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Cross Sectional and Epidemiological Studies of Bovine Babesiosis in Two Types of Geographical Regions of Andhra Pradesh, India

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Abstract: The goal of the current study was to document the prevalence of bovine babesiosis in cattle across two different geographical regions of Andhra Pradesh from January 2023 to December 2024 and to assess the factors responsible for variations. Microscopic examination of stained blood smears revealed that overall prevalence of babesiosis was 21.87%, while the polymerase chain reaction assay, which targeted the 18s rRNA gene by creating an amplified product of 733 bp, revealed an overall prevalence of 38.54%. By microscopy, the highest prevalence of babesiosis was observed in Chittoor district (31.25%) compared to YSR Kadapa district of Andhra Pradesh (12.50%). By PCR, the prevalence was 22.08% in YSR Kadapa district and 55.00% in Chittoor district. The highest prevalence of babesiosis was recorded in Chittoor district, where the number of migratory cattle moving between the borders of Andhra Pradesh, Tamil Nadu, and Karnataka is high. In contrast, YSR Kadapa district has predominantly static cattle, which showed a lower prevalence of babesiosis. This may be a consequence of sudden climatic shifts in the two locations where cattle are frequently transported across state borders. The higher prevalence could also be attributed to farmers importing a greater number of purebred cattle. In addition, unauthorized cattle sales and purchases at border areas, along with inadequate health inspections by authorities prior to interstate transportation, may further contribute to the increased prevalence.

Key words: Babesia, prevalence, geographical variations, cattle.

Babesiosis is a haemoprotozoan disease that is prevalent in tropical and subtropical regions, including in India which is transmitted by vectors. It is caused by an intra-erythrocytic protozoan parasite that infects many domestic and wild animals (Salih *et al.*, 2015). Controlling the spread of disease agents from one animal to another is a basic concept in infectious disease epidemiology, even though avoiding the types of contact that lead to transmission (Hufnagel, 2004). A comprehensive understanding of global risk patterns is essential for assessing disease risks by species and by individual states, nations, and

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Table 1. Longitudinal and Latitudinal extents of geographical locations in Chittoor district of Andhra Pradesh

Geographical location	Number of cattle	Latitudinal	Longitudinal
Thotakanuma	30	13.075110	78.526368
Sanipalle	30	13.102896	78.532886
Ramasamudram	30	13.097091	78.524574
Kambhampalle	30	13.122970	78.547410
Alapalle	30	13.151667	78.556561
Gundugallu	30	13.234244	78.583083
Gandrajupalle	30	13.193339	78.582024
Rajupalle	30	13.294975	78.58442

countries. However, literature on the prevalence of babesiosis in two distinct geographical settings: (i) an interstate border region with a high proportion of migratory cattle (Chittoor district, located between Andhra Pradesh, Tamil Nadu, and Karnataka), and (ii) a region with predominantly static cattle (YSR Kadapa district) - is very limited in India. Therefore, the present study was undertaken to document the prevalence of babesiosis in these contrasting geographical regions of Andhra Pradesh of India.

Materials and Methods

The study was conducted on the cattle presented to the various veterinary dispensaries in and around Chittoor district and YSR Kadapa district of Andhra Pradesh for a period of two years from January 2023 to December 2024. Cattle presented with clinical signs of haemoprotozoan diseases i.e., pyrexia, anaemia, tick infestation, lymphadenopathy and haemoglobinuria were included in the present study. Babesiosis was confirmed by stained blood smear examination and further confirmed by polymerase chain reaction to record the prevalence. The prevalence of babesiosis were determined by taking the total number of animals into consideration. For diagnosis of babesiosis, peripheral blood smear was prepared by collecting a drop of blood from the ear vein. Carried out the Giemsa staining after blood smears were air dried and examined for haemoprotozoans under oil immersion lens of microscope and confirmation of the *Babesia* was done in accordance with standard morphology (Sivajothi *et al.,* 2023). Further confirmed by Polymerase chain reaction (PCR) assay. Blood samples were collected in EDTA coated blood collection vials for the genomic DNA extraction (Almeria *et al.,* 2001 and Sivajothi *et al.,* 2019). All samples were individually processed for isolation of total DNA by using QIAamp DNeasy Blood & Tissue Kit (QIAamp DNA Mini Kit). All the steps were employed as per the manufacturer's instructions except at elution step where in instead of 200 µl AE buffer, 50 µl of nuclease free (dq) water was added to preserve the DNA samples. Places of samples collection from the different villages were at the interstate borders of Andhra Pradesh, Tamil Nadu and Karnataka and in YSR Kadapa district of Andhra Pradesh where static cattle population was there (Table 1 and 2) (Institutional animal ethical number S.No.14 of Proc No. 281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/057/Medicine 2022 Dated:19.11.2022).

