International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(3): 175-178
www.biochemjournal.com
Received: 08-12-2024
Accepted: 12-01-2025

Joken Bam
ICAR-Research Complex for Northeastern Hill Region Arunachal Pradesh Centre, Basar, Arunachal Pradesh, India

Pallabi Pathak
Lakhimpur College of Veterinary Science, Assam Agricultural University, Joyhing, Lakhimpur, Assam, India

Doni Jini
ICAR-Research Complex for Northeastern Hill Region Arunachal Pradesh Centre, Basar, Arunachal Pradesh, India

Corresponding Author:
Doni Jini
ICAR-Research Complex for Northeastern Hill Region Arunachal Pradesh Centre, Basar, Arunachal Pradesh, India

Babesiosis in yak: A case report

Joken Bam, Pallabi Pathak and Doni Jini

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i3c.3907

Abstract
Babesiosis is a significant disease affecting yaks and yak-cattle hybrids, caused by *Babesia bigemina*. Present study reports a case of Babesiosis in a 5-year-old female yak. The yak showed symptoms of loss of appetite, lethargy, no milk secretion, high fever exceeding 104°F and brown-colored urine. Blood sample was collected for microscopic examination and detection of *Babesia* spp. polymerase chain reaction (PCR). On the basis of the clinical presentation, the yak was promptly treated for Babesiosis with Berenil (3.5 mg/kg body weight) and ivermectin (200 mcg/kg body weight), however the animal died the next day. Post-mortem examination revealed pale and icteric mucous membranes, watery blood, an enlarged icteric liver with a distended gallbladder, and a markedly friable and enlarged spleen. The kidneys appeared black, and the urinary bladder contained brown-colored urine. Microscopic examination of Giemsa-stained blood smears revealed the presence of *Babesia* sp., which was further confirmed using polymerase chain reaction (PCR) with *B. bigemina*-specific primers.

Keywords: *Babesia bigemina*, fever, haemoglobin urea, PCR, yak

Introduction
Yaks are among the hardiest livestock species, well-adapted to extreme geoclimatic conditions, including the harsh cold of high-altitude mountainous regions. Due to their remote habitats and limited interaction with domesticated ruminants, the incidence of infectious diseases among yaks is generally lower. However, certain diseases continue to pose significant health threats and contribute to mortality in these animals. One such disease is babesiosis, a tick-borne infection caused by protozoan parasites of the *Babesia* genus, which affects domesticated animals, wildlife, and occasionally humans (Ranga Rao *et al*., 1994; Otgonsuren *et al*., 2023) [1, 2]. In yaks, babesiosis is characterized by high fever, icterus, anemia, and hemoglobinuria (Saud *et al*., 2005) [3]. The disease primarily affects adult animals, while young calves exhibit natural immunity. In India, babesiosis in yaks has been attributed to *Babesia bigemina* (Saravanan *et al*., 2013) [4]. Diagnosis is typically based on clinical signs such as a high fever, hemoglobinuria, and a history of previous infections in the region. Confirmation is achieved through the detection of intraerythrocytic *Babesia* piroplasm in blood smear examinations. If detected early, the disease responds well to treatment.
In the present study, we describe a case of babesiosis in yak with clinical presentation, investigation, post mortem findings and confirmatory diagnosis using blood smear exam and PCR.

Materials and Methods
Case presentation
A 5-year-old female yak rearer under free range in at Mandala top area of West Kameng district of Arunachal Pradesh was presented with a history of agalactia, off feed, lethargy, high fever and brown-colored urine for the past two days. The yak was part of a herd of 50 yaks that had recently down migrated from summer alpine pasture (4000 m above MSL) to Mandala top (3000 m above MSL) area grazed by hill cattle during the summer. Based on symptoms it was tentatively diagnosed as Babesiosis and was treated with Berenil (3.5 mg/kg body weight) and ivermectin (200 mcg/kg body weight), however the animal died the next day. Post mortem was performed. The herder informed of previous cases similar symptoms causing mortality only in adult females.

Blood examination

Blood samples were collected aseptically in 15 ml tube containing EDTA and transported to the laboratory. Haemoglobin levels were estimated using Sahli's Acid Hematin method. A thin blood smear was prepared and air dried and brought to the laboratory. The slide was fixed with absolute methanol and allowed to dry. The smear was stained with Geimsa for 35 minute, washed off the stained, air died and observed under microscope.

