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Research Article

Study on the Prevalence of Bovine Babesiosis and Its Associated Risk Factors in and Around LAY ARMACHIHO Woreda, AMHARA Regional State, NORTH WEST ETHIOPIA.

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Abstract

Objectives: to estimate prevalence and associated risk factors of bovine babesiosis in and around Lay Armachiho woreda.

Methods: blood samples were collected from randomly selected cattle to assess the presence of babesia species by using thin smear technique in the study woreda.

Results: 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.

Conclusions: 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.

Keywords: Cattle, Ethiopia, Lay Armachiho, Risk Factors, Prevalence

Introduction

Tick-borne hemoparasites are causing devastating losses to the livestock industry and thus pose major constraints on the livestock production throughout the world [18].

Infection is mainly transmitted by arthropod vectors like ticks, biting flies and through blood transfusion during treatment, vaccination and castration in cattle [28].

Tick-borne hemoparasites are growing steadily due to the establishment of the tick vector and the TBD including anaplasmosis, babesiosis and theileriosis which reduces livestock production in endemic areas [22,31].

The country's environmental condition and vegetation are highly conducive to 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 warmer climates, especially in tropical and subtropical areas [2]. Tick-borne hemoparasite have a serious economic impact on the livestock sector due to decreased productivity, lowered working efficiency, increased cost for control measures and death of livestock [7].

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 of 50% in susceptible herds and they are known to be transmitted in this country by *Rhipicephalus* [18].

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 ogenic and animals with chronic infections can be asymptomatic carriers [1].

Clinical signs vary in severity, depending on susceptibility, immunity, and age of the host, parasite species and load and principally maintained by carrier states [10].

The importance of ticks is principally due to their ability to transmit a wide spectrum of pathogenic microorganisms, such as *protozoa*, *Rickettsial*, *spirochetes*, and *viruses* [15]. The main effect of tick infestation in cattle is mild to severe anemia and tick infestation also results in increased calf mortality [22]. The seasonal variations within a bioclimatic zone may favour or hinder the development or activity of a tick species during certain periods [14]. Dry environmental conditions are a serious danger to ticks, particularly to the questing larvae, which are very susceptible to drying out fatally [2].

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 this 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.

Statement of the problem

Babesia infections in cattle are economically important and major impediments to the health and productive performance in tropical and subtropical parts of the world including Ethiopia. The absence of well-established research regarding the socioeconomic and public health implication of babesiosis in livestock has a negative impact on food security, animal product and byproducts. Babesiosis constitute an important constraint on the livestock production in Ethiopia including huge economic losses towards livestock production, high mortality rate in cattle, its impact on the international cattle market and causes abortion in pregnant cows [5]. In addition the influence of farming practices on the spread and maintenance of babesiosis in cattle is poorly understood.

Despite the widespread distribution of various tick species across the study area little is known about the spread of harmful hemoparasite carried by ticks, such as babesiosis. Thus, there is paucity of information on cattle babesiosis in the districts of Lay Armachiho. In order to address the above problems, a study is required to fill the gaps in knowledge about hemoparasite and their vectors to create baseline information that can be used to develop efficient disease control and prevention program.

Objectives of the study

General objectives

The overall aim of this study is to quantify the Epidemiology of bovine babesiosis in and around Lay Armachiho Woreda.

Specific objectives

The specific objectives of this study are

- To estimate the prevalence of bovine babesiosis in cattle in the study area.
- To assess the potential factors associated with the occurrence of babesiosis

Research questions

This research work will attempt to answer the following research questions.

- What is the prevalence of bovine babesiosis in cattle in the study districts?
- What are the associated risk factors for the occurrence of bovine babesiosis in cattle?

Significance of the study

No previous study has been conducted to detect bovine babesiosis as a prevailing cause of cattle disease in Lay Armachiho districts. This study may guide accurate diagnosis and early treatment before the devastating effect of the disease occurs in animals. The study will be useful in estimating the prevalence of tick borne hemoparasites Lay Armachiho districts. In this context, this study may reveal the existing status of bovine babesiosis responsible for socio-economic and public health implication in livestock has a negative impact on food security, animal product and byproducts, infertility and death. This study would provide baseline information for those investigating the disease of cattle in Lay Armachiho districts.

