

Prevalence and Risk Factors of Bovine Babesiosis in Kiltu Kara District, West Wallaga Zone, Oromia Regional State, Ethiopia

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Abstract

Babesiosis is an important protozoan parasite that causes infections in humans as well as in animals. A cross-sectional study was conducted from November 2020 to April 2021 in Kiltu Kara district, West Wallaga Zone, West Ethiopia. Blood samples were collected from 384 cattle and the prevalence of *Babesia* species was determined using Giemsa-stained blood smears. Statistical analyses were performed to evaluate the relationship between infection rates and factors, such as age, sex, body condition and tick infestation. The overall prevalence of bovine babesiosis was 18.5% (71/384), with higher rates observed in adult (20.42%) and old (33.33%) cattle compared to young animals (8.39%). Female cattle had a higher prevalence (19.9%) than males (16.94%). A significant association was found between tick infestation (27.39% infected) and *Babesia* infection ($\chi^2 = 26.83$, $P < 0.05$). Two species were identified: *Babesia bovis* (10.68%) and *Babesia bigemina* (7.81%). The study highlights a notable prevalence of bovine babesiosis in the Kiltu Kara district, with older and tick-infested cattle being more susceptible to infection. These findings underscore the need for enhanced surveillance, effective tick control measures and further research to mitigate the impact of this disease on livestock productivity in the region.

Keywords: Bovine babesiosis, Cattle, Cross-sectional study, Risk factors

Introduction:

Babesiosis is a globally distributed protozoan disease affecting many mammal species, significantly impacting cattle and humans (Pal, 2007). The disease in human was first time recorded in Yugoslavia in the year 1957 (Pal, 2007). The recognition of babesiosis as a public health issue is increasing worldwide (Schorn et al., 2011; Zanet et al., 2014). *Babesia* species cause babesiosis, protozoan parasites primarily transmitted by ticks, which infect a wide range of domestic and wild animals (Pal, 2007; Duh et al., 2008; Silva et al., 2010). After trypanosomes, *Babesia* ranks as the second most common blood parasite in mammals (Yabsley and Shock, 2013). The disease causes substantial economic losses, including direct impacts like mortality and reductions in meat and milk production, as well as indirect costs due to tick control measures (Gharbi et al., 2011; Shahnawaz et al., 2011). Clinically, babesiosis is marked by symptoms, such as anemia, icterus, hemoglobinuria and often death (Vial and Gorenflot, 2006; Pal, 2007). In cattle, the primary species responsible for bovine babesiosis are *B. bigemina*, *B. divergens*, *B. bovis* and *B. major*, with *B. bigemina* and *B. bovis* posing the most significant threats to cattle health and productivity in tropical and subtropical regions (Iseki et al., 2010).

Subclinical babesiosis and theileriosis lead to the conversion of the affected livestock to chronic carriers and, in turn, sources of infection for tick vectors and cause natural transmission of the disease. Early detection of blood parasites is highly beneficial in control. Microscopy using Giemsa-stained blood smears has been considered the “gold standard” for detecting *Babesia* (Böse et al., 1995; Nayel et al., 2012). Host factors associated with disease include breed, age and immune status of the animals (Jabbar et al., 2015).

Different breeds of animals are not equally affected by bovine babesiosis. *Bos indicus* breeds of cattle are much more resistant to babesiosis than *Bos taurus* breeds. This phenomenon is thought to be a result of the evolutionary relationship between *Bos indicus* cattle, *Boophilus* spp. and *Babesia* (Bock et al., 2004). Previous studies conducted on the distribution, abundance and prevalence of bovine babesiosis affecting cattle in different parts of Ethiopia has shown widespread existence (Hamsho et al., 2015; Lemma et al., 2016). However, the current status of bovine babesiosis has not been thoroughly studied in our Wollega area, particularly the Kiltu Kara District of West Wallaga Zone, Western Oromia Regional State, West Ethiopia, which is scanty.

Therefore, the objectives of this study were to estimate the prevalence of babesiosis in cattle, to estimate the possible correlation between age, sex, body condition and presence or absence of tick infestation and to estimate the infection rate for the purpose of prophylaxis and effective control of the disease.

