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# PREVALENCE AND ASSOCIATED RISK FACTORS OF BABESIOSIS IN CATTLE IN LAY ARMACHIHO DISTRICTS OF AMHARA REGION, ETHIOPIA

Desalegn Zemene[1], Adem Beyan[2], Nigussie Sema[2]

[1,2]Livestock Resources and Development Office Lay Armachiho, Ethiopia.
Email: desalegnzemene2008@gmail.com

**ABSTRACT:** Tick borne hemoparasites are causing devastating losses to the livestock industry and thus pose major constraints to the livestock production throughout the world. A cross-sectional study using simple random sampling was conducted from November 2022 to September 2023 in Lay Armachiho districts of Amhara region of Ethiopia with the objectives to estimate prevalence and associated risk factors of bovine babesiosis in cattle in Lay Armachiho districts. A total of 402 blood samples were collected from randomly selected cattle to assess the presence of babesia species by using thin smear technique in the study districts. The overall prevalence of bovine Babesiosis was found to be 5.73%. In this study, Babesia bigemina (3.73%) and Babesia bovis (2%) were encountered. The highest prevalence of bovine babesiosis was found in Jiha and Addisgie kebele (9%) and this difference was statistically non significant (P=0.342 and 0.268) rspectively. According to multivariable logistic regression analysis, Body condition score, agro ecology, communal grazing land health status were identified as potential risk factors. In conclusion, currently low awareness or knowledge of the livestock owners about the diseases transmitted by ticks could be attributed to a lack of treatments and shortfall of control strategies in animals and resulting in significant economic loss and increases occurrence of the diseases. In order to minimize losses attributed to bovine babesiosis in the area strategic tick control techniques should be implemented, as it is a level of control that prevents ticks from becoming a nuisance.
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**Key words**: babesiosis, cattle, Lay Armachiho, Risk Factors, Prevalence

## 1 INTRODUCTION

### 1.1 Background

Tick borne hemoparasites are causing devastating losses to the livestock industry and thus pose major constraints to the livestock production throughout the world (Khankhawash, 2018). The infection is mainly transmitted by arthropod vectors, or through blood transfusion (Salih *et al*., 2015).

Tick-borne hemoparasites are growing steadily due to establishment of the tick vector and the TBD including anaplasmosis, babesiosis and theileriosis reduce livestock production in endemic areas (Warsame et al., 2022, Narladkar, 2018). The country's environmental condition and vegetation are highly conducive for ticks and tick-borne disease perpetuation. The presence of diseases caused by hemoparasite is broadly related to the presence and distribution of their vectors.

Ticks are more prevalent in the warmer climates, especially in tropical and sub-tropical areas (Adugna and Tamrat, 2022). Tick borne hemoparasite have a serious economic impact on livestock sector due to decreased productivity, lowered working efficiency, increased cost for control measures and limiting introduction of genetically improved cattle in the area and death of livestock (Bhatnagar *et al*., 2015).

Bovine babesiosis is a disease that commonly infects cattle, sheep, goats, horses, pigs, dogs and cats and occasionally man. Babesia bovis and B. bigemina are the main species affecting cattle widely distributed in tropical and subtropical countries which are responsible for high mortality rates up to 50% in susceptible herds and it's known to be transmitted in this country by Rhipicephalus (Mohammed and Elshahawy, 2020).

Animals suffering from acute babesiosis shows symptoms such as fever, oculo-nasal discharge, increased heart rate, increased respiratory rate, abnormal mucous membrane color, and low packed cell volume (PCV) values. Although these symptoms are very typical, they are not path gnomonic and animals with chronic infections can be asymptomatic carriers (Abdela et al., 2018).

Disease signs vary in severity from silent infection to acute circulatory shock with anemia, depending on susceptibility, immunity, and age of the host and Babesia species and parasite load and bovine Babesia is principally maintained by sub-clinically infected cattle that have recovered from disease (Disassa et al., 2015).