Results and Discussion

During the research period from January 2023 to December 2024 at different veterinary

Table 2. Longitudinal and Latitudinal extents of geographical locations in YSR Kadapa district of Andhra Pradesh

Geographical location	Number of cattle	Latitudinal	Longitudinal
Payasampalli	30	14.538389	78.536636
Thallama puram	30	14.711920	78.593775
Korrapadu	30	14.821154	78.579010
Chapadu	30	14.730719	78.658162
Obul reddy peta	30	14.699805	78.695471
Chinna guruvalur	30	14.692841	78.694358
Vedurur	30	14.708324	78.681008
Sannapalli	30	14.672622	78.687768

dispensaries in and around Chittoor (Chittoor district of Andhra Pradesh), and around Proddatur (YSR Kadapa district of Andhra Pradesh) total number of 480 cattle were included based on the clinical signs suggestive of haemoprotozoans. Out of which, 105 cattle were confirmed for babesiosis based on the microscopic examination of stained blood smears. The comprehensive prevalence of babesiosis based on the Giemsa-stained peripheral blood smears examination was 21.87%. Presence of babesiosis was confirmed by the presence of intra-erythrocytic piroplasm of *Babesia* in the form of pyriform or pear-shaped. The gDNA extracted from 480 blood samples were amplified by PCR using oligonucleotide primers designed for repetitive DNA sequence probe 18 s rRNA gene. The PCR cycle conditions were optimized by setting different time-temperature combinations for annealing and extension process. Based on amplified product of 733 bp, 185 (38.54%) gDNA samples were positive for *Babesia bigemina* with specific PCR signals as a distinct 733 bp band on agarose gel. The prevalence of babesiosis was highest in Chittoor district of Andhra Pradesh (55.00%) and in YSR Kadapa district (22.08%) region of cattle by polymerase chain reaction assay. Which was significantly difference between the two separate regions in the present study (P=0.001).

The prevalence of babesiosis was 21.87% based on the demonstration of *Babesia* organisms in the Giemsa-stained peripheral blood smears examination. The prevalence of babesiosis in Chittoor district of Andhra Pradesh based on the Giemsa-stained peripheral blood smears examination was 31.25%. The prevalence of babesiosis in YSR Kadapa district of Andhra Pradesh based on the Giemsa-stained peripheral blood smears examination was 12.50%. The cattle with babesiosis were categorized into four different age groups which comprised of heifers, 1st to 3rd calving, 3rd to 5th calving and 5th to 7th calving to know the age wise prevalence of babesiosis in cattle. In the present study, 15.25% (16/105), 45.71% (48/105), 25.71%, (27/105) and 13.3% (14/105) were found positive for babesiosis in cattle of age group heifers, 1st-3rd calving, 3rd-5th calving and 5th-7th calving respectively. The higher positivity was noticed in cattle under 1st-3rd calving followed by cattle under 3rd-5th calving. The cattle with babesiosis were categorized into five different stages of pregnancy which comprised of heifers, 1st trimester of pregnancy, 2nd trimester of pregnancy, 3rd trimester of pregnancy and recently calved to know the stage wise prevalence of babesiosis in cattle. In the present study, 15.24 % (16/105), 6.66 % (7/105), 11.43 % (12/105), 40.00 % (42/105) and 26.67 % (28/105) were found positive for babesiosis in cattle of stage of heifers, 1st trimester of pregnancy, 2nd trimester pregnancy, 3rd trimester pregnancy and recently calved respectively. The higher positivity was noticed in cattle with 3rd trimester of pregnancy followed by cattle which were calved recently. The cattle with babesiosis were divided into three different groups which includes Jersey cross, Holstein Friesian cross and non-descriptive (Desi) to assess the breed wise prevalence. In the present study, 52.38% (55/105), 34.29% (36/105) and 13.33% (14/105) were found positive for babesiosis in Jersey cross, Holstein Friesian cross and non-descriptive (Desi) respectively. The higher positivity of babesiosis was noticed in Jersey cross, followed by Holstein-Friesian cross breed. Present study was carried out into four different seasons which includes winter season (December to February), summer season (March to May), monsoon season (June to September) and post monsoon season (October to November) to assess the season wise prevalence of babesiosis. In the present study, 19.04% (20/105), 26.67% (28/105), 42.86% (45/105) and 11.43% (12/105) were found positive for babesiosis in winter season, summer season, monsoon season and post monsoon season respectively. The higher positivity of babesiosis was noticed in monsoon season followed by summer season. Prevalence of babesiosis was 81.90% (86/105) and 18.10% (19/105) in cattle with tick infestation and cattle without tick infestation respectively. The higher positivity was noticed in cattle with tick infestation for babesiosis and there was statistically significant relationship observed (P=0.001) between tick infestation and occurrence of babesiosis.

