Original Research Article

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Epidemiological Study of Bovine Babesiosis in Siyari Rural Municipality, Rupandehi, Nepal

Prakash Thapa [(1)*], Dipendra Mishra [(1)], Susma Reshmi Magar [(1)], Dipendra Lamsal [(1)], Ajit Kumar Karna [(2)]

[(1)]Institute of Agriculture and Animal Science, Tribhuvan University, Paklihawa, Rupandehi, Nepal, [(2)]Postgraduate Campus, Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur, Kathmandu, Nepal

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Abstract

A cross-sectional study was conducted from November 2022 to January 2023 to determine the prevalence of Bovine Babesiosis in cattle of Siyari Rural municipality in Rupandehi district by microscopic examination of Giemsa-stained thin blood smears. A total number of 121 blood samples were collected randomly from a different farm. Microscopic examination showed an overall prevalence of 5.79% in the study area. A chi-square test was done to test the significance of various factors. Breed, age, sex, herd size, tick infestation, feeding pattern, floor type and chemical use had an insignificant relationship with the prevalence of Babesiosis. However, body condition (0.004) and PCV (0.011) had a significant association. This significant association might be due to cattle with poor body conditions having low immunity, which favours more chance of getting an infection and as *Babesia* destroys erythrocytes which leads to a decrease in PCV. The odds ratio for age (>3/≤3) was 2.1, for herd size (>10/≤10) was 3.1, for body condition (poor/good) was 7.6, for chemical use (no/yes) was 1.9 and for PCV (low/normal) was 6.26. Further research should be conducted based on an immunological and molecular level to find the real scene of this disease. The result showed Bovine Babesiosis is a potential threat to the dairy sector. Appropriate tick control and prophylactic treatment programs should be done to improve the current situation.

Keywords: Prevalence, Babesiosis, Giemsa-stained, Microscopic, Tick infestation

Introduction:

Nepal is an agriculture-based country in South Asia, where about 66% of the total population is engaged in agriculture. The livestock sector contributes to the country's socio-economic development by providing nutrition and a source of income for rural households. Nepal has 7,466,841 cattle, 5,159,931 buffalo, 793,725 sheep and 13,442,614 goats (DLS, 2020). Livestock and its products contribute 6.25% to the national GDP and 23% to the agricultural GDP (DLS, 2020). Most of the population depends on livestock farming as a major source of income and therefore cattle farming business is increasing in the country. However, scaling up of the livestock sector is limited by multiple factors that reduce productivity, including tick-borne diseases (TBD) such as bovine babesiosis.

Bovine babesiosis is a haemo-protozoan disease transmitted by a tick vector, mainly by the genera of *Boophilus* and *Hyalomma* spp. (Battsetseg et al., 2018; Ravindran et al., 2002). The loss due to bovine babesiosis is related to mortality, abortion, decreased growth, decreased milk production, control measures and cattle trade restrictions productivity (Alim et al., 2012; Ananda et al., 2009). Babesiosis in bovine, also known as Texas cattle fever, is caused by a protozoan named *Babesia* of Phylum Apicomplexa (Abdoulmoumini et al., 2017). Although *Babesia* has more than 100 species worldwide, six species - *Babesia bovis, B. bigemina, B. divergens, B. major, B. occultans* and *B. argentina* are known to cause bovine babesiosis. Among these, *B. bigemina* is widely prevalent but *B. bovis* is a highly pathogenic species (Jacob et al., 2020). Only a 1% level of parasitemia by *B. bovis* is sufficient to produce acute disease in cattle while it requires more than 10% for *B. bigemina* (Agrawal et al., 2021). *B. bigemina* and *B. bovis* are found in the tropical and sub-tropical regions while *B. divergens* are mainly prevalent in Europe (Schnittger et al., 2012). The disease is relatively more prevalent in crossbreeds than indigenous cattle species but in adults more than younger and in females than males (Alim et al., 2012).

In Nepal, the disease is prevalent and observed between March to October as the early to late monsoon season provides a suitable environment for tick propagation (Shrestha et al., 2017) Terai region of Nepal being a major cattle production area, is also suitable for vector ticks hence the disease is responsible for the greatest hindrance to the growth of cattle production in the Terai region of Nepal (Khankhawash, 2018). The geography and the climate of the Siyari Rural Municipality of Rupandehi district are similarly favorable habitats for the survival and breeding of the tick vectors.

There has been a sporadic outbreak of suspected bovine babesiosis in Siyari Rural Municipality, as confirmed by the veterinarians providing clinical services in the municipality. However, epidemiological data was not available since there has not been targeted research for the disease in the area. Hence the study was necessary to validate the suspected outbreaks, better understand the risk factors for infection in cattle and provide evidence on the current prevalence of bovine babesiosis in Siyari Rural Municipality.

