Study on the Prevalence of Bovine Babesiosis and Its Associated Risk Factors in and Around Assosa Woreda, Benishangul Gumuz Regional State, Western Ethiopia

Bihonegn Wodajnew[1*], Haimanot Disassa[1], Tadele Kabeta[1], Tilahun Zenebe[1] and Girma Kebede[1]

[1]. Wollega University, School of Veterinary Medicine, Nekemte, Ethiopia
girmakebede27@yahoo.com

Abstract: A cross-sectional study was conducted from November, 2014 to April, 2015 to assess the prevalence of bovine babesiosis in and around Assosa District, Assosa Zone of Benishangul Gumuz Regional State, western Ethiopia. A total of 402 blood samples were collected from ear vein of cattle from six peasant associations (PAs) that encompasses veterinary clinics. In this study the overall prevalence of bovine babesiosis was found 1.5% (6/402) using microscopic examination of Geimsa stained blood smear. Except sex, most of the risk factors such as PAs, age, body conditions, Babesial species and seasons of study period showed statistical significant associations at (P=0.000) with the occurrence of the disease. Six PAs were assessed and the highest equivocal (equivalent) prevalence of 2.99% was recorded in Abrhamo, Mengele 29 and Amba 12 PAs but in the other three Pas the disease was not encountered throughout this study period. Sexwise prevalence showed that a slight higher prevalence was recorded in male (1.72%) than female (1.32%) but there was no statistical significant association (P=0.739). Age wise prevalence showed that the highest prevalence among old animals (3.51%) followed by adult (0.96%) and the disease was not found in young cattle throughout study period. Body condition of the animals showed highest prevalence recorded in animals with poor body condition (4.08%) followed by 1.03% of prevalence in animals with moderate body conditions and the disease was not observed in animals with good body conditions. Two species of Babesia identified were *B. bovis* (1.24%) followed by *B. bigemina* (0.248%). Seasons of the study period revealed that the highest prevalence was compiled during the autumn season (2.99%) followed by extremely low prevalence in the winter season (0.88%) throughout the study period. In conclusion the results of this study have indicated that bovine babesiosis was extremely low prevalent in the study area. However, it has a paramount importance to conduct further study on the prevalence and epidemiological aspect of the disease via immunological based study. In order to alleviate the existing trends, it is far better to adopt appropriate tick control and strategic prophylactic treatment in the focus area.

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1. Introduction

Ethiopia is one of the countries with the largest number of livestock in Africa and livestock production plays a major role in the development of Ethiopia's agriculture. Ethiopian livestock population is estimated to be 53.38 million cattle, 25.50 million sheep, 22.78 million goats, 6.21 million donkey, 2.08 million horse, 1.10 million camels, 0.39 million mules and 49.28 million poultry (Central Statistics Authority (CSA), 2011).

Nevertheless, cattle productivity is low (CSA, 2009). This may be due to improper management, disease, nutritional deficiencies, harsh environment and genetic factors. Arthropod transmitted hemoparasitic diseases are economically important vector-borne diseases of tropical and subtropical parts of the world including Ethiopia. *Bovine babesiosis* caused by an apicomplexan haemoprotozoan parasite, *Babesia bigemina* (family Babesiidae, order Piroplasmida), is transmitted by brevirostrate tick, *Rhipicephalus microplus*, causing significant morbidity and mortality in cattle and buffaloes (Alekaw, 2000).

Babesia is the second most common parasite found in the blood of mammals after trypanosomes (Yabsley and Shock, 2013). Babesiosis is a tick born disease of domestic, wild and laboratory animals as well as humans caused by the genus *Babesia*. Almost any mammals, that serves as a host for a Babesia infected tick is a potential reservoir (Homer *et al*., 2000).

Babesia species are intra erythrocytic protozoan parasites of domestic, wild and laboratory animals. It belongs to protozoan parasites of the genus Babesia, order Piroplasmida, phylum Apicomplexa and subclass Piroplamsia and are commonly referred to as 'piroplasmas' due to the pear-like shaped merozoites which live as small parasites inside RBC of mammals. 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. Bovine babesiosis is caused by multiple species: *Babesia bigemina*, *Babesia divergens*, *Babesia bovis*, and *Babesia major*. Two species, *B. bigemina* and *B. bovis*, have a considerable impact on cattle health and productivity in tropical and subtropical countries (Iseki *et al*., 2010).

The presence of the diseases Babesiosis is broadly related to the presence and distribution of its vectors. These diseases cause negative effects on the health of the livestock including production and productivity. *Babesia* species are intra erythrocytic protozoal organisms spread by arthropods like ticks and biting flies transplacentally and by blood transfusion. 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 (Zintl *et al*., 2003).