Materials and Methods

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 located in the 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 [9].

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 [18]. 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.

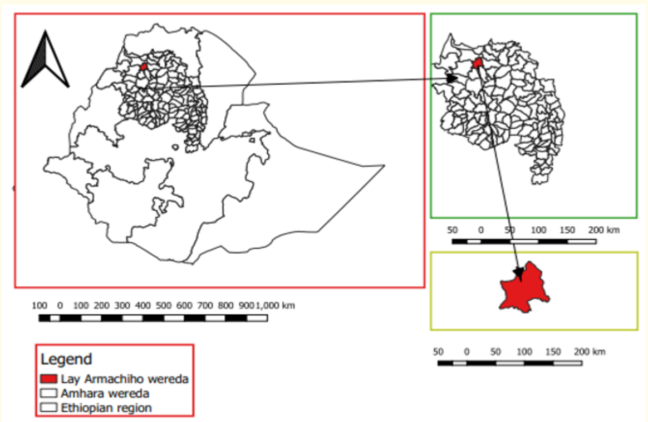

Figure 1: Maps of the study area.

Study design

A cross-sectional study design was conducted to screen the collected blood samples for the presence of tick-borne pathogen from November 2022 to June 2023.

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 [31] with 95% confidence level and 5% absolute precision.

$$n = \frac{1.96^2 (Pexp (1-Pexp)}{d^2} = \frac{1.96^2 (0.5 (1-0.5)}{0.05^2} = 384$$

Where, n = required sample size

Pexp = expected prevalence

d = desired absolute precision [31].

However, to increase the absolute precision, 402 samples were collected throughout the study period.

Study population

The study were 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 were evaluated during sample collection and the cattle was grouped as emaciated (poor), moderate (medium) and good based on anatomical parts and the flesh and fat cover at different body parts [24] (Annex 1). Animals were conveniently classified as young (<3 years) and Adult (>3 years) age categories as described by [8].

Sample collection and examination

Blood sample collection and laboratory analysis

A total of 402 blood samples were collected from the ear vein of simple randomly selected cattle from Lay Armachiho districts following the standard protocol described by [32]. 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 was 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 [25]. 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) [35]. The parasites species were identified according to the characters distinguished by their size, shape, position and location described by Soulsby [30].

Blood sample examination

- **Thin blood smear:** Giemsa staining procedures and microscopic examination of the slides were conducted according to [25].

The parasites were identified according to the characters described by [30].

Data analysis

The collected data were 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 multiplying by 100. 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 ≤ 0.05 was considered as cut off for statistical significance and odds ratio (OR) and 95% CI were also calculated.

Results

Prevalence of bovine babesiosis at the species level

The present findings indicated that bovine babesiosis had 5.73% prevalence in the study area and *Babesia bovis* and *Babesia bigemina* were identified, and a 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.

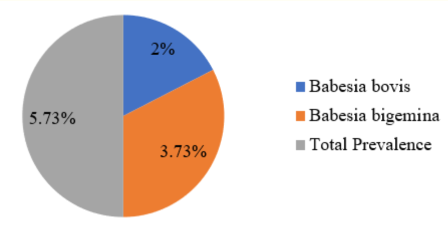

Figure 2: Prevalence of hemoparasites at species level.

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.

Variables	Categories	Number examined (%)	Babesia bovis	Babesia bigemina
Kebele	Kerker	67(4.48%)	1(1.5%)	2(3%)
	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%)

Table 1: Prevalence of tick borne hemoparasite at kebele level in cattle.

Risk Factors for bovine babesiosis

In univariate 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)

The prevalence of bovine babesiosis was not 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.

With regard to the breed of study animals, the prevalence of bovine babesiosis in cattle was estimated to be higher in local breed (7.4%) than in 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%) than in male animals (4.45%). The odd of occurrence of bovine babesiosis in male animals were 1.81 times more likely than in female animals

Variables	Categories	Number examined	Number positive (%)	OR (95%CI)	P-value
Kebeles	Kerker	67	3 (4.48%)	Ref.	
	Shumara	67	5 (7.46%)	1.72 (0.39 - 7.51)	0.47
	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

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

BCS: Body Condition Score; OR: Odd Ratio, CI: Confidence Interval; Ref: Reference

Variables	Categories	Number examined	Number positive (%)	OR (95%CI)	P-value
BCS	Good	146	3 (2.05%)	Ref.	
	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
Agro ecology	High land	135	4 (3%)	Ref.	
	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

Table 3: Final multivariable logistic regression model output of factors associated with bovine babesiosis.

