

PREVALENCE OF TROPICAL BOVINE THEILERIOSIS: AN ASSESSMENT

C. Priyanka¹, K. Jalajakshi*², P. Malakondaiah³ and T. Nagendra Reddy⁴

Department of Veterinary Parasitology
College of Veterinary Science
Sri Venkateswara Veterinary University
Tirupati-517502, Andhra Pradesh.

Abstract

Tropical bovine theileriosis poses a significant threat to livestock productivity and health, often presenting as anaemia, sharp decline in milk production, progressive weight loss, and potential mortality. Establishing effective prevention and control measures requires a thorough evaluation of the regional prevalence and its underlying epidemiological drivers. The current study investigates the occurrence of bovine theileriosis and its associated managerial risk hazards among cattle populations in the Tirupati district of Andhra Pradesh. A total of 524 cattle were investigated between December 2022 and November 2023. Blood samples and lymph node impression smears were examined to detect the presence of Theileria and confirmed by PCR. Of the 524 cattle examined, 120 were confirmed positive for Theileria annulata by polymerase chain reaction (PCR), resulting in an overall prevalence of 22.9% (120/524). Notably, animals older than three years exhibited a higher T. annulata infection rate (37.41%) than their younger counterparts. Females showed a prevalence of 24.94%, while Holstein Friesian cattle exhibited a prevalence of 26.9%. Seasonal variation was also evident, with the highest infection rate recorded during the rainy season (44.87%). The findings indicate that age, breed, and season were significantly associated with the occurrence of T. annulata infection ($p < 0.05$), whereas no significant association was observed with sex ($p > 0.05$). These findings highlight the importance of age, breed, and season-specific surveillance, along with regular tick control and improved farm management practices, for reducing the burden of bovine tropical theileriosis.

Keywords: Bovine theileriosis, prevalence, risk factors, PCR

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¹M.V. Sc Scholar, Part of M. V. Sc thesis submitted to SVVU, Tirupati.

²Assistant Professor,

*Email address of corresponding author- jalajakshikopparthi@gmail.com

³Associate Dean, College of Veterinary Science, Tirupati

⁴Assistant Professor, Department of Veterinary Microbiology, College of Veterinary Science, Tirupati.

INTRODUCTION

Theileriosis is a haemoprotozoan disease caused by the parasites of the genus *Theileria*, which belongs to the suborder Piroplasmorina (Demessie and Derso, 2015). Ticks belonging to the genera *Hyalomma*, *Amblyomma*, *Haemaphysalis*, and *Rhipicephalus* are known to transmit theileriosis in cattle Mans *et al.* (2015). *Theileria annulata* and *Theileria orientalis* are the most frequently seen species in tropical regions (Kolte *et al.*, 2017). According to Saeed (2016), bovine tropical theileriosis is primarily transmitted by *Hyalomma* ticks, leading to severe clinical signs and occasional death in cattle. The life cycle of *Theileria annulata* involves two hosts, with sexual development, zygote formation, ookinetes development, and sporogony occurring in the tick vector, while schizogony and merogony occur in the bovine host, involving development in lymphocytes and erythrocytes, respectively (Gul, 2015). Climatic and environmental factors, particularly temperature, relative humidity, rainfall, and seasonal conditions, can influence the survival, development, and activity of tick vectors and thereby affect the transmission dynamics of bovine tropical theileriosis. In India, variations in these climatic conditions across geographical regions may contribute to differences in tick abundance and disease occurrence. Regions with environmental conditions favourable for tick development may experience increased tick activity and a corresponding risk of *Theileria annulata* transmission. Seasonal differences in the occurrence of

bovine tropical theileriosis have also been reported, reflecting the influence of climatic conditions on tick population dynamics and parasite transmission (Kohli *et al.*, 2014). The epidemiology of theileriosis is multifactorial and may be influenced by environmental and climatic conditions, geographical location, cattle age and breed, management practices, and the presence and abundance of competent tick vectors (Gul, 2015). The strategies for the effective treatment and control of theileriosis, the input facilities like sensitive diagnostic tests are required (Ambrosio and Waal, 1990). Consequently, this study sought to evaluate the prevalence of bovine theileriosis and pinpoint key host and management variables influencing its occurrence.

