



Prevalence of Bovine Babesiosis in Selected District of East Wollega Zone, Western Ethiopia

Authors: Ishetu Namomsa¹, Jiregna Gari^{2*}, Amare Eshetu³

Abstract

Introduction. Bovine babesiosis is a hemoparasitic disease that severely impacts cattle, leading to significant morbidity and mortality. This study aimed to determine the prevalence of bovine babesiosis and identify associated risk factors. **Methods.** A cross-sectional study was conducted in two selected districts of East Wollega, Oromia region, Ethiopia. Blood samples were collected from a purposively selected group of 384 animals (268 females and 116 males), and thin and thick smears were prepared to identify *Babesia* parasites. Anemia was assessed using packed cell volume measurements. **Results.** Bovine babesiosis prevalence was 5.2%. *Babesia bovis* infected 3.91%, and *Bovis bigemina* infected 1.30% of animals. Significant correlations ($p < 0.05$) were found between husbandry practices and previous anti-*Babesia* drug treatment. No significant associations ($p > 0.05$) were observed with age, sex, breed, or body condition. Males (6.0%) had a higher prevalence, highest in animals of more than seven years (6.1%) and mature animals (5.2%). The lowest prevalence (4.2%) occurred in young animals. Low body condition cattle (7.1%) had higher infection rates than medium (5.0%) and high (1.2%) scores. **Conclusion.** Bovine babesiosis was prevalent in the study area and poses a significant threat to cattle production overall.

Key word: babesia, bovine, east wollega, prevalence, ethiopia.

¹College of Veterinary Medicine, Haramaya University, Dromia, Ethiopia

²Department of Veterinary Microbiology, Ambo University, Dromia, Ethiopia

³Haramaya University, College of Veterinary Medicine, Dromia, Ethiopia

*<https://orcid.org/0000-0001-5363-1023>

Corresponding author:
Jiregna Gari

Address: Ambo University, College of Agriculture and Veterinary Science, Ethiopia.

E-mail: jiregnagari@gmail.com

Institutional email: jiregnagari2023@ambou.edu.et
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Introduction

Ethiopia boasts the largest livestock population in Africa, contributing significantly to the country's economy and potential for economic growth(1, 2). The livestock population in Ethiopia consists of approximately 54 million cattle, 25.5 million sheep, and 24.06 million goats(3). Most cattle (98.95%) are local breeds, while the remaining are hybrids or exotic species(4). Despite having the highest livestock population in Africa(5), animal diseases pose a major challenge, impacting output and productivity. Hemoparasitic conditions are often associated with the prevalence and transmission of their vectors(6).

Tick-transmitted intra-erythrocytic protozoan parasites known as bovine babesiosis cause of disease on cattle brought on by the protozoan parasites *Babesia bovis*, *B. bigemina*, and *B. divergens*. The primary carriers of *B. bovis* and *B. bigemina*, *Rhipicephalus* are found across tropical and subtropical regions. *Ixodes ricinus* is the main carrier of the *B. divergens*(7, 8). *Babesia* is a protozoan parasite that is a member of the phylum *Apicomplexa*, order *Piroplasmida*,

subclass *Piroplasmia*, and genus *Babesia*. Piroplasmas are so named because their pear-shaped merozoites reside as tiny parasites inside mammals' red blood cells (RBC)(9, 10). Babesiosis is a hemolytic illness that can cause a rapid development of fever, anemia, icterus, hemoglobinuria, listlessness, anorexia, jaundice, and mortality(11). The significant anemia that causes many deaths in non-immune cow herds is the most important element of hemoparasite pathogenicity(12).

Most tropical and subtropical climates, including Ethiopia, are home to the two most common species, *B. bovis* and *B. bigemina*. *Babesia bovis* is a tiny parasite that often lives in the center of the erythrocyte(13). It is typically found in pairs at an oblique angle to one another and is around 1-1.5 μ m long and 0.5-1.0 μ m broad. The considerably longer parasite *Babesia bigemina* is frequently discovered in pairs that are acutely angled to one another. *Babesia bigemina* normally has a pearshape; however, other unique variations exist. It is 3-3.5 mm length and 1-1.5 mm broad. In paired forms, each parasite frequently has two distinct redstaining spots(14).