Worldwide, Babesia species are primarily of veterinary importance (L'Hostis *et al*., 1995) but human cases mainly reported from North America and Europe have raised the question of whether they may also be emerging human pathogens (Hildebrandt *et al*., 2013).

In Ethiopia, various surveys have been carried out on distribution, abundance and prevalence of hemoparasite species on livestock in different regions of the country by various investigations including trypanosomosis of ruminants, babesiosis in donkeys, and canine bebasiosis (Tolosa, 2010). Such investigations have been conducted in the different regions of the country like Ghibe, Asela, Wolayta, Bahirdar, Gondar, Debre Markos, Arbaminch, Tigray (Mekele), Kefa, West Gojjam, Upper Didesa valley, North Omo, Arjo, Wonago, Gore, Mizan Tefere, Debre-Zeit and so forth. However, the detailed status of the hemoparasitic diseases in ruminants particularly Bovine Babesiosis is not thoroughly studied in and around Assosa and information is so far scanty. Therefore, the objectives of this study were;

- To determine the prevalence of Bovine Babesiosis and its associated risk factors in and around Assosa Woreda.
- To laid down abaseline information on the prevalence and amplitude of bovine Babesiosis in the study area and to instigate those of future researcher.
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2. Materials and Methods

2.1. Study area

The study was conducted from November 2014 to April 2015 in Assosa woreda, Assosa zone of Benishangul Gumuz Regional State, Western Ethiopia, which is characterized by low land plane with altitude ranges of 580-1544 meter above sea level. Assosa is located between $8^0$30" and $40^0$27" N and $34^0$21" and $39^0$1" E. According to National Meteorological Service Agency (NMSA, 2007), the average annual rainfall is 1316mm with uni-modal type of rainfall that occurs between April and October. Its mean annual temperature ranges between 16.75^{0}C and 27.9^{0}C. Assosa zone of Benishangul-gumuz has 61,979 cattle, 14,253 sheep, 12,374 goats, 11,093 donkeys (CSA, 2011).

2.2. Study Population and Sample Size Determination

The study subjects were cattle of different ages and sexes which were brought to veterinary Clinics owned by each Peasant Associations (Pas). The ages, sexes, breeds 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 (2005).

$n= [1.96^2 Pexp (1-Pexp)]/d^2$

$n =1.96^2*0.5(1-0.5)/(0.05*0.05)$

$=3.8416*0.25/0.0025 =0.9604/0.0025$

n = 384. However, to increase the absolute precision 402 samples were taken throughout the study period.

Where: n= sample size

Pexp= minimum expected prevalence=50%

1.96= the value of Z at 95% confidence interval

d= desired accuracy level at 95% confidence interval

2.3. Study Design and Sampling methodology

A cross sectional survey was conducted from November, 2014 up to April, 2015 to estimate the prevalence of bovine Babesiosis and to investigate risk factors associated with the Babesiosis in and around Assosa woreda, Assosa Zone of Benishangul Gumuz Regional State. The Zone was randomly selected from the three Zones of Benishangul Gumuz Regional state based on the accessibility of veterinary health diagnostic laboratory center. The Woreda also selected randomly from seven Woredas of Assosa Zone regarding on the accessibility of laboratory center as do for the Zone. Blood samples were collected from ear vein of randomly selected 402 bovines from purposively selected Veterinary Clinics of Six peasant associations (Pas) of Assosa woreda comprising of Amba 8, Amba 12, Mengele 29, Mengele 32, Abrhamo and Barro veterinary clinic based on the cattle population density of the area, accessibility of roads and transportation systems and vegetation coverage of the focuss area.

2.4. Collection and transportation of Blood

Blood sampling was done after proper restraining of the animal according to Urquhart *et al*., (1996). For the blood collection, marginal ear vein was prepared

for disinfection with the help of methyl alcohol (5%) and the hair around the intended area was shaved with scalpel blade and a slight tearing of vein was made with lancet then the sample blood was taken with heparinized one end sealed microhaematocrit tube (capillary tube). After labeling, it was transported to Assosa Regional Veterinary Health Diagnostic Research Laboratory with ice box as soon as possible.

2.4.1. Preparation of Slides

Thin blood smear on clean and dry glass slides was prepared from the blood taken from marginal ear vein. This smear was air dried and fixed in methyl alcohol (1%) for 10 minutes and stained with working solution of Giemsa stain (1:9) ratio with phosphate buffer solution having pH 6.8 and fixed for 30 minutes. The smear was washed with tap water to remove extra stain and was air dried and Slides were examined under the oil immersion lens of a light microscopic. The parasite was identified by the characters described by Soulsby (1982).