MATERIALS AND METHODS

Blood samples and Tick samples collection

Over a one-year period, from December 2022 to November 2023, a total of 524 cattle suspected of theileriosis were examined in and around Tirupati, Andhra Pradesh. The animals were selected based on their availability and clinical suspicion of theileriosis during the study period. Relevant information regarding the animals and their management practices was collected through direct physical examination and a structured proforma administered to the respective cattle owners. From each animal, two blood samples were collected: one from the ear vein for preparation of peripheral blood smears and another from the jugular vein for further laboratory investigation. Information

on age, sex, and breed of each animal was recorded at the time of sample collection. Tick infestation was also assessed, and ticks, when present, were collected for identification.

Information collection from the owners

Clinical information was collected individually for each suspected animal using direct physical examination and a structured questionnaire administered to the respective cattle owners. The investigators assessed clinical parameters including rectal temperature, superficial lymph-node enlargement, and the presence and degree of tick infestation during physical examination. Information regarding anorexia and reduction in milk yield was obtained from the owners as part of the clinical history. All observations and owner-reported information were recorded separately for each animal in a structured proforma. The questionnaire used for collecting the relevant clinical history and management information is provided in the annexure (Fig 6).

Examination of blood smears and Lymph node smears

Following clinical examination, blood samples were collected from each animal for microscopic and molecular examination. Blood was collected from the ear vein for preparation of peripheral blood smears. A separate blood sample was collected from the jugular vein into sterile EDTA-containing tubes for molecular detection of *Theileria annulata* by polymerase

chain reaction (PCR). For microscopic examination, a drop of ear-vein blood was placed on a clean, grease-free glass slide and a thin peripheral blood smear was prepared. The smear was air-dried and subsequently stained with Giemsa stain. Lymph-node impression smears, where collected, were also subjected to Giemsa staining. The stained smears were allowed to dry at room temperature and examined under a 100× oil-immersion objective of a light microscope for the detection of *Theileria* organisms.

Processing of ticks

The collected ticks were carefully punctured on the ventral surface without damaging the ventral plates and immersed in 10% KOH solution overnight for clearing of the internal tissues. The ticks were subsequently heated in 10% KOH solution for approximately 15–20 minutes. Following KOH treatment, the ticks were thoroughly washed with distilled water to remove residual KOH. The specimens were then dehydrated sequentially through graded concentrations of ethanol (70%, 90%, and 100%) and subsequently cleared in lacto phenol for 5–10 minutes. The cleared specimens were examined microscopically for identification based on standard morphological characteristics (Soulsby 1982).

Results

Of 524 bovine blood samples screened, 33 cattle samples tested positive for *Theileria* piroplasms via microscopic examination, yielding an overall prevalence

of 6.29%. Morphological identification revealed characteristic piroplasms- ring-shaped, comma- shaped and oval structures within erythrocytes as well as schizont stages (Koch blue bodies) in lymphocytes (Fig. 4&5). All collected tick samples after processing were identified as *Hyalomma anatolicum anatolicum* based on the characteristic external traits, with particular emphasis on the subanal plates, which were positioned laterally (Soulsby 1982) (Fig.1).

Infection rates of bovine tropical theileriosis were studied across six chosen locations in the greater Tirupati area. Among the areas surveyed, Tirupati showed greatest proportion of cattle (33.33%), followed by Peruru (32.85%), Puttur (29.48%), Renigunta (23.80%), and Madanapalli (19.30%) and Kuppambadur (9.89%). Of these Kuppambadur recorded the lowest prevalence. The incidence of bovine tropical theileriosis demonstrated significant regional variation across the six locations ($p < 0.05$). The observed geographical variation may be related to differences in the environmental conditions prevailing in individual locations. Factors such as ambient temperature and relative humidity may influence the tick development, survival and population density, thereby modifying the risk of *Theileria* transmission among cattle breeds (Table 1 and 2).