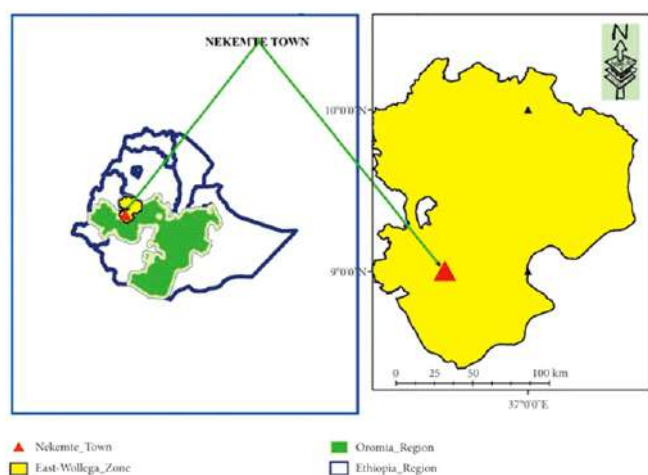
Sporozoites are introduced into the host during a tick bite and immediately infect red blood cells(15). In the host's infected erythrocyte cell, Babesia sporozoites transform into piroplasms, generating two or, rarely, four daughter cells that leave the host cell to infect additional erythrocytes(16). It has a detrimental impact on the health of the cattle as well as on their productivity and output. It has significant economic significance, particularly for cattle, because it affects adults more severely than young animals, which results in direct losses through mortality and lower output of things like meat and milk(17).

There is little data available in these study areas on the frequency of Bovine babesiosis, despite several studies being carried out across Ethiopia to assess the status and economic significance of Bovine babesiosis(18-20). This study aimed to examine the prevalence of Bovine babesiosis with its associative risk factors in the study area.

Material and Methods

Description of Study Area

This study was conducted in Guto Gida and Diga districts, located in East Wollega, Oromia, regional state of Ethiopia (Figure 1), between November 2017 and April 2018. The districts are situated at an altitude of 2,088 meters above sea level, with latitude 9° 5'N and longitude 36°33' East. Nekemte, located 331 kilometers west of Addis Ababa, is the nearest city to the study area. The climate in the region experiences a long rainy season (June to September), a short rainy season (March to April), and a dry winter season (December to February). The average daily temperatures range from 15 to 27°C, while the annual rainfall ranges from 1450 to 2150mm.



Study Design

The prevalence of bovine babesiosis and its associated risk factors were investigated using a cross-sectional research approach in selected districts of the East Wollega Zone, Oromia regional state, Ethiopia.

Sampling Methods and Sample Size Determination

Sample size determination and Sampling Technique.

The total sample size required was calculated according to the formula given by Thrusfield(21) given below, using a five percent desired absolute precision, 95% confidence interval, and 50% expected prevalence. Accordingly, the total sample size would be 384.

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

Where N = sample size, P_{exp}= expected prevalence, d = desired absolute precision (0.05). Accordingly, 384 animals were included in this study.

The study included cattle presented at the Nekemte Veterinary Clinic in the Guto Gida district and the Diga Veterinary Clinic in the Diga district. These clinics were chosen through purposive sampling, considering the number of cattle presented and their accessibility. Animals were selected from both clinics using purposive sampling techniques, taking into account clinical signs such as changes in urine color, yellowish or pale visible mucous membranes, and tick infestation. A total of 219 animals were selected from the Nekemte Veterinary Clinic, while 165 animals were selected from the Diga Veterinary Clinic.

Sample collection, transportation and laboratory investigation

Blood samples were collected from either the jugular vein or ear vein of the animals, following proper restraining techniques described by Urquhart et al.(22). Prior to blood collection, the puncture site was cleansed, hair was trimmed, and 70% alcohol was used for disinfection. Heparinized vacutainer tubes and hematocrit capillary tubes were used to collect blood samples from the jugular vein and ear vein. The samples were then transferred in ice boxes to the laboratory of Veterinary Parasitology at the College of Veterinary Medicine, Wollega University.

During the sampling process, relevant information such as the age, sex, and body condition score of the studied animals was recorded. The ages of the animals were estimated based on the information provided by the owners, following the guidelines outlined by De-lahunta and Habel (23). The animals' body condition was assessed using the method described by Nicholson and Betterworth(24).

Laboratory investigation procedures. The collected blood samples were processed to create thin and thick blood smears, which were then placed on clean, dry glass slides. Thin blood smears were air-dried and fixed in absolute methanol for 2-5 minutes, followed by staining with Giemsa for 30 minutes. After rinsing the slides with tap water to remove excess stains, they were viewed under a light microscope with an oil immersion lens. The morphological characteristics of Babesia species were identified using the key provided by Soulsby(25).

