RESEARCH ARTICLE

Molecular Prevalence of Babesiosis in Buffaloes of North Gujarat

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Abstract

Babesiosis is a tick-borne disease caused by haemo-protozoan parasites of the genus *Babesia* with four main species, *viz., Babesia bigemina, Babesia bovis, Babesia major* and *Babesia divergens* and is characterized by significant morbidity and mortality worldwide. The present study was conducted to investigate the prevalence of *B. bigemina* in water buffaloes of North Gujarat. Ticks mostly present in the study area are *Rhiphicephalus (Boophilus)* spp. and *Hyalomma* spp., etc. A total of 223 blood samples were drawn from buffaloes of different age groups during October 2022 to September 2023 and analysed by microscopic and PCR techniques. The overall prevalence of *Babesia bigemina* infection in buffaloes was 6.72 (15/223) % by conventional microscopic method and 9.86 (22/223) % by PCR. No blood samples were found positive for other *Babesia* spp. by microscopic as well as by PCR based assay. Babesiosis was found to be more common in age group of 1-3 years old followed by 3-5 and > 5 years old buffaloes, with calves below < 1 year showed the least infection. Summer season had highest prevalence followed by monsoon and winter seasons. The haematological and biochemical parameters were found to be non-significantly altered, except the levels of Hb, TEC, PCV, which were found to be significantly decreased and the levels of WBC, granulocytes were found significantly elevated.

Key word: Babesiosis, Buffalo, Molecular detection, North Gujarat, Prevalence.

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Introduction

Gujarat is a leading milk producer in India, supplying about 12.7 million tons per year whose major contributors are cattle and buffaloes. The total population of livestock in the nation is about 535.78 million, where buffaloes are 109.85 million. Gujarat alone is contributing 1,05,43,250 buffaloes of which Banaskatha district of North Gujarat stands out with 15,01,537 buffaloes. But one of the seriously threatened challenges that the state facing is the occurrence of babesiosis, with clinical or subclinical form and occasionally led to mortality (Taylor *et al.,* 2007). These chronic haemoprotozoan infections constantly pose serious health risks to animals. Haemotropic protozoa *Babesia*, belongs to the members of the phylum Apicomplexa of family Babesiidae and order Piroplasmida. Four species are mainly responsible for babesiosis in buffalo: *Babesia bigemina, Babesia bovis, Babesia major,* and *Babesia divergens,* of which *Babesia bovis* and *B. bigemina* are mainly accountable for babesiosis in buffaloes worldwide (Suarez and Noh, 2011). The primary agent responsible for babesiosis in buffaloes is the brevirostrate tick, *Rhipicephalus (Boophilus) microplus* and *Hyalomma* spp. that cyclically transmit the disease in animals (Ravindran *et al*., 2002).

The application of PCR-based assays for epidemiological studies has high sensitivity and specificity for the detection of infection (Singh *et al*., 2012) as compared to microscopy due to low sensitivity (Bose *et al.,* 1995). *Babesia* species in carrier stage can be distinguished using the PCR technique, which permits identification at parasitemia levels ranging from 0.001 % to 0.0000001 % (1 parasite in 10^9 RBCs) (Buling *et al*., 2007). Figueroa *et al*. (1992) and Fahrimal *et al*. (1992) reported first use of PCR for the detection of *B. bigemina* and *B. bovis*. This study was aimed to investigate the molecular prevalence of babesiosis in buffaloes of North Gujarat.

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Materials and Methods

Geographical Area and Sample Collection

The study was conducted in Banaskantha district of Gujarat (24.3217° N, 72.3177° E). The climate of the region is subtropical with annual rainfall of 33 to 152 cm and average maximum and minimum temperatures of 37°C to 44°C and 10°C to 15.5°C, respectively, which is conducive for the optimal development and propagation of the vector ticks involved in the transmission of various haemoprotozoan diseases.