2.4.2. Blood films examination

Giemsa staining procedures and microscopic examination of slides was conducted according to OIE, (2010). 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 (x100) of a light microscope according to (Zafar *et al.,* 2006). The parasites were identified according to the characters described by (Soulsby, 1982). The smears were recorded as negative for piroplasms (babesias) if no parasites were detected in oil-immersion fields (According to Moretti *et al*., 2010).

2.5. Body condition evaluation and estimation of age

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 (Nicholson and Butterworth, 1986). 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 (1986).

2.6. Data management and analysis

The data collected during the study period were stored in Microsoft Excel spread sheet and analyzed by the statistical software called STATA version 11 for Windows (Stata Corp. College Station, USA). The prevalence was calculated by dividing the number of cattle found to be positive for Babesia by the total number of cattle examined for Babesia spp. The association of risk factors like age, sex, body condition, peasant associations and seasons of study period for babesiosis was 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.

3. Results

From a total of 402 cattle examined, the overall prevalence was found 1.5% with 95% CI of 0.5-2.7. The highest equivalent prevalence of 2.99% (2/67) was recorded in Abrhamo, Mengele 29 and Amba 12 PAs but the disease was not found in Mengele 32, Amba 8 and Barro peasant associations that owned veterinary clinics as indicated (Table 1). Highest infection rates was observed in old cattle (3.51%) followed by adults (0.96%) and the young age group were negative for bovine babesiosis. Body condition of the animals, age, peasant associations and seasons of study period showed a significant association (P= 0.000) with positivity for babesiosis, while sex was not significantly associated (P=0.739) as shown in (Table 2). Two species of Babesia were identified with the prevalence of 1.24% (5/402) and 0.249% (1/402) for *B. bovis* and *B. bigemina* respectively (Table 3).

Table 1: Prevalence of bovine Babesiosis in six Peasant Associations

Peasant association	No of tested animals	Positive	Prevalence (%)	X^2	P-value
Mengele 32	67	0	0	3.50	0.000
Abrhamo	67	2	2.99		
Amba 8	67	0	0		
Barro	67	0	0		
Mengele 29	67	2	2.99		
Amba 12	67	2	2.99		
Total	402	6	1.5		

As shown from the above table 1, the highest equivalent prevalence (2.99%) of bovine babesiosis was compiled in Abrhamo, Mengele 29 and Amba 12 peasant associations while in the other remaining PAs the disease has not yet found throughout the study period.

Table 2: Prevalence of Babesiosis on the basis of age, sex, BCs and seasons of study period

Risk factors	No. of tested animals	Positive animal	Prevalence (%)	X^2	P-value
Age					
Young	79	0	0	4.756	0.000
Adult	209	2	0.96		
Old	114	4	3.51		
Sex					
Male	174	3	1.73	0.112	0.739
Female	228	3	1.32		
BCs(Body Conditions)					
Good	109	0	0	3.270	0.000
Medium	195	2	1.03		
Poor	98	4	4.08		
Season of study period					
Late spring	39	0	0	3.218	0.000
Winter	229	2	0.88		
Autumn	134	4	2.99		

Note: as shown from the above table 2, the potential risk factors such as age, body conditions, and seasons of study period revealed a statistical significant associations at (P= 0.000) with the occurrence of the disease while sex of the animal did not show any statistical associations (P= 0.739) with the occurrence of the disease. Animals of old aged group and those with poor body conditions were the highest affected group with 3.51% and 4.08% prevalence rate respectively. Autumn is the season of highest infection rate with 2.99% prevalence during the study period.

Table 3: Prevalence of Babesiosis on the basis of Babesia species identified

Total animals tested	Babesia spp. Identified	Positive	Prevalence (%)
402	*Babesia bovis*	5	1.243%
	Babesia bigemina	1	0.248%
	Total	6	1.5%

As indicated from the above table 3, the two species of bovine Babesiosis identified were babesia bovis and B. bigemina. Babesia bovis was more prevalent in this study than B. bigemina with known prevalence of 1.243% and 0.248% respectively.

4. Discussion

In this study, the overall prevalence rate of bovine babesiosis was found to be 1.5% out of which two species of Babesia comprising of *B. bovis* (1.24%) and *B. bigemina* (0.248%) were identified using Giemsa stained microscopic examination. This finding was lower than the earlier 42% prevalence reports from Malaysia (Rahman *et al*., 2010), 40% prevalence reports from Hazem *et al*., (2014), the prevalence of 26.6% from a cattle raised nearby forest in Salakpra Wildlife Sanctuary in Kanchanaburi province (Patcharathorn *et al*., 2013), from previous findings 16.9% by Hamsho *et al*, (2015), 9.9% from study conducted in Khyber Pakhtunkhwa, Pakistan (Ayaz *et al*., 2013) and 6.6% from Malakand Agency (Ahmad and Hashim, 2007) and Sargodha District, Pakistan (Farhan *et al*., 2012). However, this current study finding was slightly higher than the prevalence (0.6%) reported in Debre zeit, Addis Ababa University by Sitotaw *et al*., (2014).