For the preparation of thick blood films, a small droplet of blood was applied to a clean glass slide, spread over a small area using the edge of another slide, air-dried, and then stained with Giemsa. Thick blood films are more sensitive for detecting *Babesia* species as they concentrate the parasites, but it can be more challenging to differentiate between different species.

To assess the level of anemia in each animal, the packed cell volume (PCV) was measured. This was done by filling a hematocrit capillary tube with blood up to three-quarters of its volume, sealing it with clay or wax, and placing it in a hematocrit centrifuge at 3000 rpm(26).

Data Management and Analysis

The collected data were accurately recorded and entered into a Microsoft Excel spreadsheet. Statistical analysis was performed using SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA) after coding the data. Descriptive statistics were used to summarize the data. Logistic regression analysis was conducted to evaluate the relationships between the risk variables (age, sex, and body condition score) and the prevalence of Bovine *Babesia* infection.

To examine differences in mean packed cell volume values among *Babesia* infection statuses, Student's t-test was applied. Analysis of variance (ANOVA) was used to compare the mean PCV values of infected animals with different *Babesia* species. A significance level of $p < 0.05$ was employed to determine the statistical significance of differences at the 95% confidence level.

Results

In this study, the prevalence of bovine babesiosis was investigated in a sample of 384 cattle. Most animals were local breeds (91.9%), and the majority were female (69.8%). Most cattle had poor body conditions, and a large proportion (86.7%) were managed under an extensive system. Among the 384 examined cattle, 20 (5.2%) were found to be infected with *Babesia*. The predominant *Babesia* species identified in the study area was *Babesia bovis* (15; 3.91%), followed by *Babesia bigemina* (5; 1.30%).

A univariable logistic regression analysis was conducted to explore the associations between age, sex, breed, body condition score, and management system categories of the cattle with the prevalence of *Babesia* (see Table 1). The prevalence of babesiosis was higher in crossbred cattle (19; 5.4%) compared to local breed cattle (1; 3.2%), but this difference was not statistically significant ($P > 0.05$) among cattle breeds. The analysis indicated that cattle with poor body condition scores were more susceptible to *Babesia* infection than animals in good condition (OR = 6.25; 95% CI: 0.74-53.1%), as shown in Table 1.

Table 1

Results of logistic regression model analysis to evaluate the odds of cattle infected by *Babesia* based on the risk factors

Risk factors		No. of animals		MLE			Odds	
		N	Positive (%)	Coef. (β)	SE	P	OR	95% CI
BCS	Good [†]	80	1(1.2)	-	-	-	1	-
	Medium	120	6(5)	1.46	1.1	0.2	4.29	0.46-40.3
	Poor	184	13(7.1)	1.83	1.1	0.1	6.25	0.74-53.1
Management	Intensive [†]	32	1(3.1)	-	-	-	1	-
	Semi intensive	19	4(21.1)	3.37	1.4	0	29	1.81-46.4
Breed	Local [†]	31	1(3.2)	-	-	-	1	-
	Cross	353	19(5.4)	1.09	1.2	0.4	2.97	0.27-32.7
Age	Young [†]	96	4 (4.2)	-	-	-	1	-
	Adult	174	9 (5.2)	0.05	0.7	0.9	1.06	0.28-3.95
	Old	114	7(6.1)	0.51	0.7	0.5	1.66	0.42-6.64
Sex	Female [†]	268	13 (4.9)	-	-	-	1	-
	Male	116	7 (6)	0.34	0.5	0.5	1.41	0.49-4.03
Constant				-3.29	0.4	0		

N, Number examined; BCS, Body condition score; †, Reference category; MLE, Maximum Likelihood Estimate; SE, Standard Error; OR, odds ratio; CI, confidence interval

PCV Results

This study's overall mean PCV of the sampled animals was $24.6\% \pm 1.8$. The mean PCV values between *Babesia*-infected and uninfected cattle were compared using a student's t-test (refer to Table 2). The analysis revealed a significant difference ($P < 0.05$) in the mean PCV values between the two groups. The mean PCV value of *Babesia*-infected cattle ($22.3\% \pm 1.3$) was significantly lower ($P < 0.05$) than that of uninfected animals ($24.7\% \pm 1.7$), as shown in Table 2.