Presence of pear shape piroplasm inside red blood cells is confirmative of diagnosis especially in acute stages of the disease. Ganzinelli *et al.* (2019), Saini *et al.* (2022), Masih *et al.* (2022), Ahmad *et al.* (2023) examined Giemsa-stained blood smears under a light microscope and identified structures morphologically consistent with *Babesia* piroplasms within erythrocytes for confirmation of *Babesia bigemina* in cattle. The gDNA extracted from 480 blood samples were amplified by PCR using oligonucleotide primers designed for repetitive DNA sequence probe pMUTec 6.258 gene. The PCR cycle conditions were optimized by setting different time-temperature combinations for annealing and extension process. Based on amplified product of 733 bp, 185 (38.54%) gDNA samples were positive for *Babesia bigemina* with specific PCR signals as a distinct 733 bp band on agarose gel. Sharma *et al.* (2016) determined the prevalence by using PCR targeting the specific 18S rRNA gene to differentiate between clinically and sub-clinically babesia infected animals. Hashem *et al.* (2018) conducted PCR testing newly designed primer set successfully amplified a 733 bp region of the 18S rRNA in all piroplasms of *B. bigemina*.

Khan *et al.* (2016) recorded the overall prevalence of *Babesia bigemina* as 20.66%. Maharana *et al.* (2016) reported, out of 254 and 226 suspected blood samples the prevalence was 37.00% and 38.93% in cattle and buffaloes respectively. Krishna Murthy *et al.* (2016) reported, out of 300 blood samples examined of which babesiosis accounted positive for 27 (12.5%). Vetrivel *et al.* (2017) documented the overall prevalence of babesiosis as 20.27%. Matheus *et al.* (2019) conducted a study on 392 blood samples from Sanga cattle in the Ohangwena region of Namibia, they found that the overall prevalence of *B. bigemina* in cattle was 36.5%.

Rather *et al.* (2015) reported highest prevalence in 1-5 years age group cattle. Kumar *et al.* (2018) found that cattle older than 3 years were more susceptible to infection (45.42%). Barman *et al.* (2018) prevalence mostly seen in age around 2.5-7.5 years cattle (10.48%). Shah *et al.* (2020) infection was in 2-5 years age group (9.48 %) followed by 7.47% in above 5 year age group, 6.48% in 6 months to 2 year age group while 4.18% in calves below 6 months. Khan *et al.* (2016) investigated the prevalence of babesiosis in cattle aged 0 to 2 years. The results were consistent with those of Ayaz *et al.* (2013) from Pakistan, who found that the prevalence was highest in elderly animals (13.4%) followed by adult animals (11.7%) and lowest in young animals. The findings of this study, however, are at odds with those of Amorim *et al.* (2014), who found that calves were more vulnerable to *Babesia* species than adult cows. This discrepancy may result from the fact that young animals, especially younger than six months have pre-immunity, maternal immunity from colostrum feeding making them marginally more resistant to infection than older animals. However, the lower prevalence in young animals is ascribed to their restricted grazing, which is likely to lessen their exposure to disease vectors (Kamani *et al.,* 2010). Because older animals can maintain their immunity and are less likely to contract infections again, the higher significant infection rate seen in animals younger than three years old can be explained. Since acquired immunity makes animals resistant to infection and produces a fully protected adult herd, it plays a critical role in maintaining enzootic stability (Awad *et al.,* 2011). Young calves are said to have a robust innate immunity against *Babesia bovis* infection, which lasts for about six months after birth and is eliminated when the spleen is removed.

