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Cover: Oil painting of Humpback Whale *Megaptera novaeangliae*. © R. Mahesh.



Theileriosis in a captive Indian Gaur *Bos gaurus*: a rare encounter

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Abstract: This study describes fatal theileriosis in two captive Indian Gaurs *Bos gaurus* housed at Sanjay Gandhi Biological Park, Patna, Bihar, India. Both animals exhibited high fever, anorexia, lethargy, congested mucous membranes, subcutaneous haemorrhages and enlarged lymph nodes and subsequently succumbed to the disease. Post-mortem examination revealed enlarged lymph nodes, characteristic punched-out abomasal ulcers and petechial haemorrhages in multiple visceral organs. Diagnosis was confirmed by detection of Koch's blue bodies in lymph node smears, histopathological lesions consistent with Theileria infection and PCR analysis. The findings highlight the occurrence of severe, fatal theileriosis in captive gaur and emphasize the need for timely diagnosis and effective control measures in zoological settings.

Keywords: Abomasum, anorexia, histopathology, Koch blue bodies, liver, lymph node, lymphadenopathy, petechial haemorrhages, polymerase chain reaction, zoo animal.

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Author contribution: VS, DK, KK—conception and design of the study. VS, DK—conducted post-mortem examination and sample collection. VS, DK, IA—histopathological examination and interpretation. PK—PCR analysis and microbiological investigations. RT, AK—clinical management and case investigation. VS—drafted the manuscript. DK, KK, IA—reviewed and finalized the manuscript.

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INTRODUCTION

Theileriosis is a tick-borne parasitic disease caused by protozoa of the genus *Theileria* primarily affecting cattle and buffaloes but also capable of infecting other wild animals including the Indian Gaur *Bos gaurus*. This haemoprotezoan disease transmitted by arthropods mainly impacts calves leading to significant illness, mortality and substantial economic losses in the livestock sector worldwide. Among the pathogenic species, *Theileria annulata* and *T. parva* are most notable, transmitted by *Hyalomma* and *Rhipicephalus* ticks, respectively.

The Indian Gaur, the largest bovid confined to the oriental biogeographic region has approximately 85% of its current global population occurring in India (Ashok et al. 2011) and is vulnerable to epidemic diseases such as foot & mouth disease, anthrax, and rinderpest. Although there is scanty representation of haemoprotezoan diseases like theileriosis and babesiosis yet theileriosis is an economically significant vector-borne disease spread by arthropods and blood protozoa in tropical and subtropical regions of India (Sitotaw et al. 2014). Although haemoprotezoan infections such as theileriosis are relatively under-reported in some regions, theileriosis, affects a range of domestic ruminants primarily cattle (bovine tropical theileriosis caused mainly by *Theileria annulata*), Water Buffalo *Bubalus bubalis*, sheep, and goats and it has also been detected in wild ungulates (like Sambar, Spotted Deer, and other cervids) in India.

Molecular and meta-analytic studies indicate *T. annulata* is the dominant pathogenic species in Indian cattle while *T. orientalis* and other *Theileria* spp. occur in both bovines and small ruminants (Krishnamoorthy et al. 2021). It infects many domestic and wild animals through ixodid ticks of the genera *Amblyomma*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus* (Mans et al. 2015). With cattle, water buffalo, waterbucks, and African Buffalo susceptible to *T. parva* infection, although symptomatic illness is mainly observed in cattle and water buffalo (Spickler et al. 2010). Among the many species of *Theileria* infecting cattle, *T. parva* and *T. annulata* are the most pathogenic with economic impacts making them the most prevalent causes of bovine theileriosis worldwide (Kohli et al. 2014; Abdel-Rady et al. 2023).

A systematic review and meta-analysis of studies across India estimated an overall theileriosis prevalence of ~20% in cattle and buffaloes (Krishnamoorthy et al. 2021). Regionally, microscopy-based surveys recorded *Theileria* prevalence of 2.2% (Cauvery Delta, Tamil Nadu; Jayalakshmi et al. 2019) and ~13% (western Tamil

Nadu; Velusamy et al. 2014) and sensitive molecular qPCR work from an endemic area in Odisha showed high *T. annulata* parasitaemia among clinical cases and detectable carriers (Sahoo et al. 2022).

Following attachment of infected ticks to the host, the incubation period ranges 4–14 days and in the acute phase, the disease duration can vary 3–4 days or extend up to 20 days (Bakor et al. 2008). The clinical signs include fever (>103°F), enlargement of superficial lymph nodes (acute form), anorexia, pale or congested mucous membranes, conjunctivitis, severe ocular congestion, excessive lacrimation, corneal opacity, respiratory symptoms such as serous nasal discharge, cough, purulent nasal discharge, dyspnea, weakness, and death (Mahmmod et al. 2011).

In wild animals, the prevalence of theileriosis varies significantly depending on species, geographical location, and surveillance methods.

These data suggest that while theileriosis is widely distributed among wild ungulates, its prevalence in Gaur appears to be rare, underreported or possibly underestimated due to limited surveillance.