Molecular diagnosis using PCR

Genomic DNA was isolated from blood sample using DNeasy Blood and Tissue kit (Quiagen® Kit). PCR was performed using published *Babesia bigemina* specific primers (Guido *et al*., 2002) [5]: GFU5 (F): TGG CGG CGT TTA TTA GTT CG and GFU6 (R): CCACGCTTGAAGCACAGGA. The PCR reaction was set up in 25 µl reactions containing 2.5 µl PCR Master Mix (Promega), 0.5 µl of 10 mM dNTP (Promega), 0.5 µl of Forward Primer, 0.5 µl of Reverse Primer and 0.25 µl of Taq Polymerase (5U/ µl) (Promega), 1 µl of Template DNA and 19.75 µl of Nuclease Free water. The cyclic conditions involved Initial denaturation at 94 °C for 2 minutes and 40 cycles of denaturation at 94 °C for 30 second, annealing at 55 °C for 30 second, extension at 72 °C for 1minute and final extension set at 72 °C for 5 min. The PCR amplicon were then visualised in 0.8% agarose gel electrophoresis stained with ethidium bromide (0. 5 µg/ml) and analyzed on a UV transilluminator in Tris Boric acid EDTA (TBE) as running buffer with 1kb DNA ladder (Promega).

Results, Discussion and Conclusion

Babesiosis continues to be one of the most important diseases of the large ruminants impacting their health and production across the world, including yaks. Extensive literature with clear depiction of the diseases, morphological forms of the parasite, post mortem finding and diagnostic advances is available on Babesiosis in cattle however the information is scanty when it comes to yaks.

Acute babesiosis is a significant cause of economic losses in the dairy and beef industries (Bock *et al*., 2004) [6]. The clinical symptoms of acute cases include anemia, fever, icterus, hemoglobinuria, and high morbidity. In the present study, the observed clinical symptoms were indicative of babesiosis, characterized by a high fever exceeding 104°F, brown-colored urine, and pale, icteric sub-conjunctival mucous membranes. The hemoglobin level was recorded at 6.5 g/dL, consistent with previous findings of 6.08±0.66 g/dL reported by Saud *et al*., 1995) [3].

Blood smear examination revealed a heavy infection of *Babesia* piroplasms in red blood cells, exhibiting varied morphological forms (Fig. 1). Necropsy findings were characteristic of babesiosis, as described by Soulsby (1982) [7]. The mucous membranes appeared pale and icteric. Multiple *Rhipicephalus microplus* ticks (Fig. 2) were recovered from the inner thigh and udder regions. The liver was markedly enlarged and congested (Fig. 3), while the spleen also showed significant enlargement with rounded borders (Fig. 3). The kidneys were enlarged and darkened (Fig. 4). The blood appeared thin and watery, and the urinary bladder was filled with reddish-brown urine, indicative of hemoglobinuria (Fig. 5).

Babesiosis remains a critical tick-borne parasitic disease in yaks. The disease has been reported in India (Saravanan *et al*., 2013) [4] and China (Qin *et al*., 2015) [8]. The *Babesia bigemina*-specific PCR assay successfully amplified a 1124 bp product (Fig. 6), confirming *B. bigemina* as the causative agent in this case. Saravanan *et al*., 2013 [4] reported a 5.32% prevalence of *B. bigemina* in yaks from Dirang, India, using a PCR assay based on the small-subunit rRNA (SS rRNA) gene, demonstrating the assay's sensitivity. *B. bigemina* has also been reported in yaks from Xinjiang Province (Niu *et al*., 2015) [9] and Mongolia (Otgonsuren *et al*., 2023) [2]. A gene analysis based on the 18S rRNA of *Babesia* in 409 blood samples from white yaks in Tianzhu, China, identified three bovine *Babesia* species-*B. bigemina*, *B. bovis*, and *B. ovate*, as well as a new *Babesi*a sp. closely related to *B. venatorum* and B. sp. Akita (Liu *et al*., 2017) [10]. The prevalence of *B. bovis* in yaks has also been reported in China (He *et al*., 2021) [11] and Mongolia (Otgonsuren *et al*., 2023) [2].

Yaks are raised under a transhumance system, migrating to higher alpine pastures during the summer and returning to lower-altitude grazing areas in late autumn. These pastures are often previously occupied by cattle, cattle-mithun crosses, and yak-cattle hybrids, which serve as reservoirs for ticks and other vectors, posing a significant health threat to yaks. A meta-analysis of bovine babesiosis in India over 30 years (1990-2019) estimated a prevalence of 10.9% (Borthakur *et al*., 2023) [12], indicating that the disease is widespread across the country and severely impacts bovine populations. A case study from an organized farm in Meghalaya (2012) reported a loss of 51.6 liters of milk over 30 days in a milch crossbred cow due to *B. bigemina* infection (Laha *et al*., 2012) [13].

With global warming facilitating the expansion of arthropod vectors into previously colder regions, vector-borne diseases such as babesiosis are becoming increasingly significant for cold-adapted species like yaks. Therefore, effective mitigation strategies for babesiosis in yaks must include targeted prevention and control measures for ticks and reservoir hosts, including other high-altitude ruminants.