A total of 223 blood samples were drawn from buffaloes of different age groups during October 2022 to September 2023 from sites including organized farms, Gausalas, Panjrapole, and veterinary clinics. Regardless of age, breed, or season, all animals received comprehensive physical and clinical examinations, with careful noting down of any clinical signs. Appropriate consent was obtained from each owner prior to blood collection. Animals were divided into four age groups, <1 year, 1-<3 years, 3-<5 years, and > 5 years to determine the prevalence of babesiosis. The year was divided into 3 seasons according to local climatic conditions, *viz.,* summer, monsoon and winter.

From the collected blood samples, thin blood smears were made, air dried, fixed in methanol for 5 min and stained with working dilution of 10 % Giemsa stain for 30-45 min and washed with tap water, air dried and examined under oil immersion for demonstration of *Babesia* piroplasms, if any. Whole blood samples of 30 infected and 30 healthy animals were also used for haematological analyses on auto-analyzer. In order to separate serum, blood samples were also taken in clot activator vials and centrifuged for 15 min at 2000 g. The serum samples were stored at -20°C until used for biochemical examination using standard procedures and assay kits on biochemistry analyzer. Blood samples were further stored at -20° C for DNA extraction.

Genomic DNA Isolation

DNA was extracted from whole blood samples using the QIAamp® DNA Blood Mini Kit (Thermo Scientific, Lithuania; Qiagen, Germany) according to the manufacturer's instructions. Initially, 200 µL of whole blood was mixed with 200 µL of RBC lysis solution and 20 µL of proteinase K to disrupt the red blood cells and release the parasites into the medium. After vortexing and incubating the mixture in a water bath for ten min, ethanol was added, vortexed again, and the solution was loaded into a QIAamp Mini spin column provided in the kit. The column was then centrifuged at 6000 g with buffers AW1 and AW2 for one min, and the supernatant was discarded, leaving the residual liquid containing the parasites of interest (e.g., *Babesia* spp.). Subsequently, 100 µL of elution buffer AE was added to the column, incubated at room temperature for 1 min, and centrifuged again at 6000 g for one min. The extracted genomic DNA was then stored at -20°C for further analysis. As a positive control, genomic DNA from *B. bigemina* was isolated from infected animals blood showing high parasitemia and typical symptoms such as fever and coffee-colored urine etc. Genomic DNA was also isolated from the whole blood of infection free buffalo and used as a negative control.

PCR Assays

The classical PCR assays were carried out using the sequences of oligonucleotide primers specific for *B. bigemina* as described by Figueroa *et al*. (1992). The 278 bp nucleotide sequence of *Babesia bigemina* was amplified using specific primers, Forward (Bbig278F): 5'CATCTAA TTTCTCTCCATACCCCTCC3' and Reverse (Bbig278R): 5'CCTCGGCTTCAACTCTGATG CCAAAG3' (Bhat *et al*., 2015).

Two rounds of PCR in a final volume of 25 µL were carried out in a PCR thermal cycler (Eppendorf, Germany). In the classical PCR assay, the master solution consisted of 12.5 µL, 2 x Dream *Taq* Green PCR master mix (2X Dream *Taq* DNA Polymerase, 2X Dream *Taq* Green buffer, dNTPs, and 4 mM $MgCl_2$), 1 µL each (15 pmol) of the external forward and external reverse primers and 2 µL of template DNA isolated from field samples. The volume was made up to 25 µL with 8.5 µL nuclease-free water. The cycling conditions were: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 45s, and extension at 72°C for 30s and the final extension was performed at 72°C for 4 min. The PCR products were checked for amplification by electrophoresis on 1.2 % agarose gel and visualized using gel documentation system (Life Technologies, USA).

The data on prevalence were analyzed using descriptive statistics, and those on haemato-biochemical parameters by paired 't' test (Snedecor and Cochran, 1994).