The importance of ticks is principally due to the ability to transmit a wide spectrum of pathogenic microorganisms, such as protozoa, Rickettsial, spirochetes, and viruses (Jongejan and Uilenberg, 1994). The main effect of tick infestation in cattle is mild to severe anemia, loss of appetite, leading to a reduction in growth rate and decreased productivity and tick infestation also results in increased calf mortality (Mohsen et al., 2013). The seasonal variations within a bioclimatic zone may favour or hinder the development or activity of a tick species during certain periods (Getahun et al., 2016). Dry environmental conditions are a serious danger to ticks, particularly to the questing larvae, which are very susceptible to drying out fatally (Adugna and Tamrat, 2022).

Although quite a lot of similar studies on bovine babesiosis in cattle have been conducted in different areas of Ethiopia, it is worth nothing that Ethiopia is a large country with a huge number of livestock populations, mostly cattle. Information regarding bovine babesiosis in cattle in the study area is scarce. A study is required in the area to generate baseline information on bovine babesiosis for developing disease control and prevention programs. Therefore, the objectives of this study were to quantify the Epidemiology of bovine babesiosis of cattle in the study area.

## 3 MATERIALS AND METHODS

### 3.1 Study Area

The study was conducted in Lay Armachiho districts in Central Gondar administrative zone in Amhara regional state, North western Ethiopia from November 2022 to June 2023. Lay Armachiho district is found in Central Gondar zone with an area of 1,059.33 square kilometers. It is located at a latitude of 13° north and longitude of 37° 10′ 0.1” east at an elevation of 1730 meters. It is located 749 kilo-meters away from Addis Ababa, the capital city of the country, and 207 kilometers away from Bahir Dar, the capital city of the region (Demeke et al., 2020).

The administrative center of the district is Tekle Dingay, with 29 rural and two town administrative units/kebeles. The livestock populations of the district were 483522 (cattle 172,733, sheep 40917, goat 72247, horse 330, mule 295, donkey 20219 and poultry 197000). While cattle serve as sources of drought power and milk, small ruminants (sheep and goats) are important cash sources. Pack animals (donkeys, horses and mules) are major means of transportation (LALRDO, 2023). The altitude of the district ranges from 1500 - 2800 meters above sea level (m.a.s.l), with climatic zone of 7 % highland, 61 % midland and 32 % lowland with a temperature ranges from 23 - 25°C.

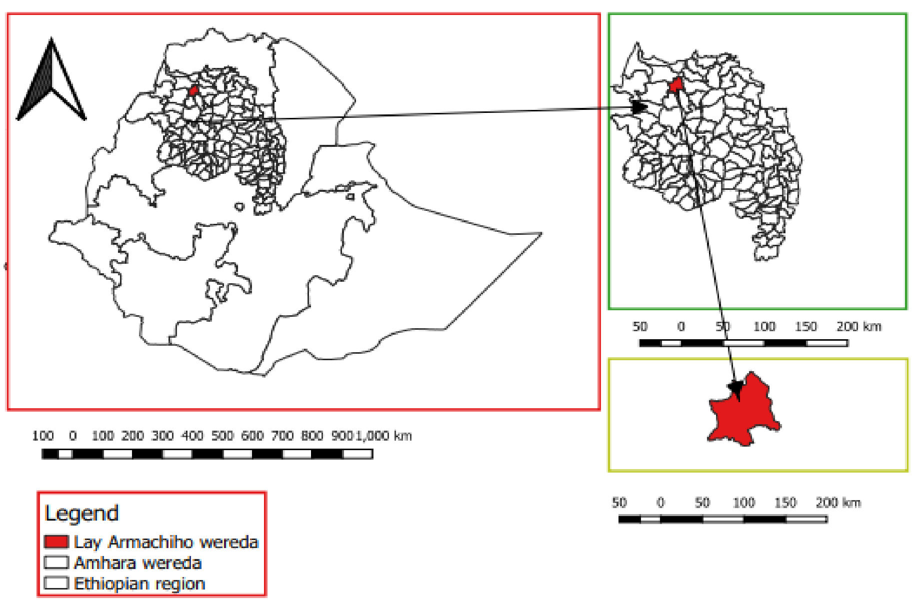


Figure 1**: Maps of the study area**

**3.2 Study Design**

A cross-sectional study was conducted from November 2022 to June 2023 to estimate the prevalence of bovine babesiosis, to identify associated risk factors and to estimate the packed cell volumes in Lay Armachiho district in cattle.