Materials and Methods:

Description of the study area

The study was conducted in the Siyari Rural municipality of the Rupandehi district, Nepal, between November 2022 and January 2023. This municipality is located at an altitude of 100 to 127 m above sea level with latitude 27º 31' 0" to 27º 37' 30" N and longitude 82º 20' 0" to 83º 28' 0" E. It covers an area of 66.17 square kilometers and consists of 38,466 population. The average daily temperature varied between 8.78º C and 43.4º C. It has a total of 230 registered cattle farms.

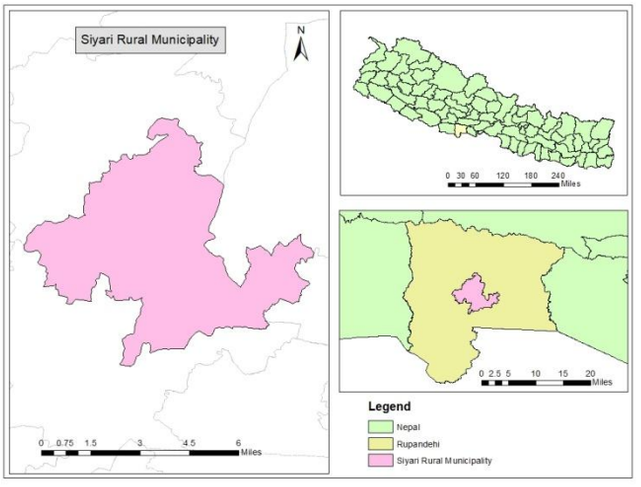

Figure 1: Map of the study area

Study design and sample size determination

A cross-sectional study was conducted to estimate the prevalence of bovine babesiosis in the study area. The sample size was calculated using the Daniel formula.

Sample size(n) = $Z^2P(1-P)/d2 = 1.96^2 \times 0.0602(1-0.0602)/0.05^2 = 87$

Where, n = sample size, Z = statistic for the level of confidence (for the level of confidence of 95%, the Z value is 1.96), P = expected prevalence (6.02%) (Shrestha et al., 2017), d= desired precision at 0.05.

Sampling procedure

A list of the cattle farms in the municipality was obtained from Siyari Rural Municipality office. A total of 121 samples were collected from all seven wards of the municipality by simple random sampling method of the farms. More number of samples than the estimated sample size was obtained for better precision. Three ml of blood was drawn from each cattle and collected blood was brought to the Veterinary Physiology Laboratory of the Institute of Agriculture and Animal Sciences (IAAS) in Paklihawa. During the sampling process, information on age, sex, body condition, history of the disease and health status of animals was gathered from the owner in addition to directly observing the sanitation situation of the farm at the time of the visit. The body condition of the cattle was scored following the Elanco method (Isensee et al., 2014).

Calculation of Packed Cell Volume (PCV)

The microhematocrit tubes were filled up to 75% of their length and centrifuged at 10,000 rpm for 5 minutes (Stephen et al., 2017). Centrifuged tubes were placed over the hematocrit reader and Packed Cell Volume (PCV) for individual samples was determined. The value between 24-46 % was taken as a normal range (Balcha and Dima, 2017; Ghimire et al., 2022).

Microscopic examination of blood smear

A standard process was followed to prepare a thin blood smear and Giemsa staining of the slides (Ibeh et al., 2021). The slides were observed under a 10X objective lens and further observed using immersion oil at 100X. A total of fifty fields from each Giemsa-stained slide were examined to identify the piroplasm of *Babesia*, a pear-shaped, located in pairs and round, oval, or irregular inside erythrocytes depending on the stage of development of the parasite (Yadav, 2015).

Statistical analysis

The data were entered in Microsoft Excel. The apparent prevalence of *Babesia* was estimated by dividing the number of positive samples by the total number of samples. The association between breed, age, sex and other factors was statistically compared by Chi-square test using SPSS version 25 with a significance level defined at $P<0.05$.

Results and Discussion:

Estimates of packed cell volume

Upon examining the PCV of the blood samples obtained from cattle, the mean PCV (%) ± SEM was 34.2 ± 0.8. (Table 1).

Table 1: Mean PCV values of cattle examined

Description	Number (%) of cattle examined	Mean PCV (%) ± SEM
Lower than the normal range	14 (11.6%)	19.4 ± 1.01
Within the normal range	98 (81.0%)	34.9 ± 0.6
Higher than the normal range	9 (7.4%)	49.9 ± 1.3
Total	121 (100%)	34.2 ± 0.8

Microscopic examination of slides

The microscopic examination of the blood smears revealed that 7 (5.8%) of 121 cattle had piroplasm of *Babesia* species (Fig. 2).