Table 2

The mean PCV value among the examined animals

Infection status	No. examined	Mean PCV \pm SD	t test	P value
Parasitemic	364	22.3 ± 1.3	6.2	0
Aparasitemic	20	24.7 ± 1.7		
Total	384	24.6 ± 1.8		

Discussion

The prevalence of bovine babesiosis in the selected district of East Wollega was 5.2%, which is markedly lower than the earlier findings of 12.8% (50/390) reported from Jimma, Southwestern Ethiopia (27), and 16.9% (65/384) reported from Teltele district, northwest Borana zone, southern Ethiopia (28). On the other hand, it is higher than the report of Wodajnew et al. (29) and Sitotaw et al. (30), who reported a prevalence of 1.5% (6/402) and 0.9% in Benishangul Gumuz of Assosa, Western Ethiopia, and at Bishoftu, respectively. This difference in the prevalence of bovine babesiosis might be caused by various factors, including animal husbandry practices, anti-parasitic drug use for vector control, parasite variation in carriers of the disease over time, test sensitivity, distribution of vectors, and interaction of wildlife sanctuaries

with animals and forests where Babesia vectors are present (31).

This study shows that the prevalence of Babesia infection is greater in animals with poor body condition scores than in animals with medium body condition and good body condition scores, with no statistically significant difference ($P>0.05$). This finding is in agreement with the earlier study by Kamani et al. (32). The present study revealed that the prevalence of Babesia infection was higher in males (6.0% or 7/116) than in females (4.9% or 13/268) cattle, but the difference was not statistically significant ($P>0.05$). This result agrees with findings by Fakhar et al. (33), Choramo and Ibrahim (27), and Wodajnew et al. (29), who noted a higher prevalence in male cattle than in females. Additionally, a higher prevalence of hemoparasitic disease in male animals may be related to the stress these animals experience at work, suppressing their immune systems (29).

According to a current investigation, the prevalence of bovine babesiosis varies depending on the animal's age, resulting in 6.1% (7/144) in old animals, 5.2% (9/174) in adults, and 4.2% (4/96) in young animals, with no statistically significant variation ($P>0.05$). These results align with the findings of Ayaz et al. (34) from Pakistan. Furthermore, these current findings are in agreement with the findings of Choramo and Ibrahim (27), who reported prevalence rates of 12.4% (28/226) in old animals, 14.2% (19/133) in adults, and 10% (3/30) in young animals in Jimma. However, these findings disagree with the report of Amorim et al. (35), who reported that calves were more susceptible to Babesia species compared to adult cattle. These variations may exist because young animals experience tick infection at a lower incidence than older animals. Conversely, restricted grazing of young animals, which tends to lessen their probability of interaction with the vectors of the disease (30), and passive immunity acquired from their mothers through colostrum (27), are thought to be the causes of the reduced incidence in young animals.

In the current study, packed cell volume (PCV) was measured for individual animals to determine the level of anemia and to compare the mean PCV of anemic (22.3 ± 1.3) and non-anemic (24.7 ± 1.7) animals ($P<0.05$). This variation in PCV might be due to the parasite's effect on blood through the hemolysis of red blood cells (36).

Conclusion

The study revealed a total prevalence of bovine babesiosis of 5.2% in the study area. This highlights the significant economic losses of bovine babesiosis, including poor weight gain, reduced productivity, treatment costs, and animal deaths. The prevalence of bovine babesiosis was found to increase with the age of the animals, and variables such as husbandry systems and the history of anti-Babesia drug treatment in the animals were significantly associated with the prevalence of the disease. Therefore, it is crucial to implement routine prevention measures for Babesia in the study area, and further comprehensive studies are warranted

to gather complete data on the epidemiology of babesiosis infections and their economic impact in the area.

Author Contribution Statement

Study conception and design: IN; data collection: JG; analysis and interpretation of results: IN, JG and AE; draft manuscript preparation: IN and JG. All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

Ethics statement

The authors declare that the published work reflects an investigation and analysis carried out truthfully and completely. This research received permission by the Haramaya University College of Veterinary Medicine: Haramaya University Ethical committee, Haramaya University, HRU2017C4, October 2017. The district's veterinary clinics received the official letter. It was agreed upon that samples would be taken from animals after the purpose of the research had been made clear.

Conflict of Interest

It is stated by the authors that they have no competing interests.

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None.

Availability of data

The corresponding author will provide the datasets used and/or analyzed during the current work upon reasonable request.

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