Materials and Methods:

Study area:

The study was conducted from November 2020 to April 2021 in and around Kiltu Kara district, West Wallaga Zone of Oromia regional state, West Ethiopia. Kiltu Kara is located in the Oromia region of the West Wallaga zone. The district is located 550 km away from Addis Ababa. The district is bounded by Leta Sibu district at the eastern, Mana Sibu district at the western, Babo Gambel district at the northern and B/Gumuz at the southern. The human population of the district is 67566. It receives 900mm to 1500 mm of annual rain on average. While the maximum and minimum temperatures are 240°C and 170°C, respectively.

The livestock population of the district was estimated to be 47,193 cattle, 15,649 sheep, 16,850 goats, 44 mules, 9,213 donkeys and 84,460 avians (CSA, 2017).

Study population and sample size determination:

The study subjects were cattle of different ages and sexes, which were owned by each peasant association (PA). The ages, sexes, presence or absence of ticks and body condition scores were recorded. Taking an estimated prevalence of 50%, the minimum sample size at a 95% confidence interval and a 5% precision or accuracy level, 384 samples were taken throughout the study period by using the formula given by (Thursfield, 2007).

$$n = \frac{1.96^2 \text{Pexp} (1 - \text{Pexp})}{d^2}$$

$$n = \frac{1.96^2 * 0.5(1 - 0.5)}{(0.05 * 0.05)} = \frac{3.8416 * 0.25}{0.0025} = 0.9604 / 0.0025; n = 384.$$

Where: n= sample size

Pexp= minimum expected prevalence=50%

1.96= the value of Z at a 95% confidence interval

d= desired accuracy level at 95% confidence interval

Study Design and Sampling methodology:

A cross-sectional study was conducted from November 2020 up to April 2021 to estimate the prevalence of bovine babesiosis and to investigate risk factors associated with babesiosis in cattle at Kiltu Kara district, West Wallaga Zone of Oromia Regional State, West Ethiopia. Blood samples were collected from the ear

veins of randomly selected 384 bovines from purposively selected four peasant associations (PAs) based on the cattle population density of the area, accessibility of roads and transportation systems.

Collection and transportation of blood

Blood sampling was carried out following proper animal restraint. The marginal ear vein was disinfected using 5% methyl alcohol and the hair around the collection site was shaved with a scalpel blade. A slight incision was made in the vein using a lancet and blood samples were collected in heparinized sealed micro haematocrit (capillary) tubes. After labeling, the samples were promptly transported to the Kiltu Kara Woreda Veterinary Laboratory in an icebox to ensure proper preservation.

Preparation of Slides

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

Blood slide examination

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

Body condition evaluation and estimation of age

During sample collection, the body condition of the animals was evaluated subjectively. Based on the assessment of various anatomical parts and the coverage of flesh and fat, animals were categorized into three body condition scores: emaciated (poor), moderate (medium) and good. Additionally, the animals were classified into age categories using a combination of dental and horn evaluation and information provided by the owners. The age categories were young (≤ 3 years), adult (4-6 years) and old (≥ 7 years).

Data management and analysis

The data collected during the study period were stored in a Microsoft Excel spreadsheet and analyzed using Statistical package for social science (SPSS) version 20. The prevalence of *Babesia* infection was determined by dividing the number of positive cattle by the total number of cattle examined for *Babesia* spp. To assess the association between risk factors, such as age, sex, body condition, peasant associations and tick infestation and the prevalence of *Babesia*, a Chi-square (χ^2) test was employed. Statistical significance was set at $P < 0.05$, with a 95% confidence interval (CI) used in all analyses.

Results and Discussion:

Out of 384 cattle examined, 71 (18.5%) were found to be infected with *Babesia*, with a 95% confidence interval (CI) of 14.9% to 22.7%. The prevalence of babesiosis was higher in female animals (19.9%) compared to males (16.94%). In terms of age, the infection rate was 8.39% in young animals, 20.42% in adults and 33.33% in old animals (Table 1). A significant difference ($P < 0.05$) was observed between age groups, with older animals being more susceptible to infection than younger and adult animals.