Shah *et al.* (2020) recorded haemoprotozoan infections higher in lactating (10.30%) cows than pregnant cattle (7.46%). Babesiosis was more common in nursing animals than in non-lactating animals. Hormonal and immunological changes that occur during lactation are most likely the cause of higher infection rates in nursing animals (Kumar *et al*., 2010).

The higher positivity was noticed in Jersey cross with babesiosis, followed by Holstein-Friesian cross breed. Rather *et al.* (2015) found highest 56.71% prevalence in exotic cattle. Kumar *et al.* (2018) observed crossbred Holstein Friesian cattle were more prone to the disease (47.03%). Shah *et al.* (2020) identified Crossbred cattle (10.19%) were found to be more susceptible to haemoprotozoan infections than indigenous cattle (5.92%).

The higher positivity of babesiosis was noticed in monsoon season followed by summer season. Ibrahim *et al.* (2012) reported the prevalence of *Babesia bovis* in cattle highest prevalence in summer (39.13%) followed by autumn (9.24%) and least in winter (8.80%). Atif *et al.* (2012) reported the month wise prevalence was recorded and it was highest in June month. Chaudhri *et al.* (2013) recorded the season wise, *B. bigemina* were recorded in higher percentage of cross-bred cows during summer (1.60%) and rainy season (1.10%). Jyothisree *et al.* (2013) recorded the with high seasonal prevalence during monsoon (10.76%) followed by summer (7.40%) and least during winter (4.65%). Kumar *et al.* (2018) conducted highest prevalence was observed during the rainy season (58.55%). Barman *et al.* (2018) conducted season wise prevalence mostly seen monsoon season (13.54%). Malyar and Farid (2018) high during summer (68.75%), followed by moderate in autumn and spring (62.50%), (64.58%) and compare to winter (56.25%) seasons. Shah *et al.* (2020) conducted winter season (9.96%) followed by post monsoon (9.38%), summer (8.03%) and monsoon (4.48%). Khan *et al.* (2020) identified the higher prevalence during the summer season. Masih *et al.* (2022) showed that in summer, 24.05% of buffaloes and 29.40% of cows were infected with *B. bovis*. However, in the winter, the infection rates dropped to 5.06% in buffaloes and 8.88% in cows. The rainy season showed the highest prevalence and it was noticed that babesiosis was significantly influenced by the season. This may be because high temperatures and high humidity during the rainy season encourage the growth of ticks, which makes it easier for *Babesia* to spread to uninfected animals quickly and easily. The current results are consistent with those of studies that reported similar findings regarding seasonal prevalence, such as Kumar *et al.* (2010). These findings were consistent with those of Kamani *et al.* (2010), who found that cows had the highest rates of babesiosis infection in the summer and fall, while the lowest rates occurred in the spring and winter. This suggests that temperature, high humidity, and other critical factors have recently affected the rate and seasonal variation of vectors. According to Solomon *et al.* (2003), the prevalence and intensity rate of tick-borne hemoparasitic disease infestation were generally higher during the rainy season and lower during the dry season. The higher positivity was noticed in cattle with tick infestation for babesiosis but, there was statistically significant relationship observed between tick infestation and occurrence of babesiosis. Furthermore, it was frequently observed during this study that animals with poorer body conditions had a higher burden of ectoparasites, or ticks, than animals with better body conditions. This can lead to an increased risk of *Babesia* infection.