A seasonal investigation revealed fluctuations in the occurrence of bovine tropical theileriosis. For the purpose of analysis, the study duration was divided into three seasonal periods: winter (November–

February), summer (March–June), and the rainy season (June–September). The infection rate was markedly higher during the rainy season (44.87%), whereas moderate during summer (15.02%), and the lowest incidence observed during winter (12.00%). This seasonal investigation showed a significant relationship between infection rates and the three distinct seasons ($p < 0.05$) (Table 6).

At the time of blood sampling cattle were stratified into three age groups (0–6 months, 7 months–3 years, and >3 years). Infection rates for *Theileria annulata* varied significantly depending on the age category ($p < 0.05$). Cattle aged more than 3 years showed the highest prevalence (37.41%), followed by animals aged 0–6 months (21.78%), whereas the 7 months to 3 years group exhibited the lowest prevalence (12.10%) (Table 3). The occurrence of bovine tropical theileriosis was evaluated in relation to the sex of the animals. A comparatively higher prevalence was observed among female cattle (24.94%) than males (10.66%) (Table 3).

The occurrence of bovine tropical theileriosis was evaluated in relation to the sex of the animals. A comparatively higher prevalence was observed among female cattle (24.94%) than males (10.66%) (Table 6). Nevertheless, the observed difference in infection prevalence between male and female cattle did not reach statistical significance (Table 4).

Breed-wise analysis revealed variation in the occurrence of bovine tropical theileriosis among the cattle examined. Holstein Friesian animals showed the highest prevalence (26.90%), followed by Jersey cattle (22.55%), while non-descript cattle had the lowest prevalence (10.60%). Theileriosis infection rates showed significant variation across cattle groups ($p < 0.05$) (Table 5).

The ticks collected from the examined cattle were preserved and examined under a stereomicroscope for morphological identification. Adult ticks were identified to species level using standard morphological taxonomic keys and published descriptions (Hoogstraal, 1956; Walker *et al.*, 2003; Soulsby 1982). Identification of *Hyalomma anatolicum anatolicum* was based on a combination of diagnostic characters rather than on a single feature. These included the relatively small, elongated body; long mouthparts; presence of eyes; short lateral grooves and distinct cervical grooves; sparse scutal punctation; pale annulations on the legs; characteristic morphology of coxae, particularly coxa I; and the configuration of the ventral plates. In male specimens, the subanal plates were positioned approximately in alignment with the adanal plates, which was used as an important distinguishing character. These characters were compared with descriptions of morphologically similar *Hyalomma* species to confirm the species identification. The collected specimens were identified as *H. Anatolicum anatolicum* based on

the concordance of these morphological characteristics with the standard taxonomic descriptions.

Discussion

Infection rates of bovine tropical theileriosis were studied across six chosen locations in the greater Tirupati area. A comparatively higher prevalence was observed among female cattle (24.94%) than among males (10.66%) however, the difference was not statistically significant. The higher occurrence in females may be associated with differences in physiological or managerial factors, although these factors were not specifically evaluated in the present study. The predominance of females in the study population, owing to their greater importance in dairy production, may also have contributed to the observed pattern. Similar sex-related variations in tick-borne infections have been reported by Sutherst *et al.* (1983), Kabir *et al.* (2011), Atif *et al.* (2012), Saleem *et al.* (2014), Naik *et al.* (2016), Sinha *et al.* (2021), and Patel *et al.* (2022). Lloyd (1983) proposed that hormonal influences, including changes in prolactin and progesterone, may affect susceptibility to theileriosis; however, these hormonal and reproductive factors were not assessed in the present study and therefore cannot be directly attributed to the observed difference.