The data analysis conducted during the study period showed no significant variation ($\chi^2 = 2.68$, $P > 0.05$)

between animals from different villages. Despite of that, the highest prevalence of bovine babesiosis was compiled in Wato dalle, followed by Dandi gudi, Guyo jirma and Sarbi peasant associations throughout the study period (Table 2).

Out of 384 cattle, 219 had tick infestations. Among these, 60 (27.39%) were infected with *Babesia*. In contrast, only 11 (6.66%) of the 165 cattle without tick infestations tested positive for *Babesia*. A statistically significant relationship was identified between tick presence and *Babesia* infection ($\chi^2 = 26.83$, $P < 0.05$). The study also examined the body condition of the cattle with *Babesia* infection. Of the 149 cattle classified as having poor body condition, 33 (22.15%) were infected. The prevalence among cattle with medium body condition (115 animals) was 22.61%, while only 10% of those with good body condition (120 animals) were infected. A significant association was also observed between body condition and *Babesia* infection ($\chi^2 = 8.35$, $P < 0.05$) Table 3.

Table 4 summarizes the identification of *Babesia* species in the studied cattle population. The following key findings are reported: Two species of bovine *Babesia* were identified: *B. bovis* and *B. bigemina*. *B. bovis* showed a higher prevalence at 10.68% compared to *B. bigemina*, which had a prevalence of 7.81%.

Table 1: Prevalence of babesiosis in cattle, stratified by sex and age

Categories	No.of animal examined	No.Positives	Prevalence (95% CI)	X ²	P-value
Sex					
Female	201	40	19.9 (15.41-26.50)	0.55	0.45
Male	183	31	16.94 (12.2- 23.1)		
Age					
Young	155	13	8.39 (6.7-18.64)	23.56	0.000
Adult	142	29	20.42(14.61-27.79)		
Old	87	29	33.33 (24.32-43.75)		

Table 2: prevalence of bovine babesiosis in four peasant associations

Peasant association	No.animal examined	Positive	Prevalence (95C %)	X ²	P-value
Wato dalle	101	22	21.78 (14.8- 30.8)	2.638	0.451
Sarbi	102	14	13.72 (8.3- 21.7)		
Dandi gudi	91	19	20.88 (13.8- 30.5)		
Guyo jirma	90	16	17.78 (11.2- 26.9)		

Table 3: Prevalence of *Babesia* in relation with animal body condition and tick infestation

Category	No.of animals examined	Positives	Prevalence (95 CI%)	X ²	P-value
Body condition					
Poor	149	33	22.15	8.356	0.015
Medium	115	26	22.61		
Good	120	12	10		
Tick infestation Status					
Yes	219	60	27.397	26.834	0.000
No	165	11	6.667		

In this study, the overall prevalence rate of bovine babesiosis was found to be 18.5%. This result was nearly similar to the results obtained by (Hamsho et al., 2015), who recorded that 16.9% of the cattle were infected with *Babesia* in the Teltele district of the Borena zone. However, the overall prevalence obtained in this study is very low as compared to the recent reports of (Lemma et al., 2016) in Jimma (23%), South Western Ethiopia and elsewhere in the world, such as the reports of Rahman and others (2010) (42%), from Malaysia; and Simking and co-investigators (2014) reported a prevalence of 46.7% from cattle raised nearby in a forest in Salakpra Wildlife Sanctuary in Kanchanaburi province in Thailand. On the contrary, the overall prevalence recorded in this study was slightly higher than the prevalence reported at Debre Zeit (0.6%) by Sitotaw and others (2014); 9.9% from a study conducted in Khyber Pakhtunkhwa, Pakistan (Ayaz et al., 2013); 6.6% from the Malakand Agency (Ahmad and Hashim, 2007) and Sargodha District, Pakistan (Atif et al., 2012); and 1.5% that was reported in Assosa Woreda, Western Ethiopia, by Wodajnew and co-investigators (2015).

The variation in the prevalence of bovine babesiosis might be due to different factors like management condition of the focus area, use of acaricides during tick infestation, farming system and proper use of ant parasitic drugs, fluctuations of parasites during the chronic course of the disease and in carriers animals, sensitivity of tests used, distribution of infected vectors and accessibility of animals to wildlife sanctuary and parks and forest areas harboring the *Babesia* vectors (Calder et al., 1996; Gubbels et al., 1999; Homer et al., 2000). Other causes of variation may be due to different geographical conditions or to different breeds of cattle studied.