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 (Homer *et al*., 2000; Gubbels *et al*., 1999). Other cause of variation may be due to different geographical conditions and or due to different breeds of cattle studied (Nasir *et al*., 2000).

The highest equivalent prevalence (2.99%) of bovine babesiosis was recorded in Abrhamo, Mengele 29 and Amba 12 peasant association (PA) among the study areas considered for this study but in the remaining three PAs, the disease has not yet encountered throughout the study period. The possible

explanation for this might be associated with that Abrhamo, Mengele 29 and Amba 12 PA have a wide ranging area covered with dense savannah grassland with a varieties of herbs and bushes which is believed to be conducive environment for the vector of the Babesia species but the three remaining kebeles (Mengele 32, Amba 8 and Barro) have grazing area with scattered trees of Savanah grasslands which is less likely to be inhabitant for vectors of Babesia species and those disease free study area are sited near the district where strict measures of tick control has been employed. Besides on this semi-intensive management system has been adopted in those disease free study area so that cattle are allowed either for open grazing or zero-grazing system. Therefore, cattle are experienced less exposure to vectors of Babesiosis as compared to those cattle pursued solely open grazing. This finding concurs with the study conducted in Uganda that the prevalence of infection with tick-borne parasites varied with livestock production and/or grazing system being higher in open grazing system (39.1%) compared to zero-grazing system (6.9%)Angwech *et al*., (2011).

In the present study slightly higher infection rate was recorded in male 1.72% (3/174) as compared to female animals 1.32% (3/228). Even though this difference was not statistically significant, this finding was in disagreement with the report of Kocan *et al*. (2010) who found higher prevalence of babesiosis in female 11.2% (184/1639) compared to male cattle 6.96%. 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.

In the present study the highest prevalence of babesiosis was noted among old age (3.51%) followed by adult (0.96%) but the young aged cattle were free from disease throughout the study period. This result was in line with the finding of Ayaz *et al*. (2013) 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*. (2014) 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 (Kamani *et al*., 2010).

The prevalence of the disease based on the body condition of the animals was 1.03% and 4.08% for medium and poor scoring respectively but animals with good body conditions did not show any confirmed case. Body conditions of the animal confers significant association (P = 0.000) 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 study 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.

The results of the present study showed slightly higher B. bovis (1.24%) infection rate compared to *B. bigemina* (0.248%) which coincides with the earlier prevalence 17% B. bovis and 16% B. bigemina from Malaysia as reported by Rahman *et al*. (2010). This is may be due to higher concentration of the former parasite in the capillary and veins than the latter 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 (Bock *et al*., 2004).

Respecting to the seasonal incidence of piroplasmosis (babesiosis), the seasonal prevalence of Babesiosis using blood smears examination showed that the peak of infection of Babesiosis in cattle was recorded in autumn (2.99%).These results were similar with results of Kamani *et al*,2010 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 sample collected from late spring to early autumn season. 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 (Solomon *et al*., 2003).

5. Conclusion and Recommendation

In conclusion the present findings indicated that bovine babesiosis had extremely less prevalence in the study area that might be due to low tick infestation rate and enhanced managmental practices. *B. bovis* and *B. bigemina* were identified as the species responsible for bovine babesiosis with greater prevalence of B. bovis. In order to keep it up and alleviate the existing problem and to promote the status of livestock production more feasible in these

areas, regular strategic prophylactic treatment and use of acaricides should be enhanced in order to control Babesia parasite. Microscopic examination is not suitable for detecting the carrier or chronic phases of piroplasmosis. However, it remains the most rapid confirmatory method for detecting this infection in acute phase of the disease. Furthermore, Babesiosis is one of the most important diseases in our countries because it occurs sometimes in acute forms with serious recognized clinical manifestations yet lowering the productive performance of the affected animals. Therefore, further research should be conducted to elucidate the impacts and epidemiology of bovine babesiosis using immunological methods to implement better control measure against ticks and tick borne diseases of cattle and to validate the present study.

Corresponding Author: Girma Kebede, Department of Microbiology and Public Health, School of Veterinary Medicine, Wollega University, Nekemte, Ethiopia, Telephone: 0929038290, P.O. Box: 395,E-mail: girmakebede27@yahoo.com.

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