Singh *et al.*, (2000) and Ogden *et al.*, (2002), highlighted the role of favourable macroclimatic conditions, particularly rain fall, humidity and temperature in determining

tick propagation and epidemiology of tick-borne diseases. Favourable climatic conditions increase the exposure of susceptible cattle, as reported from Ludhiana district of Punjab. Singh *et al* (2012), and Tuli (2015) reported that the occurrence of theileriosis is closely influenced by the geographic distribution of major tick vector, *Hyalomma anatolicum anatolicum*, especially across the arid and semi-arid areas of Punjab. Shashi kala and Bhawesh Gopal Deo (2018) indicated that vector abundance, reservoir hosts, and seasonal changes were important determinants of hemoprotozoan transmission in cattle. Regional differences in the occurrence of theileriosis have also been reported within Andhra Pradesh. Ram Naresh (2018) recorded prevalence values ranging from 0.81% in Vizianagaram to 46.15% in Anantapur and proposed that variation in vector distribution, environmental conditions, host susceptibility, and husbandry practices could account for the differences observed between districts. Muniraja *et al.*, (2021) reported a relatively high occurrence of haemo-parasitic infections among bovines in the Chittoor region of Andhra Pradesh and associated this pattern with favourable temperature and humidity conditions and increased tick activity. Thus, the differences observed among the study regions may reflect the combined effects of local climate, vector ecology, host-related factors, and cattle management practices.

The significantly higher occurrence of theileriosis in older cattle may be attributed to cumulative exposure to tick vectors and

repeated infection over time. This effect may be more pronounced in crossbred cattle, which may have comparatively lower resistance to tick-borne infections than indigenous breeds. In addition, increased exposure to managerial and environmental stressors may further predispose adult cattle to infection. A similar association between cattle age and the occurrence of bovine theileriosis has been reported by Kundave *et al.*, (2015), Abaker *et al.*, (2017), Sinha *et al.*, (2021), and Patel *et al.*, (2022).

Breed-wise analysis revealed variation in the occurrence of bovine tropical theileriosis among the cattle examined. Holstein Friesian animals showed the highest prevalence (26.90%), followed by Jersey cattle (22.55%), while non-descript cattle had the lowest prevalence (10.60%). Theileriosis infection rates showed significant variation across cattle groups ($p < 0.05$). Crossbred cattle may exhibit greater susceptibility to tick-borne haemoparasite infections than indigenous cattle under certain environmental, nutritional, and management conditions (Bock *et al.*, 2004). The observed breed-related variation is consistent with the findings reported by Naik *et al.* (2016) and Sinha *et al.* (2021).

This seasonal investigation showed a significant relationship between infection rates and the three distinct seasons ($p < 0.05$). The study area was characterized by three distinct seasons: winter (October to January), summer (February to May), and monsoon (June to September). The winter

season is associated with relatively lower temperatures and rainfall, which may reduce tick activity and vector abundance. The summer season is characterized by higher temperatures and variable humidity, whereas the monsoon season is marked by increased rainfall, warm temperatures, and high humidity. These environmental conditions may favour tick survival, proliferation, and activity, thereby facilitating the transmission of tick-borne pathogens. In the present study, the highest prevalence of bovine tropical theileriosis was observed during the monsoon season. This finding agrees with Vahora *et al.*, (2009), who reported the highest prevalence of theileriosis during the monsoon season (82.94%) in Gujarat. Similarly, Kohli *et al.*, (2014) recorded a prevalence of 45.4% during the rainy season in Uttarakhand, India. Brahma *et al.*, (2018) also reported the highest prevalence during the monsoon season (53.85%), followed by pre-monsoon (48.84%), winter (45.74%), and post-monsoon (42.27%) seasons in Assam. However, the present finding differs from reports in which the highest prevalence was observed during summer. Datta *et al.*, (1988) reported the highest incidence of theileriosis during summer (March–June; 9.34%), followed by the monsoon (July–October; 4.62%) and winter (November–February; 1.56%). Arun Raj *et al.*, (2019) reported the highest prevalence during summer (15.64%), followed by monsoon (10.53%) and winter (3.42%) in the sub-Himalayan region of northern India. Farooq *et al.* (2019) similarly observed the highest prevalence during summer (7.57%), followed by autumn (3.51%).