The prevalence of babesiosis in different villages in this study was not statistically significant. The possible explanation for this might be due to their location in the same agroecology. But the slight difference in prevalence might be due to management differences like tick control.

Also, these villages (PAs) are located at a different distance from the Woreda clinic, which might be the reason why there is slight variation in the infection rate of *Babesia*. In the present study, a slightly higher infection rate was recorded in females 19.9% (40/201) as compared to male animals 16.94% (31/183) with no statistically significant variation, which is in agreement with the findings of Kocan and co-investigators (2010).

The slightly higher prevalence of babesiosis recorded for female animals may be; moreover, the higher prevalence of tick-borne diseases in female animals may be due to the fact that female animals are kept longer for breeding and milk production purposes (Kamani et al., 2010). On the other hand, higher prevalence in female animals might be due to hormonal disturbances due to its use in milk production and breeding system, which lowers the immune system of the animal.

In the present study, the highest prevalence of babesiosis was noted among the elderly (33.33%), followed by adults (20.42%), but the lowest (8.39%) was found in young animals 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. In addition, this study agreed with the findings of Lemma and others (2016) from Jimma Zone of Ethiopia, who reported a high prevalence in old animals (27%), followed by adult animals (21.7%) and 20.7% young animals.

However, the results of this study disagree with the observation of Amorim and co-investigators (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 is attributed to restrict grazing of young animals, which is likely to reduce their chance of contact with vectors of these diseases (Kamani et al., 2010). In the current study, the prevalence of the disease based on the body condition of the animals was 22.61% and 22.15% for medium and poor scoring, respectively.

The body conditions of the animal confer statistically significant association ($p < 0.05$) for the occurrence of babesiosis. The animals with good body conditions were slightly affected when compared to those of medium and poor. This could be due to the fact that animals with poor and medium body conditions have lower immunity, which encourages infection of animals by different organisms like *Babesia*. The prevalence of the disease based on the presence of ticks was statistically significant ($p < 0.05$), those cattle having ticks have 27.397%, while those that do not have tick have 6.667%, which agrees with that reported from Jimma, 41% of those cattle having ticks and 2.6% of those that do not have ticks (Lemma et al., 2016). This shows a strong association between *Babesia* infection and tick vector, which supports that the tick is responsible for the transmission of the parasite.

Respecting *Babesia* species, the present study showed a slightly higher *B. bovis* (10.68%) infection rate compared to *B. bigemina* (7.81%), which coincides with the earlier prevalence of 17% *B. bovis* and 16% *B. bigemina* from Malaysia as reported by Rahman and co-investigators (2010) and 1.24% *B. bovis* and 0.248% *B. bigemina* from Assosa Woreda, Western Ethiopia, by (Wodajnew et al., 2015).

This may be due to a higher concentration of the former parasite in the capillary and veins than the latter parasite, which is 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).

Conclusion:

This study demonstrated an 18.5% prevalence of bovine babesiosis among cattle in the Kiltu Kara district of the West Wallaga Zone, Oromia Regional State, Ethiopia. The findings indicated that older cattle exhibited significantly higher infection rates compared to younger and adult animals. The results highlight the need for increased awareness and monitoring of bovine babesiosis within the region, particularly in relation to the factors that contribute to its transmission and impact on cattle health.

- ✓ Regular surveillance programs should be implemented to monitor the prevalence of bovine

babesiosis and other tick-borne diseases in cattle populations.

- ✓ Local farmers and veterinary professionals should receive training on the signs of bovine babesiosis, the importance of regular tick control measures and best practices for managing cattle health.
- ✓ Integrated pest management strategies, including the use of acaricides, biological control agents and improved husbandry practices, should be promoted to reduce tick populations and consequently the risk of *Babesia* transmission.

Conflict of interest:

There was no conflict of interest among the authors.

Contribution of authors:

All the authors contributed equally in the preparation of the manuscript.

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