Case Presentation

Two zoo-born Indian Gaurs *Bos gaurus* housed at Sanjay Gandhi Biological Park died within an interval of seven days. One animal was approximately five years and nine months old while the other was seven months old and both were males. The animals exhibited clinical illness for approximately two weeks prior to death, characterized by high fever, anorexia, lethargy, dyspnoea, weakness, congested mucous membranes, subcutaneous haemorrhages and marked enlargement of pre-scapular lymph nodes and eventually succumbed to the disease. Despite being maintained under generally well-managed conditions with adequate hygiene, accumulation of faecal material was observed in the surrounding area. At the time of clinical examination and necropsy, ticks were not prominently observed. No similar clinical signs or mortality were recorded among other wild ruminants housed in adjacent enclosures during this period. Both affected animals were confirmed to be zoo-born and maintained under captive conditions since birth. Necropsy was performed under aseptic conditions following standard procedures (Maxie 2015). External examination revealed enlarged pre-scapular lymph nodes, congested mucous membranes, subcutaneous haemorrhages, abdominal distension, and rigor mortis in the hind limbs.

During necropsy, impression smears were prepared from the cut surface of the lymph nodes, fixed in

methanol for 2 minutes, air-dried and stained with Giemsa stain for 45 minutes for cytological examination. Tissue samples from the abomasum, lymph nodes, liver, and lungs were collected and fixed in 10% neutral buffered formalin for histopathological analysis. The tissues were processed using the standard paraffin embedding technique, sectioned at 4–6 µm thickness and stained with haematoxylin and eosin (H&E) following established protocols (Luna et al. 1968; Maxie 2015).

For molecular confirmation, genomic DNA was extracted from lymph node tissue using the HiPurA™ Multi-Sample DNA Purification Kit (MB553, HiMedia Laboratories, Mumbai, India) following the manufacturer's protocol and subsequently subjected to polymerase chain reaction (PCR) targeting the 18S rRNA gene of *Theileria* spp. using genus-specific primers. Genomic DNA extracted from a previously confirmed *Theileria* spp. positive sample was used as the positive control, while nuclease-free water served as the negative control in the PCR reaction. PCR amplification was carried out using genus-specific primers: forward primer 5'-CCTGAGAAACGGCTACCACATCT-3' and reverse primer 5'-GGACTACGACGGTATCTGATCG-3' as described by Yang et al. (2014). The thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 58 °C for 30 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min and the reaction was then held at 4 °C. PCR amplification yielded a distinct product of approximately 591 bp corresponding to the expected size for *Theileria* spp., which was visualized by agarose gel electrophoresis along with appropriate positive and negative controls.

Following confirmation of theileriosis, the remaining three adult Gaurs were treated with buparvaquone (Butalex™, MSD Animal Health, Pune, India) at a dose of 2.5 mg/kg body weight administered via dart gun. Supportive management and vector control measures were simultaneously implemented. Carcass disposal of deceased animals was carried out by deep burial with lime application in accordance with MoEFCC (2017) guidelines. Routine disinfection of the enclosure and contaminated materials was performed. The treated animals showed clinical improvement within 3-4 days post-administration, with resolution of fever, and improved appetite.

RESULT & DISCUSSION

Gross Findings and Interpretation

At necropsy, the abomasum exhibited prominent, well-demarcated punched-out ulcers characterized by discrete, circular mucosal defects (Image 2a,b), which are considered characteristic lesions of theileriosis and have been previously reported in bovines affected with *Theileria* infection (Omer et al. 2003). The spleen was markedly enlarged and congested with a mottled appearance and multifocal petechial to ecchymotic haemorrhages on the capsular surface (Image 2c), indicative of systemic vascular damage and haemolytic processes. The liver was mildly enlarged with diffuse congestion and multiple small pale focal areas (Image 2d), suggesting hepatocellular injury associated with systemic infection. Lymph nodes were markedly enlarged, edematous and congested, reflecting lymphoproliferative and inflammatory responses typical of theileriosis. The kidneys showed multifocal haemorrhages (Image 2e) while the lungs were congested, edematous and emphysematous, consistent with respiratory compromise. The heart exhibited petechial and ecchymotic haemorrhages on both epicardial and endocardial surfaces, further supporting a systemic haemorrhagic condition.

Cytological, histopathological and molecular findings

Impression smears from the pre-scapular lymph nodes stained with Giemsa revealed the presence of Koch's blue bodies, a diagnostic feature of *Theileria* infection.



Image 1. Indian Gaur during postmortem examination exhibiting clinical signs of theileriosis found in recumbent position. © Authors.

