in carriers animals, sensitivity of the test used, distribution of infected vector and accessibility of animals to wildlife sanctuary and parks and forest area harboring the *Babesia* vectors [28]. Other cause of variation may be due to different geographical conditions and or due to different breeds of cattle studied [29].

As indicated from Figure 1, the two species of bovine babesiosis identified were *B.bovis* and *B. bigemina*. *Babesia bigemina* was more prevalent in this study than *B. bovis* with known prevalence of 3.38% and 0.26% respectively. The difference was statistically highly significant ($P = <0.001$). The results of the present research showed higher *B. bigemina* (3.38%) infection rate compared to *B.bovis* (0.266%) which disagree with the earlier prevalence 17% *B. bovis* and 16% *B. bigemina* from Malaysia as reported by Rahman *et al*. [21] 9.9% *B. bovis* and 7% *B.bigemina* from Borena Ethiopia as reported by Hamisho *et al*. [25] and 1.24 *B.bovis* and 0.29 *B.bigemina* from Asossa Ethiopia as reported by Wodajnew *et al*. [19]. 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 [4].

In the present study the highest prevalence of Babesiosis was noted among old age (7.5%) followed by adult (3.3%) and 2.9 young. This result was in line with the finding of Ayaz *et al*. [26] from Pakistan who reported high prevalence in old animals with 13.4% (61/452) followed by adult animals with 11.7% (48/409) while the lowest was found in young animals. However, the results of this study disagree with Amorim *et al*. [30] who identified that calves were more susceptible to *Babesia* species when compared to adult cows. This variation can 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 old 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 to vectors of these diseases [31].

In the present study slightly higher infection rate was recorded in female 4.4% as compared to male animals 3%. Even though these proportional rates of prevalence of *Babesia* infection in female animal slightly higher than male animal, there was no statistically significant difference observed, which indicated that both sexes were equally susceptible for the infection. This finding was in agreement with the report of Kocan *et al*. [32] who found higher prevalence of babesiosis in female 11.2% compared to male cattle 6.96%.

Respecting to the seasonal incidence of Babesiosis, the seasonal prevalence of Babesiosis using blood smears examination showed that the peak of infection of Babesiosis in cattle was recorded in autumn (3.8%). These results were similar with results of Kamani *et al*. [31] who recorded that, the highest infection rate of Babesiosis was recorded in both summer and autumn and was less in spring and low in winter in cows. The earlier studies were conducted during the late dry and early rainy season but in the present study all samples were collected from early winter up to mid-autumn. This implies that crucial factors have just influenced the rate and seasonal variation of vectors, high humidity and temperature. In general, prevalence intensity rate of tick born hemoparasitic disease infestation were generally low during dry season and higher in rainy season [33]. The prevalence of the disease based on the presence of tick was statistically significant ($p<0.05$) those cattle having tick has 11.5% while those does not have tick was 0.4%. This shows strong association between *Babesia* infection and tick vector. The presence of diseases caused by hemoparasites is broadly related to the presence and distribution of the vectors [34].

The mean PCV value between infected cattle and non-infected cattle are statistically significant ($p<0.05$). The mean PCV value of infected cattle was 19.29% which shows below normal value and this suggesting that anemia is clinical sign of the infection due to the parasite invade in red blood cell cause hemolytic. This agrees with the previous report of Sitotaw *et al*. [1]. 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 [35].

During this study, a total of 113 cattle were found to be infested with different genera of ticks. The prevalence of ticks genera were found to be *Amblyomma* (36.13%), *Rhipicephalus* (23.16%) and Hyalomma (40.71%). Regarding to tick infestation in relation to age groups, from the total of 384 examined cattle for tick infestation 29.43% (113) of cattle under different age groups were found positive for one or more ticks genera. Of which had over all prevalence of 37.5%, 30.4% and 28.0% of old, young and adult respectively.

The difference in prevalence among the age groups were statistically not significant ($p>0.05$). The calves were kept apart from adult animals at population density and were thus possibly exposed to lower parasite burden on the pasture and almost graze on zero grazing. Calves generally more resistant to infestation of tick than adult [36].

The difference in prevalence of tick infestation was found statistically insignificant ($P>0.05$) between sex of cattle. This result is in line with the other author in Benchi Maji [18], but it disagreed with the previous works in Assosa by Bossen and Abdu [37] that the difference in prevalence was found statistically significant between sex groups. The difference in prevalence among body condition groups were statistically insignificant ($P>0.05$). The result was disagreeing with other researcher's results performed in different parts of Ethiopia [38], but in line with the statement given by Kassa and Yalew [17] and Tesfaheywet and Simeon [18] because there existed no statistical significant difference ($P>0.05$) in the prevalence of ticks between the different sex, age and body condition score categories of cattle. Prevalence of tick was higher in rainy (autumn) season (38.8%) than in dry winter (season) (22.7%) which agree with Doube and Wharton [39, 40] had reported that tick infestation was higher in summer than in winter, O`Kelly and Spiers [40, 41] had demonstrated that animals maintained in the sun carried considerably fewer ticks than animals allowed access to shade and Stuti *et al*. [42, 43] who reported that maximum tick infestation was experienced by cattle during rainy season.

CONCLUSION

In conclusion the present findings indicate that bovine babesiosis is to be one of the important hemoparasitic protozoan diseases of cattle that circulate in and around Meki and Batu towns. Tick vectors were also found in the study area. *Babesia bovis* and *Babesia bigemina* were identified as the species responsible for bovine babesiosis with greater prevalence of *B. bigemina*. Since the prevalence of babesiosis is directly related to its tick vectors, seasonal cattle and pasture treatment before and after rainy season and awareness creation on routine investigations of tick vectors and their control measures should be adopted by various groups of cattle producers.

In order to keep the good management practices, alleviate the existing problems and to promote high status of livestock production more feasible in these areas, it is essential to customize appropriate and integrated tick control measures with improved management practice and tactical treatment of overt bovine Babesiosis clinical cases. Strategic prophylactic treatment of bovine Babesiosis is not advocated for fear of disturbing the enzootic stability or equilibrium that exists between the host and parasite in indigenous breed of cattle. Thus, only tactical treatment of overt bovine Babesiosis clinical cases is highly recommended. Finally, Since the accurate identification of the causative organisms of disease is fundamental to the study of prevalence and epidemiology of Babesiosis further research should be conducted to elucidate the epidemiology and impacts of tick borne diseases using immunological methods and PCR technique to implement better control measures against tick borne diseases of cattle and to validate the present study.

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