**3.4 Sampling Method and Sample Size Determination**

The study districts were selected purposively based on their livestock population, agro ecology representation and accessibility. Simple random sampling techniques were used to select study kebeles, villages and animals.
Sample size was calculated according to the formula given by Thrusfield, (2007) with 95% confidence level and 5% absolute precision.

$$n=\frac{1.96^2\ (Pexp\ (1-Pexp)}{d^2} = 384$$

Where, n = required sample size
Pexp = expected prevalence
d = desired absolute precision
However, to increase the absolute precision, 402 samples were taken throughout the study period.

**3.5 Study Population**

The study will be conducted on local and cross breed cattle of different age, sex and body score condition (BSC) reared under different farming system. Body condition scores of each cattle will be evaluated during sample collection and the cattle will be 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) (Annex 1). Animals were conveniently classified as young (<3 years) and Adult (>3 years) age categories as described by Delahunta and Habel (1986).

**3.6 Sample Collection and Examination**

3.6.1 Blood sample collection and laboratory analysis

A total of 402 blood samples will be collected from ear vein of simple randomly selected cattle from Lay Armachiho districts following the standard protocol described by (Urquhart *et al*., 1996). Briefly, after proper restraining of the animal, ear vein will be disinfected with alcohol (70%) and the hair around the intended area will be shaved with scalpel blade followed by a slight tearing of the vein with sterile, pyrogen free and non toxic disposable blood collection needle. Take drop of blood on a grease free clean slide and spread the blood by another clean slide at angle of $45^0$ then dry it quickly and labeled. After labeling, it will be transported to University of Gondar, Veterinary laboratory room to fix with methyl alcohol for 2 minutes, dry and stain the slide with 0.76% Giemsa for 30 minutes. After staining wash the slide with distilled water till it assumes a bluish purple color. Finally allow it to dry by standing upright on rack and examine under the microscope (X100).

3.6.2 Blood sample examination

**Thin blood smear**: A thin blood smear will be prepared by taking drops of blood and placed on frosted microscopic slides and spread by using other clean slides at an angle of 45°, air dried and fixed with methyl alcohol for 2 minute. Giemsa staining procedures and microscopic examination of the slides will be conducted according to OIE (2010). The slides will be immersed in 0.76% Giemsa stain solution for 30 minutes according to (Zafar *et al*., 2006). The excess Giemsa solution willl be drained and washed using distilled water, allowed to dry by standing up right on the rack and examined under the microscope with oil immersion objective lens (Hendrix and Robinson, 2006). Fields from each stained slides will be examined for identification of blood parasite at genus and species level. (Urquart *et al*., 1996).

**3.8 Data Analysis**

The collected data was entered into Microsoft Excel, coded and summarized using descriptive statistics. The prevalence was calculated for all data by dividing positive samples over the total number of examined samples and multiplied by hundred. All statistical analyses was done using Stata 17 statistical software.

Kebeles, breed, sex, age category, body condition score, agroecology, tick infestation, health status, tick season occurrence, packed cell volumes, communal grazing land and communal watering point were the predictor variables where associations were examined. Univariable logistic regression was used to assess if there is a statistically significant association between the occurrence of bovine babesiosis and potential risk factors. Statistical significance was considered to exist if p-value less than or equal 0.25. Correlation, confounding and interaction tests was checked. In the multivariable mixed effect logistic regression, P-value $\leq$ 0.05 was considered as cut off for statistical significance and odds ratio (OR) and 95% CI were also calculated.

## 4. RESULTS

### 4.1 Prevalence of bovine babesiosis at species level

The present findings indicated that bovine babesiosis had 5.73% of prevalence in the study area and Babesia bovis and Babesia bigemina were identified and the greater prevalence of Babesia bigemina was identified. Out of the total positive cattle 2% and 3.73% animals were infected with a species of Babesia bovis and Babesia bigemina respectively.