Results and Discussion

Examination of Giemsa-stained thin blood smears revealed 6.72 % (15/223) animals positive for *B. bigemina* (Fig. 1). Further, in order to assess the true status of *B. bigemina* infection in buffaloes, all the samples were analysed by classical PCR. Of the total samples subjected to classical PCR, 9.86 % (22/223) were found to be positive for *B. bigemina* infection with an amplification size of 278-bp product (Fig. 2). The review of literature has shown varying degree of babesiosis ranging from 0.76 to 18.50% in India. The present observations of 15 (6.72 %) buffaloes found positive for *Babesia* spp. by using a conventional microscopic method and 22 (9.86 %) through PCR techniques were in line with the findings of Sharma *et al*. (2016), Sawitri *et al.* (2021), Borthakur *et al.* (2023) and El-Alfy *et al.* (2023). However, Khetran *et al.* (2019), Zaman *et al* (2022) and Muraleedharan *et al.* (2005) found higher prevalence rate of *Babesia* spp. infection in buffaloes than present findings. Buffaloes are comparatively less susceptible to tick infestations than cattle, which leads to a lesser chance of haemoprotozoan infections, such as babesiosis.

In developing countries like India considerable economic losses occurs in large and small holding livestock farming system due to haemoprotozoan diseases. In the present study, high prevalence of *Babesia bigemina* (9.86 %, 22/223) can be attributed to *Rhipicephalus (Boophilus) microplus* being the only tick which infests the buffaloes of Banaskantha district of Gujarat. The PCR primers used in the present assay did not amplify any product when the genomic DNA of *T. annulata*, were used as template revealing the specificity of these primers for detection of *B. bigemina* infections in buffaloes

In the present study, it was observed that the rate of babesiosis declined as the age of animals advanced. Data presented in Table 1 revealed that age wise highest infection rate was observed in 1-<3 year old buffaloes followed by 3-5 years-old age group and 5 years and above age group by microscopy and PCR methods According to Benitez *et al.* (2018), buffaloes have inherent genetic features to have less parasitic infection. Older animals were less likely to be affected with babesiosis than younger animals. An inversely proportionate association between the age of the animal and the prevalence of infection was also reported by Simking *et al.* (2013) and Choramo and Ibrahim (2017). According to Zaman *et al*. (2022), babesiosis was more common in calves over 16 months of age (23.67 %) than adult animals (16.40 %), and the prevalence was lowest in the physiologically non-active (acyclic, non-pregnant, weaned) group (19.7 %) and highest in the immature group (41.4 %).

Table 1: Prevalence of *Babesia* infections in buffaloes according to age

Age group	No. of samples	No. of sample positive by	
		Microscopically	By PCR
1-<3 year	102	08 (7.84%)	11 (11.22%)
3-<5 year	63	04 (6.34%)	07 (11.11%)
>5 year	51	03 (5.88%)	04 (7.84%)
Overall	**223**	**15**	**22**

Season wise, both microscopy and PCR gave highest incidence during summer followed by monsoon. Interestingly, animals showed no incidence of babesiosis in winter in either of the techniques (Table 2). The high incidence rate during the summer can be ascribed to the hot, muggy weather, which is ideal for tick reproduction (Khetran *et al*., 2019; Ahmad *et al*., 2023).

Table 2: Prevalence of *Babesia* infections in buffaloes according to season

Particulars	Total samples collected	Winter	Summer	Monsoon
Total No. of samples collected	223	40	120	63
PCR positive buffalo	22	00 (0%)	16 (72.73%)	6 (27.27%)
Microscopic positive buffalo	15	00 (0%)	11 (73.33%)	4 (26.67%)