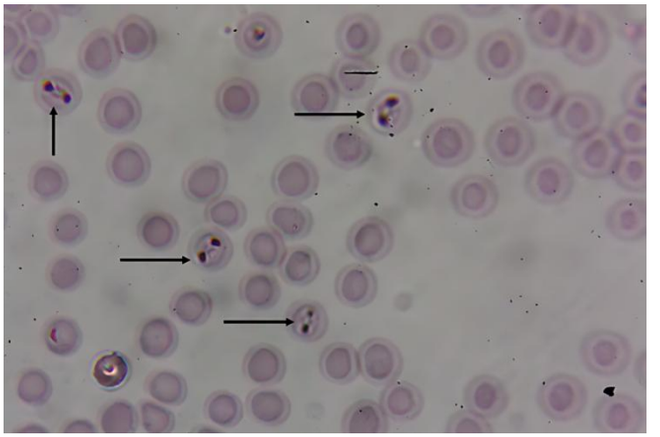

Figure 2: Piroplasm of *Babesia species* as observed under 100X objective lens (arrow indicates piroplasm)

Table 2. Risk factors of babesiosis in cattle

Risk factors	Category	Sample tested	Positive frequency	Positive %	Odds ratio (CI at 95%)	P- value
Breed	Exotic	113	7	6.2%		0.5
	Indigenous	8	0	0 %		
Age	>3 yrs	67	5	7.5%	2.1 (0.4 -16.1)	0.4
	≤ 3 yrs	54	2	3.7%		
Sex	Female	111	7	6.3%		0.4
	Male	10	0	0 %		
Body Condition	Poor	21	4	19.1%	7.6 (1.6 - 37.1)	0.004*
	Good	100	3	3.0%		
Tick infestation	Yes	99	7	7.1%		0.2
	No	22	0	0 %		
Pattern of feeding	Stall	103	7	6.8%		0.2
	Both stall & grazing	18	0	0 %		
Floor-type	Cemented	109	7	6.4%		0.4
	Muddy	12	0	0 %		
Chemical use	No	92	6	6.5%	1.9 (0.3-46.8)	0.5
	Yes	29	1	3.4%		
PCV	Low	24	4	16.7%	6.3 (1.2-35.2)	0.01*
	Normal	97	3	3.1%		

Risk factors of babesiosis in cattle

A Chi-square test was performed to characterize the association of babesiosis in the cattle with breed, age, sex, body condition, tick infestation, pattern of feeding, barn floor type and use of chemicals in the barn and packed-cell volume. In this study, the prevalence of bovine babesiosis in cattle was found highest in the exotic breed (7, 6.2%) compared to the indigenous breed. The study found that there was a higher infection rate among cattle older than 3 years of age 7.5% and 3.7% among cattle younger than 3 years (P = 0.4). The odds of developing *Babesia* among cattle of more than 3 years were 2.1 times higher compared to cattle of ≤ 3 years of age. There was no prevalence of disease in ox (P = 0.413).

The prevalence of the disease was 3% for cattle having a good body condition score within a normal range (24-46) (Ghimire et al., 2022) and 19.04% for poor body condition score (<24) with a statistically significant association (P = 0.004). The odds ratio of poor to good body condition score was found 7.6. The study of prevalence according to tick infestation showed (Table 2) a high prevalence of the disease among the cattle having a past or present history of tick infestation on the body of cattle. There was zero prevalence among the cattle never having tick infestation. While only a 3.4 % prevalence

was found in farms that used chemicals for cleaning and sanitation. The study also showed that the infected animal had lower PCV than non-infected animals.

The overall prevalence of bovine babesiosis in our study is similar to the result obtained by Shrestha et al. (2017), Yadav (2015) and Barman (2018) in different geographies who estimated a prevalence of 6.02%, 6.08% and 5.85%, respectively. However, the prevalence estimated in our study is higher than the prevalence estimated by Bohara and Shrestha (2016), Rahman et al. (2015), Laha et al. (2015), Harkirat and Jyoti (2012) and Kundave et al. (2018) who estimated prevalence of 0.64%, 1.50%, 3.60%, 1.56% and 3.25% respectively in cattle in different geographies. Contrary to our study, others reported a higher prevalence of Babesiosis; Tembue et al. (2011) estimated 78.80% *Babesia bovis* and 76.0% *Babesia bigemina* in Southern Mozambique by ELISA, Battsetseg et al. (2018) estimate 22.40% *Babesia bigemina* and 18% *Babesia bovis* in Mongolia using ELISA, Chaudhry et al. (2010) estimated 29.00% positive in Pakistan by PCR, Marufu et al. (2010) estimated 45.00% seroprevalence in cattle in South Africa. The variation in the prevalence of the disease in the study areas might be due to the variations in geography, climate, breed, animal husbandry practice and exposure of vectors.