Out of 480 cattle examined in the present study, the prevalence detected by microscopic examination of stained smears was 21.87%, whereas polymerase chain reaction (PCR) detected a prevalence of 38.54% through the development of an amplified product of 733 bp. The highest prevalence was recorded in Chittoor district of Andhra Pradesh (31.25%) compared with YSR Kadapa district (12.50%) by microscopy. By PCR assay, the prevalence was 55.00% in Chittoor district and 22.08% in YSR Kadapa district. The higher prevalence of babesiosis in cattle from Chittoor district may be attributed to the larger number of migratory cattle in this region, compared with YSR Kadapa district, where most cattle are stationary. It could also be the result of abrupt climatic changes in the two areas and the movement of cattle across state borders. The higher prevalence may further be associated with the frequent importation of pure breeds by farmers. Cattle sales and purchases at the borders often occur without proper permission or adequate health assessment conducted by authorities prior to interstate transportation. Infectious diseases can spread from one host to another through several mechanisms, including direct contact, airborne transmission, and vector-borne routes. Although preventing the types of contact that facilitate transmission may be a more sensible strategy, controlling the spread of disease agents from one animal to another remains a fundamental principle in infectious disease epidemiology (Hufnagel, 2004). Understanding disease risks by species and by nation requires a comprehensive knowledge of risk at the global level. This understanding must be supported by an effective worldwide surveillance network that regularly screens various animal species, particularly before they leave their regions of origin. Disease threats should ideally be controlled locally, rather than after movement has occurred, which necessitates fine-scale information on the point of origin as well as the species and volume of animals moved. Predicting patterns of human infectious disease spread requires knowledge of movement routes, and similar information is essential for understanding the risks of animal diseases (Thiermann, 2004). Pathogens, hosts, and likely emergence risks are already listed in databases (Ecker, 2005); additional layers of data on host species movement and their influence on emergence risks should be incorporated, and these data should be made publicly accessible. Such international collaboration and disease control at the global level will lead to improved risk management and mitigation.

Host factors associated with disease include age, breed and immune status (Jabbar *et al.,* 2015). *Bos indicus* breeds of cattle are more resistance to babesiosis than *Bos taurus*. Because of natural selection pressure, indigenous populations, having lived for a long time with local ticks and tick-borne diseases, have developed either an innate resistance or an innate ability to develop a good immune response to the tick or tick-borne hemoparasitic disease in question. It is frequently stated that there is an inverse age resistance to babesia infection in that young animals are less susceptible to babesiosis than older animals; the possible reason is passive transfer of maternal antibody via colostrum. The severity of the clinical babesiosis increases with age (Taylor *et al.,* 2007). There is a seasonal variation in the prevalence of clinical babesiosis, the greatest incidence occurring soon after the peak of the tick population. From the climatic factors, air and temperature is the most important because of its effect on tick activity; higher temperatures increase its occurrence. Heaviest losses occur in marginal areas where the tick population is highly variable depending on the environmental conditions and babesiosis infection in cattle mostly reaches peak in summer (El Moghazy *et al.,* 2014).

Conclusion

The present study demonstrates that babesiosis is prevalent among cattle in Chittoor and YSR Kadapa districts of Andhra Pradesh, with PCR-based detection (38.54%) revealing a higher prevalence than microscopy (21.87%), highlighting the superior sensitivity of molecular diagnostics. The prevalence was significantly higher in Chittoor district compared to YSR Kadapa, which may be associated with the larger population of migratory cattle, frequent importation of pure breeds, and regional climatic variations. Host factors such as age, breed, and reproductive status influenced susceptibility, with higher infection rates observed in cattle under the 1st - 3rd calving stage, during the 3rd trimester of pregnancy, and in Jersey cross breeds. Seasonal trends were evident, with the highest prevalence recorded during the monsoon season, likely due to increased tick activity. Tick infestation was strongly correlated with babesiosis occurrence, underscoring the role of vectors in disease transmission.

The findings emphasize the importance of regular surveillance, early diagnosis using sensitive molecular tools, and effective tick control measures to mitigate the spread of babesiosis. Additionally, controlling animal movement, monitoring crossbred cattle, and strengthening local and regional disease management strategies are crucial to reduce infection risk. These results provide essential epidemiological insights to support targeted prevention and control programs for babesiosis in Andhra Pradesh and similar endemic regions.

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