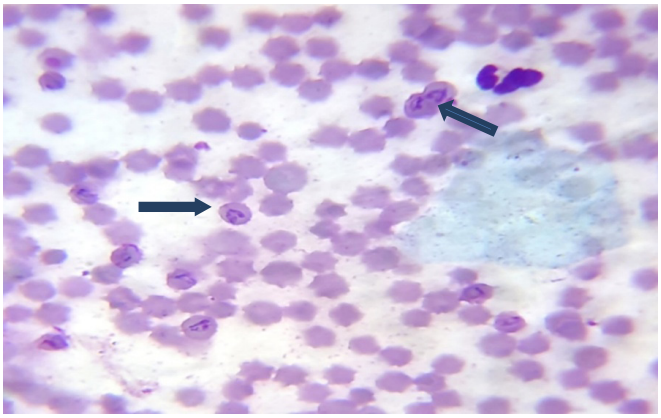

Fig.1: *B. bigemina* within infected RBCs under Giemsa staining

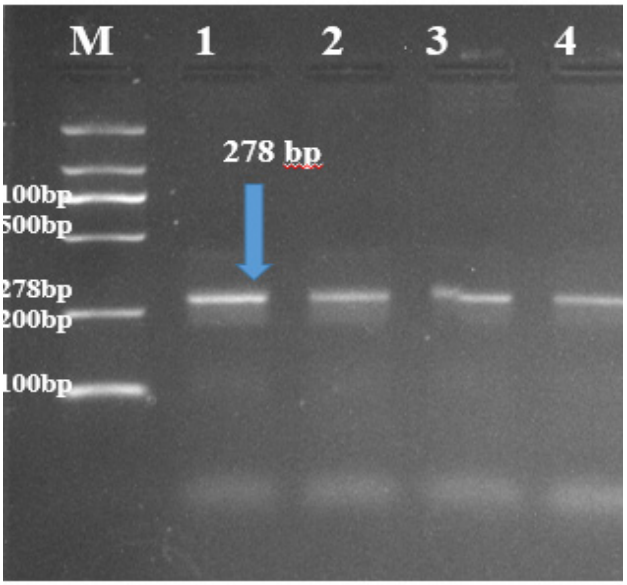

Fig. 2: PCR amplicons resolved in 1.2 % agarose gel showing *B. bigemina* positive [M= Marker (100 to 1000 bp), No. 1, 2, 3, 4 Positive samples of buffaloes at 278 bp

In the present study, the levels of haemoglobin, red blood cells (RBC) and packed cell volume (PCV) were found to be significantly ($p<0.05$) lowered, and the levels of WBC and granulocytes were significantly higher in infected buffaloes than the non-infected ones. Similar findings were also reported by Khinchi *et al.* (2022) in Udaipur, India and by Ahmad *et al.* (2023) in Pakistan. However, the levels of monocytes, platelets, MCV, MCH, MCHC, and lymphocytes did not alter significantly in babesiosis infected animals compared to healthy ones (Table 3). The piroplasm may have destroyed the erythrocytes, which leads to the lowered erythrocyte count. These findings were in agreement with Rani *et al.* (2010). Significantly lowered mean Hb and PCV levels observed in the present study, diseased animals might be due to erythrophagocytosis and erythropoietic suppression as opined by Mahmoud *et al.* (2015), while increased mean white blood cells could be due to the stimulation of phagocytic cells like lymphocytes and monocytes associated with breakdown of RBC for removal of the toxic remnants of damaged

Table 3: Haemato-biochemical alterations in buffaloes infected with *B. bigemina* (Mean ± SE)

Parameters	Infected animals (N=30)	Healthy animals (N=30)	P value
Hematological parameters			
Hb (g/dL)	9.74±0.41	11.1±0.47	<0.05
RBC $\times 10^6$ /µL	6.25±0.49	7.78±0.41	<0.05
PCV (%)	22.17±0.49	24.22±0.57	<0.05
WBC $\times 10^3$/µL	10.99±0.57	8.46±0.51	<0.05
Lymphocytes (%)	6.83±0.57	6.25±0.58	NS
Granulocytes (%)	5.6±0.39	3.74±0.48	<0.05
Monocytes (%)	3.01±0.55	2.71±0.54	NS
PLT $\times 10^3$/µL	123.7±8.67	110.13±8.38	NS
MCV (fL)	51.19±1.52	52.44±1.54	NS
MCH(pg)	16.90±0.43	16.57±0.32	NS
MCHC (g/dL)	40.27±0.72	39.23±0.64	NS
Serum biochemical parameters:			
ALT (IU/L)	27.22±2.53	26.22±2.15	NS
AST (IU/L)	111.49±6.95	102.03±5.41	NS
GGT (IU/L)	12.53±3.54	10.38±2.89	NS
BUN (mg/dL)	23.23±1.82	19.26±1.44	<0.05

P value <0.05= Significant at 5%, NS= Non-significant

erythrocytes (Guglielmone *et al.,* 1996) and activation of body defense mechanisms for antibodies production against *Babesia* infection (Court *et al.,* 2001).