The breed-wise prevalence estimated in our study is in agreement with Alim et al. (2012). In Chittagong division of Bangladesh; Hosen et al., 2020 in Sylhet district of Bangladesh; Velusamy et al., 2014 in Tamil Nadu, India and Kaur et al., 2021 in Jammu, India, who estimated infection of *Babesia* spp. higher in the cross-breed cattle compared to indigenous breed.

Age-wise prevalence was in line with the findings from the Chittagong division, Bangladesh (Alim et al., 2012). However, the result of this study disagrees with the result of a study by Amorim et al., 2014 and Abdoulmoumini et al., 2017 who estimated younger animals were more susceptible than adults. The reason for the higher prevalence in adults than young animals might be due to prior immunity by the younger animal as compared to the waned immunity of the older animal. There was an inverse age resistance in the case of Babesiosis through rapid immune response to primary infection by calves through a complex immune mechanism (Ananda et al., 2009; Zintl et al., 2005).

This finding of sex-wise prevalence is in agreement with Hosen et al. (2020) estimated a higher prevalence in females (13.4%) than males (5.5%) and Kaur et al., 2021 estimated 9.7% in females and 8.3% in males. However, this result disagreed with Amorim et al. (2014) and Zhou et al. (2019). The high prevalence in females might be due to females having hormonal disturbances and they have low immunity due to milk production and the breeding system (Hamsho et al., 2014). Our study indicated cattle of a herd size more than 10 had increased odds of positivity for babesiosis. This finding is in agreement with Perez et al. (1994) who estimated greater seropositivity of Babesiosis in farms of more than 10 heads. Similarly, the significant association between body condition and prevalence might be due to an animal having poor body conditions being weak and having low immunity as compared to an animal with good body conditions (Hamsho et al., 2014). Our findings are in agreement with Amorim et al. (2014) who found a 17.3 odds ratio of Babesiosis in tick-infested cattle and not-infested cattle. In this study, for the prevalence of this disease according to chemical use, a high prevalence (6.52%) was estimated in farms where only water was used for farm sanitation (P= 0.536). The reason for this result might be due to the use of chemicals to break the life cycle of tick vectors (Iqbal et al., 2013).

The result obtained from our study on prevalence and pattern of feeding was in line with Siddique et al. (2020) who estimated a higher prevalence of babesiosis in tethered animals than in open animals. They also demonstrated a higher prevalence in closed housing systems than in semi-closed and open housing systems. The possible reason for this might be due to reduced immune response due to tethering stress, lesser movement of the animal in confinement increases the probability of getting attacked by ticks and low exposure to sunlight in a closed housing system resulting humid environment which favors better breeding place for ticks (Iqbal et al., 2013).

The association between PCV and *Babesia* infection was statistically significant (P value= 0.01) in our study. This might be due to Babesia infection causing intravascular hemolysis destroying erythrocytes and decline PCV (Marufu et al., 2010). The odds of developing the disease were 6.3 times greater for the cattle which had low PCV as compared to those having normal PCV.

Conclusion:

Babesiosis is one of the potential threats to the livestock and dairy industry of Nepal. Knowledge about the present situation of disease is essential for effective control of the disease. Hence, the study was focused on estimating the prevalence of babesiosis in cattle of Siyari Rural Municipality. The present findings indicated a significant level of prevalence of *Babesia* spp. in the study area. Among the different risk factors studied only body condition and PCV were found to be significant and all others were found non-significant. To improve the status of cattle-dependent farmers in the area, regular prophylactic treatment and the use of acaricides should be promoted. As the microscopic examination of blood

smears has low sensitivity and specificity, further research should be conducted based on the immunological and molecular level to estimate the true status of bovine babesiosis in the area.

Conflict of interest:

The authors have no conflict of interest.

Authors' contribution:

Prakash Thapa designed the study under the guidance of Dipendra Mishra and conducted the analysis and prepared the first draft of the manuscript. Susma Reshmi Magar and Dipendra Lamsal assisted Prakash Thapa in the research and drafting of the manuscript. Ajit Kumar Karna did a thorough review of the manuscript. All authors have read and approved the final version of the manuscript.

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***Corresponding author's email ID:** parkasmagar013@gmail.com

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