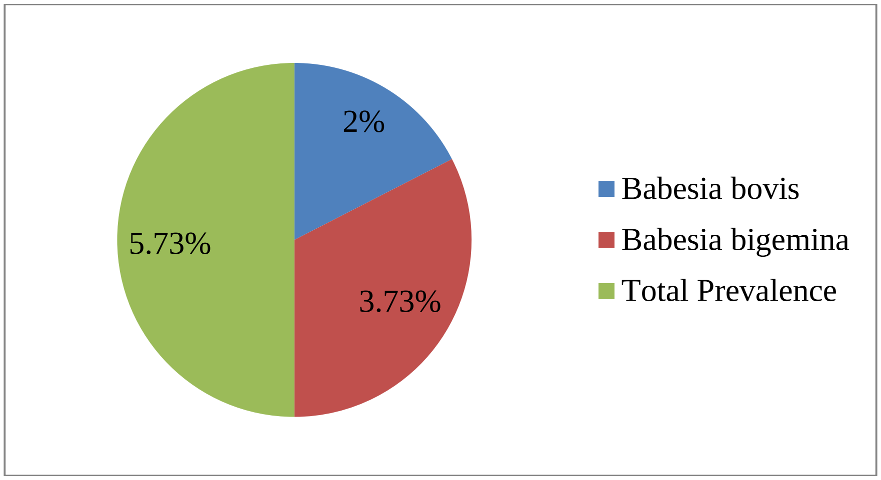


**Figure 2**: Prevalence of hemoparasites at species level

### 4.2 Prevalence of bovine babesiosis at kebele level

A total of 402 blood samples were collected from ear vein and examined using a thin blood smear and an overall Prevalence of bovine babesiosis 23(5.73%) was recorded at 95% confidence interval in the study areas. Out of the total animals exposed to bovine babesiosis 3(0.75%), 5(1.24%), 6(1.5%), 1(0.25%), 2(0.5%) and 6(1.5%) were from Kerker, Shumara, Jiha, Chira, Endivina and Addisgie kebele respectively.

Table1: Prevalence of tick borne hemoparasite at kebele level in cattle.

| Variables | Categories | Number examined (%) | Babesia bovis | Babesia bigemina |
|---|---|---|---|---|
| | Kerker | 67(4.48%) | 1(1.5%) | 2(3%) |
| kebele | Shumara | 67(7.46%) | 1(1.5%) | 4(6%) |
| | Jiha | 67(9%) | 2(3%) | 4(6%) |
| | Chira | 67(2%) | 0(0%) | 1(1.5%) |
| | Endivina | 67(4 %) | 0(0%) | 2(3%) |
| | Addisgie | 67(9%) | 4(6%) | 2(3%) |
| **Overall prevalence** | | 402(5.73%) | 8(2%) | 15(3.73%) |

### 4.3 Risk Factors for bovine babesiosis in cattle

In univariable logistic regression, the risk factors such as kebeles, breed, sex, age, body condition score; season, health status, tick infestation and communal grazing land were analyzed. Among these factors breed, sex, age, body condition, tick infestation, health status and communal grazing land were found to be significantly associated with positivity for bovine babesiosis infection (P<0.25) effects on the occurrence of these infections. However, the factors considered in the initial univariable logistic regression analysis only kebele and season were removed for multivariable logistic analysis in which p-value greater than 0.25.

Table 2: Univariable logistic regression analysis of risk factors associated with bovine babesiosis