Serum biochemical parameters analysed revealed significantly ($p<0.05$) higher mean value of BUN with other parameters exhibiting non-significant alterations (Table 3). The elevated level of BUN was attributed to the degraded byproducts of damaged RBCs, which were deposited in the kidneys during glomerular filtration and hampered the excretion of BUN (Ahmad *et al*., 2023).

Conclusion

The overall prevalence of *Babesia bigemina* in buffalo population of North Gujarat was 6.72 (15/223) % by conventional microscopic method and 9.86 (22/223) % by PCR. Microscopy is a less sensitive and less specific test which needs to be replaced with novel molecular diagnostic tests such as PCR. The prevalence of *babesiosis* was found to be highest in 1-3 year age group followed by 3-5 and > 5 years and lowest in calves < 1 year old. Summer showed the highest prevalence followed by monsoon and none in winter. Infected buffaloes showed significant alteration in haematological parameters with increased concentration of BUN.

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References

Ahmad, A., Ali, Z., & Lashari, M.H. (2023). Study on spatio-temporal prevalence and hematological attributes of bovine babesiosis in cattle population of Layyah, Southern Punjab, Pakistan. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *75*, 787-799.

Benitez, D., Mesplet, M., Echaide, I., de Echaide, S. T., Schnittger, L., & Florin-Christensen, M. (2018). Mitigated clinical disease in water buffaloes experimentally infected with *Babesia bovis*. *Ticks and tick-borne diseases*, *9*(5), 1358-1363.

Bhat, S.A., Singh, H., Singh, N.K., & Rath, S.S. (2015). Molecular detection of *Babesia bigemina* infection in apparently healthy cattle of central plain zone of Punjab. *Journal of Parasitic Diseases*, *39*, 649-653.

Borthakur, U., Ram Verma, M., Singh, Y. P., Kumar, S., Kumar, D., Bangar, Y. C., & Dhama, K. (2023). Prevalence of babesiosis in bovines of India: A meta-analytical approach for 30 years (1990-2019). *Veterinary Quarterly*, *43*(1), 1-9.

Bose, R., Jorgensen, W.K., Dalgliesh, R.J., Friedhoff, K.T., & De Vos, A.J. (1995). Current state and future trends in the diagnosis of babesiosis. *Veterinary parasitology*, *57*(1-3), 61-74.

Buling, A., Criado-Fornelio, A., Asenzo, G., Benitez, D., Barba-Carretero, J.C., & Florin-Christensen, M. (2007). A quantitative PCR assay for the detection and quantification of *Babesia bovis* and *B. bigemina*. *Veterinary Parasitology*, *147*(1-2), 16-25.

Choramo A. & Ibrahim N. (2017). Prevalence of Babesiosis in Cattle in and Around Jimma Town, Oromia Region, South Western Ethiopia. *Acta Parasitologica Globalis*, 8(2), 96-100.

Court, R.A., Jackson, L.A., & Lee, R.P. (2001). Elevated anti-parasitic activity in peripheral blood monocytes and neutrophils of cattle infected with *Babesia bovis*. *International Journal for Parasitology*, *31*(1), 29-37.

El-Alfy, E.S., Abbas, I., Elseadawy, R., Saleh, S., Elmishmishy, B., El-Sayed, S.A.E.S., & Rizk, M.A. (2023). Global prevalence and species diversity of tick-borne pathogens in buffaloes worldwide: a systematic review and meta-analysis. *Parasites & Vectors*, *16*(1), 115.

Fahrimal, Y., Goff, W.L., & Jasmer, D.P. (1992). Detection of *Babesia bovis* carrier cattle by using polymerase chain reaction amplification of parasite DNA. *Journal of Clinical Microbiology*, *30*(6), 1374-1379.