Limitations of the study

The present study has some limitations that should be considered when interpreting the findings. First, the study was conducted in and around Tirupati, Andhra Pradesh, and therefore the findings may not be directly generalizable to other geographical regions with different agro-climatic conditions, cattle populations, and tick distributions. Second, although the study covered a complete 12-month period from December 2022 to November 2023 and included seasonal observations, surveillance over multiple years would be required to assess inter-annual variation in *Theileria* transmission and tick population dynamics. Third, the cross-sectional nature of the study allows assessment of associations between infection and the factors evaluated but does not establish causal relationships. The study evaluated selected factors including age, sex, breed, season, and tick infestation; therefore, other potentially relevant epidemiological factors that were not assessed may also influence the occurrence of bovine tropical theileriosis. Information obtained from cattle owners through questionnaires may also be subject to recall or reporting bias, particularly for owner-reported management and clinical history. In addition, the study did not quantify tick infestation intensity or evaluate tick population dynamics or acaricide susceptibility. These factors may contribute to the epidemiology and transmission of *Theileria annulata* and warrant further investigation. Future studies involving larger and more representative cattle populations, multi-year surveillance,

detailed assessment of tick burden and seasonal tick dynamics, evaluation of acaricide susceptibility, and advanced molecular characterization of *Theileria* and tick populations are recommended to provide a more comprehensive understanding of the epidemiology and transmission of bovine tropical theileriosis in the region.

CONCLUSION

The present study demonstrated the occurrence of bovine tropical theileriosis in cattle in and around the Tirupati region of Andhra Pradesh, with molecular diagnosis confirming infection with *Theileria annulata*. The findings revealed variations in infection occurrence with respect to geographical area, age, breed and season, with a comparatively higher occurrence observed in older cattle, Holstein Friesian cattle breed and during the rainy season. These findings highlight the importance of continued local epidemiological surveillance for early detection and better

understanding of the distribution of bovine tropical theileriosis in the region. Regular clinical and molecular monitoring, timely diagnosis and appropriate treatment may contribute to reducing the impact of the disease. Further studies incorporating tick abundance, species distribution, infestation intensity and acaricide susceptibility are warranted to better characterize the role of tick vectors and to support evidence-based strategies for disease prevention and control in the region.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest for this manuscript

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Table.1. Details of blood samples collected from cattle in and around Tirupati.

Sanok	Characters		Number of blood samples collected	Total
1.	Age	0-6 months	179	524
		7 months -3 years	190	
		>3 years	155	
2.	Sex	Male	75	524
		Female	449	
3.	Breed	Holstein Friesian	223	524
		Jersey	235	
		Nondescript cattle	66	
4.	Season	Winter	175	524
		Summer	193	
		Rainy	156	
		Total		524

Table.2. Area-wise prevalence of bovine tropical theileriosis in cattle in and around Tirupati

S. No	Places	Number of samples screened	Found positive	Percentage
1.	Peruru	70	23	32.85 %
2.	Renigunta	63	15	23.8 %
3.	Puttur	78	23	29.48 %
4.	Tirupati	51	17	33.33 %
5.	Madanapalli	171	33	19.29 %
6.	Kuppambadur	91	09	9.89 %
	Total	524	120	22.9 %
Chi-square value		18.9898		
df		5		
P value		0.0019		

Table.3. Age-wise prevalence of bovine tropical theileriosis in cattle

Age	Number of blood samples screened	Found positive	Percentage
Calf (0-6 months)	179	39	21.78%
Heifer (7 months - 3 years)	190	23	12.10%
Adult (>3 years)	155	58	37.41%
Total	524	120	22.9%
Chi-square value	31.1628		
df	2		
P value	0.0001		

Table.4. Sex-wise prevalence of bovine tropical theileriosis in cattle

Sex	Number of blood samples screened	Found positive	Percentage
Male	75	08	10.66%
Female	449	112	24.94%
Total	524	120	22.9%
Chi-square value	1.14		
df	1		
P value	0.286		