| Variables | Categories | Number examined | Number positive (%) | OR (95%CI) | P-value |
|---|---|---|---|---|---|
| | Kerker | 67 | 3(4.48%) | Ref. | |
| | Shumara | 67 | 5(7.46%) | 1.72(0.39 - 7.51) | 0.47 |
| Kebeles | Jiha | 67 | 6(9%) | 2 (0.48 - 8.34) | 0.342 |
| | Chira | 67 | 1(1.5%) | 0.32(0.32 - 3.14) | 0.327 |
| | Endivina | 67 | 2(3%) | 0.66(0.11 - 4.06) | 0.651 |
| | Addisgie | 67 | 6(9%) | 2.25(0.54 – 9.40) | 0.268 |
| Breed | Cross | 132 | 3(2.27%) | Ref. | |
| | Local | 270 | 20(7.40%) | 3.44(1.00 -11.79) | 0.049 |
| Sex | Female | 289 | 13(4.45%) | Ref. | |
| | Male | 113 | 10(8.85%) | 1.81(0.479 - 4.28) | 0.156 |
| Age | Young | 117 | 6(5.13%) | Ref. | |
| | Adult | 285 | 17(5.96%) | 1.11(0.43-2.90) | 0.824 |
| Season | Dry | 207 | 10(4.83%) | Ref. | |
| | Wet | 195 | 13(6.67%) | 1.56(0.67-3.66) | 0.301 |
| BCS | Good | 146 | 3(2.05%) | Ref. | |
| | Medium | 152 | 11(7.24%) | 3.70(1.01- 13.52) | 0.048 |
| | Poor | 104 | 9(8.65%) | 4.44(1.17 - 18.81) | 0.028 |
| Agro ecology | High land | 135 | 4(3%) | Ref. | |
| | Mid land | 134 | 7(5.22%) | 1.81(0.52 - 6.32) | 0.355 |
| | Low land | 133 | 12(9.02%) | 3.25(1.02 - 10.34) | 0.046 |
| Tick infestation | None infested | 181 | 5(2.76%) | Ref. | |
| | Infested | 221 | 18 (4.14%) | 2.77(1.01 - 7.63) | 0.048 |
| Health status | App healthy | 341 | 13(4.98%) | Ref. | |
| | Sick | 61 | 6(9.83%) | 2.08(0.79 - 5.50) | 0.141 |
| Communal grazing land | Absent | 170 | 5(2.94%) | Ref. | |
| | Present | 232 | 66(7.76%) | 2.75(1.00-7.55) | 0.05 |

BCS= body condition score, OR= odd ratio, CI= confidence interval, Ref. = Reference

The prevalence of bovine babesiosis was none significant (P=0.268) based on the kebele of the study animals. The highest prevalence was recorded in Jiha and Addisgie kebele with the same prevalence (9%) followed by shumara and Kerker with the prevalence (7.46%) and kerker (4.48%) respectively. According to univariable logistic regression analysis, the odd of tick borne hemoparasite occurrence in Addisgie, Jiha, shumara, Endivina and Chira were 2.25, 2.0, 1.72, 0.66 and 0.32 times more likely than kerker kebele positive for bovine babesiosis respectively.

The breed of study animals, the prevalence of bovine babesiosis in cattle was estimated to be higher in local breed (7.4%) compared to Cross breed (2.27%). The odd of occurrence of bovine babesiosis in Local breed were 3.44 times more likely than in cross breed and there was statistically significant difference between the two groups local and cross breed (OR=3.44; CI=1.00 - 11.79; P = 0.049) (table 3).

The sex of study animals, the prevalence of bovine babesiosis in cattle was estimated to be higher in male animals (8.85%) compared to male animals (4.45%). The odd of occurrence of bovine babesiosis in male animals were 1.81 times more likely than in female animals and there was statistically significant difference between the two groups female and male animals (OR=1.81; CI=0.79-4.28; P =0.156 (table 3)).

The age of study animals, the prevalence of bovine babesiosis in cattle was estimated to be higher in adult animals (5.96%) than young animals (5.13%). The odd of the occurrence of bovine babesiosis in adult animals were 1.11 times more likely to be positive for bovine babesiosis than young animals, respectively. This difference was not statistically significant (OR=1.11; 95% CI=0.43-1.23; P=2.90) (Table 3).

The prevalence of bovine babesiosis in cattle were determined to be higher in wet season (6.67%) compared to dry season (4.83%). The odd of occurrence of bovine babesiosis in wet season were 1.56 times more likely than in dry season and there was statistically significant difference between the two groups wet and dry season (OR=1.56; CI=0.67-3.66; p-value=0.156) (table 3).

The prevalence of bovine babesiosis was significant (P=0.028) based on the body condition score of the study animals. The highest prevalence was recorded in animals with a poor body condition (8.65%) followed by medium body condition animals were (7.24%) and the lowest in animals with good body condition scores were (2.05%). According to univariable logistic regression analysis, the odd of bovine babesiosis occurrence in poor and medium body condition score of animals were 4.44 and 3.70 times more likely than good body condition score of animals positive for bovine babesiosis respectively.