Figueroa, J.V., Chieves, L.P., Johnson, G.S., &B uening, G. (1992). Detection of *Babesia bigemina*-infected carriers by polymerase chain reaction amplification. *Journal of Clinical Microbiology*, *30*(10), 2576-2582.

Guglielmone, A.A., Gaido, A.B., & Mangold, A.J. (1996). Light microscopy diagnosis of *Babesia bovis* and *Babesia bigemina* kinetes in the haemolymph of artificially infected *Boophilus microplus* engorged female ticks. *Veterinary Parasitology*, *61*(1-2), 15-20.

Khetran, I.B., Arijo, A.G., Buhtto, M.B., Gadahi, J.A., & Laghari, Z.A. (2019). Prevalence and molecular detection of *Babesia bigemina* in water buffalo from southeastern region of Pakistan. *Veterinaria*, *68*(1), 1-6.

Khinchi, R.K., Saini, K.K., Solanki, S., Manju., Sharma, S.K., & Gaurav, A. (2022). Molecular prevalence of babesiosis in cattle in southern Rajasthan. *Journal of Animal Research, 12*(6), 885-888.

Mahmoud, M.S., Kandil, O.M., Nasr, S.M., Hendawy, S.H., Habeeb, S.M., Mabrouk, D.M., ... & Suarez, C.E. (2015). Serological and molecular diagnostic surveys combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. *Parasites & Vectors*, *8*, 1-15.

Muraleedharan, K., Ziauddin, K.S., Hussain, P.M., Pattabyatappa, B., Mallikarjun, G.B., & Seshadri, S.J. (2005). Incidence of *Anaplasma* sp., *Babesia* sp. and *Trypanosoma* sp. in cattle of Karnataka. *Journal of Veterinary Parasitology*, *19*(2), 135-137.

Rani, N.L., Sreedevi, C., Annapurna, P., & Kumar, A. (2010). Clinical management and haemato-biochemical changes in babesiosis in buffaloes. *Buffalo Bulletin*, *29*(2), 92-94.

Ravindran, R., Mishra, A.K., & Rao, J.R. (2002). On the high seroprevalence of bovine babesiosis in Wynyard district of Kerala. *Journal of Applied Animal Research*, *22*(1), 43-48.

Sawitri, D.H., Wardhana, A.H., Ekawasti, F., & Dewi, D.A. (2021). Parasitological and molecular detection of babesiosis in cattle and buffalo in west and central Java. In: *9th International Seminar on Tropical Animal Production*. Atlantis Press, pp. 135-140.

Sharma, A., Singla, L.D., Batth, B.K., & Kaur, P. (2016). Clinicopatho-biochemical alterations associated with subclinical babesiosis in dairy animals. *Journal of Arthropod-Borne Diseases*, *10*(2), 258.

Simking, P., Saengow, S., Bangphoomi, K., Sarataphan, N., Wongnarkpet, S., Inpankaew, T., & Igarashi, I. (2013). The molecular prevalence and MSA-2b gene-based genetic diversity of *Babesia bovis* in dairy cattle in Thailand. *Veterinary parasitology*, *197*(3-4), 642-648.

Singh, N.K., Singh, H., Jyoti, Haque, M., & Rath, S.S. (2012). Prevalence of parasitic infections in cattle of Ludhiana district, Punjab. *Journal of Parasitic Diseases*, *36*, 256-259.

Suarez, C. E. & Noh, S. (2011). Emerging perspectives in the research of bovine babesiosis and anaplasmosis. *Veterinary Parasitology*, 180(1-2), 109-125.

Taylor, M.A., Coop, R.L., & Wall, R.L. (2007). *Veterinary Parasitology*, 3rd edn. Black Well.

Zaman, M.A., Atif, F.A., Abbas, R.Z., Shahid, Z., Mehnaz, S., Qamar, W., & Hussain, K. (2022). Climatic regions based molecular prevalence of babesiosis and theileriosis in cattle and water-buffalo in Pakistan. *Research Square*, Preprint (Version 1), DOI:10.21203/rs.3.rs-1519513/v1.