Figures

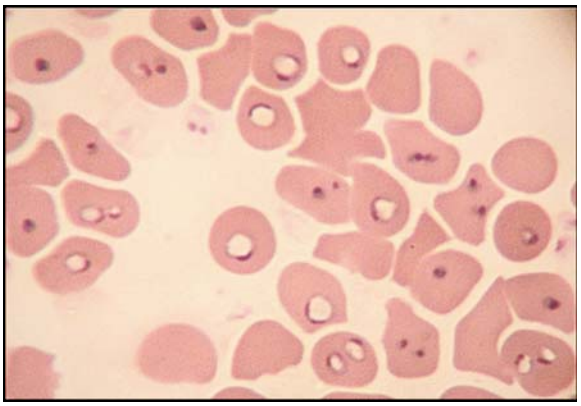

Fig 1: Blood smear showing intra-erythrocytic *Babesia bigemina* piroplasm

Fig 2: Ticks recovered from the carcass of the yaks

Fig 3: Figure showing liver enlarged, icteric liver with gall bladder fully distended with bile and enlarged spleen with rounded borders.

Fig 4: Figure showing swollen and blackened kidney at necropsy

Fig 5: Urinary bladder of filled with reddish brown urine at necropsy

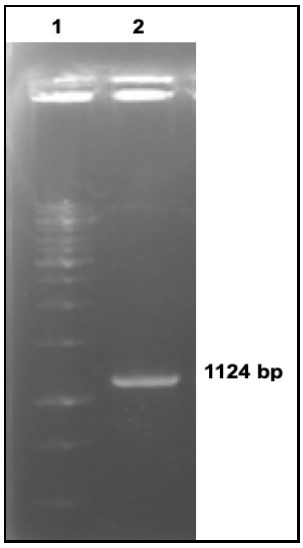

Fig 6: Agarose gel electrophoresis of PCR product. Lane 1 = 1 kb DNA ladder and Lane 2 = 1124 bp *B. bigemina* amplicon.

Acknowledgement
The authors are thankful to the Head, ICAR-Research Complex for Northeastern Hill Region Arunachal Pradesh Centre, Basar-791101, Arunachal Pradesh, India for the support and encouragement.

Conflict of Interest
The authors declare that they have no conflict of interest.

Reference

1. Rangarao GS, Sharma RL, Hemaprasanth. Parasitic infections of Indian yak *Bos* (*Poephagus*) *grunniens*-An overview. Veterinary Parasitology. 1994;53:75-82.
2. Otgonsuren D, Myagmarsuren P, Zoljargal M, Ahedor B, Sivakumar T, Battur B, *et al*. The first survey of bovine *Babesia* species infecting yaks (*Bos grunniens*) in Mongolia. Journal of Parasitology. 2023;109(5):480-485.
3. Saud N, Sheikh IU, Pourouchottamane R, Bhattacharya M. Haematology of babesiosis in yaks. Indian Veterinary Journal. 2005;82:1110-1111.
4. Saravanan BC, Das S, Siju SJ, Tewari AK, Sankar M, Kataktalware MA, *et al*. *Babesia bigemina* infection in yak (*Poephagus grunniens* L.): Molecular detection and characterization. Veterinary Parasitology. 2013;194:58-64.
5. Guido FCL, Angela PS, Lloyd HL, Cláudio RM. Assessment of primers designed from the small ribosomal subunit RNA for specific discrimination between *Babesia bigemina* and *Babesia bovis* by PCR. Ciência Animal Brasileira. 2002;3(2):27-32.
6. Bock R, Jackson L, de Vos A, Jorgensen W. Babesiosis of cattle. Parasitology. 2004;129(Suppl.):S247-S269.
7. Soulsby E. Helminths, Arthropods and Protozoa of Domesticated Animals. Bailliere Tindall: London, UK; 1982.
8. Qin S, Wang J, Ning H, Tan Q, Yin M, Zhang X, *et al*. First report of *Babesia bigemina* infection in white yaks in China. Acta Tropica. 2015;145:52-54.
9. Niu Q, Liu Z, Yu P, Yang J, Abdallah MO, Guan G, *et al*. Genetic characterization and molecular survey of *Babesia bovis, Babesia bigemina* and *Babesia ovata* in

cattle, dairy cattle, and yaks in China. Parasites & Vectors. 2015;8:518.
10. Liu J, Guan G, Li Y, Liu A, Luo J, Yin H. A molecular survey of *Babesia* species and detection of a new *Babesia* species by DNA related to *B. venatorum* from white yaks in Tianzhu, China. Frontiers in Microbiology. 2017;8:419.
11. He Y, Chen W, Ma P, Wei Y, Li R, Chen Z, *et al*. Molecular detection of *Anaplasma* spp., *Babesia* spp. and *Theileria* spp. in yaks (*Bos grunniens*) and Tibetan sheep (*Ovis aries*) on the Qinghai-Tibetan Plateau, China. Parasites & Vectors. 2021;14:613.
12. Borthakur U, Verma MR, Singh YP, Kumar S, Kumar D, Bangar YC, *et al*. Prevalence of babesiosis in bovines of India: A meta-analytical approach for 30 years (1990-2019). Veterinary Quarterly. 2023;43(1):1-9.
13. Laha R, Das M, Goswami A, Singh P. A clinical case of babesiosis in a crossbred cow of Meghalaya. Indian Journal of Animal Research. 2012;46(3):302-305.