Table.5. Breed-wise prevalence of bovine tropical theileriosis in cattle

Blood	Number of blood samples screened	Found positive	Percentage
Holstein Friesian	223	60	26.9%
Jersey	235	53	22.55%
Non descriptive cattle	66	07	10.6%
Total	524	120	22.9%
Chi-square value	7.8779		
df	2		
P value	0.00195		

Table.6. Season-wise prevalence of bovine tropical theileriosis in cattle

Season	Number of blood samples screened	Found positive	Percentage
Winter (Nov-Feb)	175	21	12 %
Summer (March-June)	193	29	15.02 %
Rainy (June -September)	156	70	44.87 %
Total	524	120	22.9%
Chi-square value	64.61		
df	2		
P value	<0.001		



Fig .1. Microscopic picture of *Hyalomma anatolicum anatolicum*

- a. Longirostrate mouth parts
- b. Sub traingular basis capitulum
- c. Deeply bifid first coxa
- d. Sub anal plates -away from mid axis



Fig.2. Pre scapular Lymph node enlargement



Fig.3. Severe tick infestation

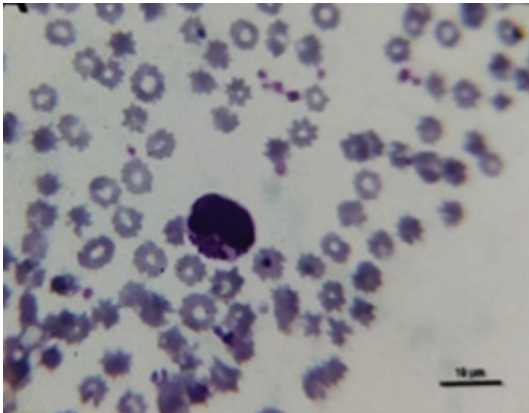


Fig.4.Intra-erythrocytic *Theileria* organisms

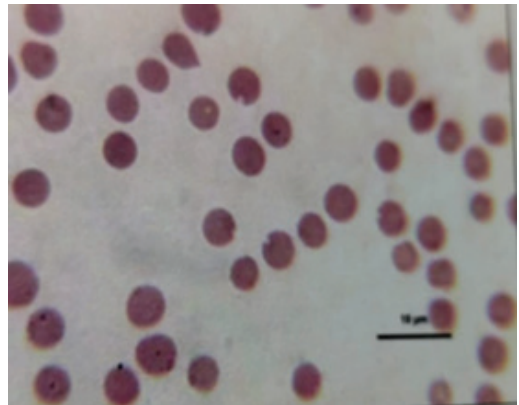


Fig.5.Koch blue bodies in Lymphocytes

ANNEXURE	
PROFORMA / QUESTIONNAIRE	
Name of the Owner	::
Location & Date	::
Breed Name	::
Sex	:: Male /Female
Age	:: Young (<one year) Adult (>1yr)
Presence of Ticks at the time of examination	:: Yes/No
Past History of Medication if any given	::
Body condition	:: Good /Fair/Thin
Blood smear examination	:: Yes /No
Tick	:: present /Absent
Tick control measure followed	:: Yes/No
Feeding History	:: Normal / Anorectic
Clinical Symptoms	::
Lymph node enlargement	:: Yes /No
Pallor mucous membrane	:: Yes/No
Pyrexia: Yes/No Respiratory distress	:: Yes /No
prick:yes/No Coughing	:: Yes /No
Lacrimation:yes /No Exophthalmia	:: Yes /No
Petechiae /ecchymoses	:: Yes /No
Latent recumbency	:: Yes /No
Submandibular / Ventrals edema	:: Yes /No
Diarrhea	:: Yes /No
Haemoglobinuria	:: Yes/No
Yellow mucous membrane	:: Yes /No

Fig.6. Annexure/ Proforma/Questionnaire

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