BCS: Body Condition Score; OR: Odd Ratio; CI: Confidence Interval; Ref: Reference and there was a 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 in young animals. 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, in the 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 was recorded in animals with good body condition scores (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 agroecology of the study animals. The highest prevalence was recorded in animals with a low land agroecology (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 agroecology of animals were 3.25 and 1.81 times more likely than high land agroecology of animals positive for bovine babesiosis respectively and there was a 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 was 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 communal grazing land (7.76%) had the highest prevalence of bovine babesiosis than animals not 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 a 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 were not statistically significant. It can be removed for multivariable logistic regression analysis in which p-value greater than 0.25.

Discussion

Tick borne hemoparasites are growing steadily because of the establishment of thetick vector and the TBD including anaplasmosis, babesiosis and theileriosis reduce livestock production in endemic areas [32,33].

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.

In the present study, the overall prevalence of bovine babesiosis was 5.73%. The result of bovine babesiosis is in line with the finding of [1], who indicated 6.1% prevalence of bovine babesiosis in Southwestern, Ethiopia, and higher than [10], who reported 1.5% prevalence of bovine babesiosis in Western Ethiopia. This result was found to be low compared with the result of [20] who reported 59.3% babesiosis prevalence in Darfur, Sudan. This variation in the prevalence of babesiosis may be due to the variation of the geographical conditions and tick burden.

In the present study, slightly higher infection was recorded in males 8.65% as compared to female animals 4.45%. Although difference was not statistically significant (p = 0.156), it indicated that both sexes were equally susceptible to the infection. This finding is not in-line with the report of [17] who found a higher prevalence of babesiosis in male 6.96% compared to female cattle (11.2%). 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, highest prevalence of bovine babesiosis was observed among adult animals (5.96%) compared with young animals (5.13%), and there was no statistically significant difference observed (P = 0.824) among the age groups.

This result was not in line with the finding of [1], from Ethiopia who reported the highest prevalence in adult animals (7.5%) followed by young animals in (3.3%) and [6] in Pakistan who reported in young animals with 14.4% followed by adult animals, 13.7%. However, the results of this study concurred with [3] from Brazil who reported a high prevalence in young 5.4% followed by adult animals 28%.

The present study results reviled that young animals had relatively less prevalent bovine babesiosis and were more prevalent in adult cattle even if they were kept in the same grazing area. The lowest occurrence of pathogen among young animals may be due to acquired immunity through colostrums feeding and they regenerate red blood cells faster than adults. Adult animals are a slightly free of pathogens than young animals (12–36 months) [11], which could be due to used up passive immunity and inexperienced to active immunity.

The higher prevalence of TBPs in adult cattle in the present study agree with [26] due to endemic stability developed in adult cattle between host, agent, vector and environment for vector borne diseases is such that clinical disease occurs rarely or not at all, and it could be due to immunosuppression. Strong immunity occurs after natural infection with TBPs [26]. Additionally, the present result was in line with the idea of the larger size of adult animals; providing a high chance of contact with the vectors including TBD vectors and management practices of the farmers [4]. This may contribute to the increase in prevalence of hemoparasites as age increases.

This study showed a significant association of positivity for babesiosis with the body condition of the study animals (P = 0.028). This result is not in-line with the prevalence of babesiosis with reported by [12] from Ethiopia, in that highest prevalence was recorded in medium body condition animals 35.96% followed by poor body condition animals 15.57% and least in good body condition animals (9.5%). Moreover, this difference could be due to the fact that animals with poor body conditions have lower immunity which encourages infection by different organisms like Babesia.

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 the 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. 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, on the basis of the above conclusion the following recommendations are forwarded.

- Awareness creation and improved management systems should be practiced by stakeholders.
- Further research works should be carried out at on molecular level.
- The livestock sector should play an important role in designing and implementing tick and babesiosis control and prevention programs.
- Further studies 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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