The prevalence of bovine babesiosis was significant (P=0.046) based on the agro ecology of the study animals. The highest prevalence was recorded in animals with a low land agro ecology (9.02%) followed by med land agro ecology of animals were (5.2%) and the lowest in animals with high land agro ecology were (3%). According to univariable logistic regression analysis, the odd of bovine babesiosis occurrence in low land and mid land agro ecology of animals were 3.25 and 1.81 times more likely than high land agro ecology of animals positive for bovine babesiosis respectively and there was statistically significant difference (OR=3.25; CI=1.02 -110.34; p-value=0.046) (table 3).

Regarding the prevalence of bovine babesiosis in tick-infested and non-tick-infested cattle the higher prevalence was found in tick-infested (4.14%) cattle than non-tick infested (2.76%) cattle. The risk of occurrence of bovine babesiosis in tick-infested cattle was 2.77 times more likely than non- tick infested cattle. This difference was statistically significant (OR=2.76; 95% CI=1.01- 7.63; P=0.048) (table 3).

The prevalence of bovine babesiosis in cattle were determined to be higher in sick animals (9.83%) compared to apparently healthy animals (4.98%). The odd of occurrence of bovine babesiosis in sick animals were 2.08 times more likely than in apparently healthy animals and there was statistically significant difference between the two groups of sick and apparently healthy animals (OR=2.08; CI=0.79 – 5.5; p-value=0.141) (table 3).

Animals on the communal grazing land (7.76%) had the highest prevalence of bovine babesiosis than animals no grazing on communal land (2.94%) animals, which were 2.75 times more likely to be positive for bovine babesiosis than animals no grazing on communal land. This difference was statistically significant (OR=2.75; 95% CI=1.00-7.55; P=0.05) (Table 3).

The factors considered in the initial univariable logistic regression analysis kebele and age was not statistically significant. It can be removed for multivariable logistic regression analysis in which p-value greater than 0.25.

Table 2: Final multivariable logistic regression model output of factors associated with bovine babesiosis in cattle.

| Variables | Categories | Number examined | Number positive (%) | OR (95%CI) | P-value |
|---|---|---|---|---|---|
| | Good | 146 | 3(2.05%) | Ref. | |
| BCS | Medium | 152 | 11(7.24%) | 3.70(1.01- 13.52) | 0.050 |
| | Poor | 104 | 9(8.65%) | 4.44(1.17 - 18.81) | 0.033 |
| | High land | 135 | 4(3%) | Ref. | |
| Agro ecology | Mid land | 134 | 7(5.22%) | 1.81(0.52 - 6.32) | 0.274 |
| | Low land | 133 | 12(9.02%) | 3.25(1.02 - 10.34) | 0.047 |
| Communal grazing land | Absent | 170 | 5(2.94%) | Ref. | |
| | Present | 232 | 66(7.76%) | 2.75(1.00-7.55) | 0.043 |
| Health status | App healthy | 341 | 13(4.98%) | Ref. | |
| | Sick | 61 | 6(9.83%) | 2.08(0.79 - 5.50) | 0.05 |

BCS= body condition score, OR= odd ratio, CI= confidence interval, Ref. = Reference

## 6 CONCLUSION AND RECOMMENDATION

The present findings indicated that bovine babesiosis had 5.73% of prevalence in the study area that might be due to abundance of tick infestation and presence of communal grazing land. Both Babesia bovis and Babesia bigemina were identified and Babesia bigemina has a greater prevalence to be identified. The main risk factors found to be significantly associated with bovine babesiosis were body condition, communal grazing land, and agro ecology and health status. Moreover, the current low awareness or knowledge of the livestock owners about the diseases transmitted by ticks could be attributed to a lack of treatments and shortfall of control strategies in animals and resulting in significant economic loss and increases occurrence of the diseases. In order to minimize losses attributed to ticks and tick-borne disease in the area strategic tick control techniques should be implemented, as it is a level of control that prevents ticks from becoming a nuisance.

Therefore, based on the above conclusion the following recommendations are forwarded.

- Awareness creation and improved management systems should be practiced by the stakeholders.
- Further research works on molecular level should be carried out.
- The livestock sector should be played an important role in designing and implementing tick and babesiosis control and prevention programs.
- Further study on the epidemiology of the disease, the biology and ecology of the ticks are useful in planning and programming control strategies.

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