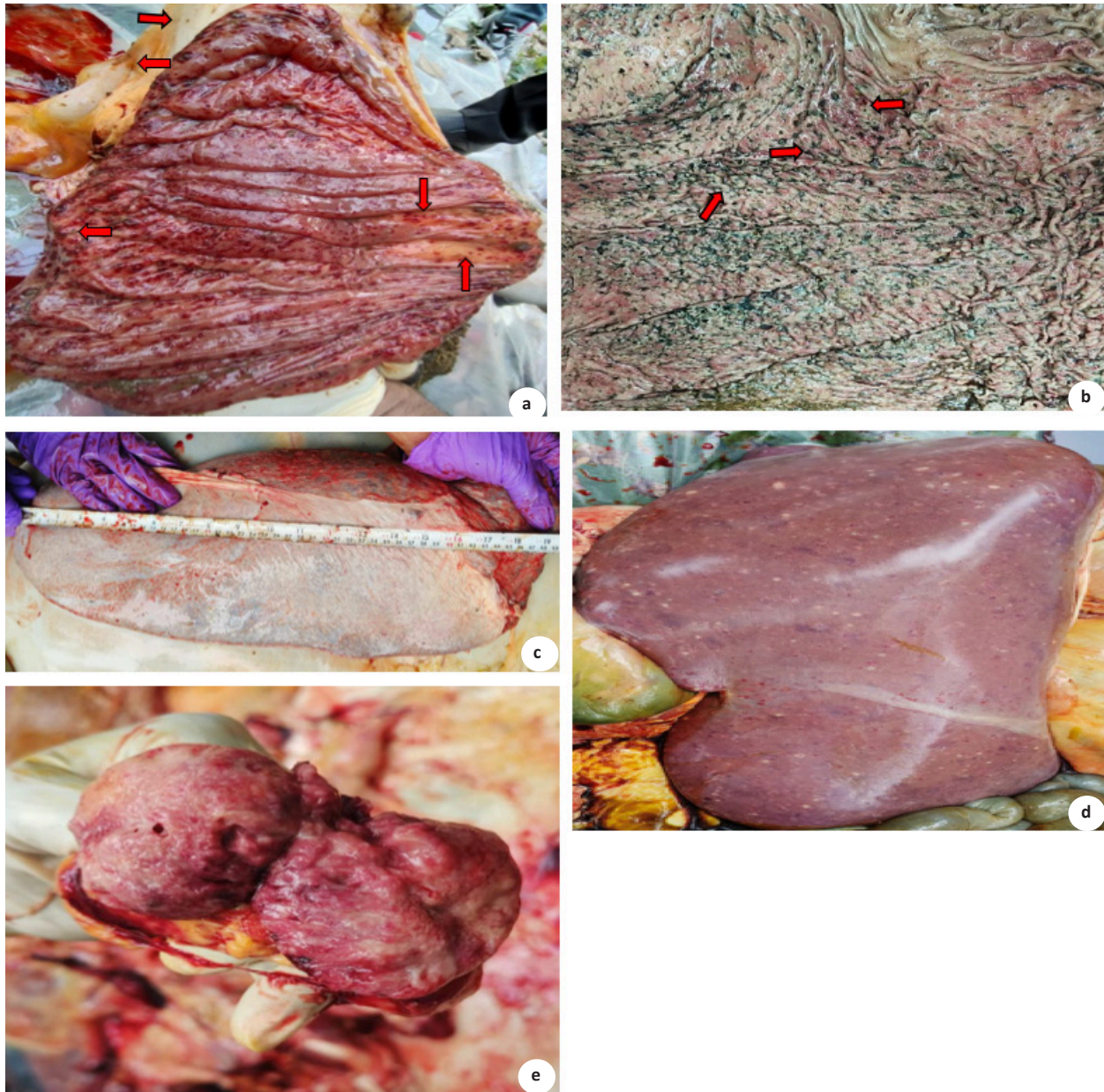


Image 2. a,b—Punched-out ulcerative lesions on the abomasal mucosa of and infected calf (left) and adult Indian Gaur (right) | c—Enlarged, congested spleen with mottled appearance and scattered multiple petechial to ecchymotic hemorrhages | d—Mildly enlarged liver with smooth capsule, and diffuse congestion along with multiple small pale focal lesions throughout the parenchyma | e—Markedly enlarge edematous and congested lymph nodes with a tense capsule and diffuse reddish discoloration. © Authors.

Histopathological examination of the abomasum (H&E, $\times 100$) revealed focal to multifocal mucosal ulceration with epithelial necrosis, loss of mucosal architecture and infiltration of lymphocytes and macrophages (Image 3a), corresponding to the gross lesions. Lymph node sections showed lymphoid depletion with macrophage infiltration containing intracytoplasmic Koch's blue bodies (Image 3b), indicating active parasitic infection and immune response. The liver exhibited moderate hepatocellular

vacuolar degeneration, sinusoidal congestion and mononuclear inflammatory infiltration with prominent Kupffer cell activation (Image 3c), reflecting systemic inflammatory insult. Lung sections revealed diffuse interstitial pneumonia with thickened alveolar septa due to mononuclear infiltration and vascular congestion, along with eosinophilic proteinaceous fluid in alveolar spaces suggestive of pulmonary edema (Image 3d). The myocardium showed focal myofibre degeneration with

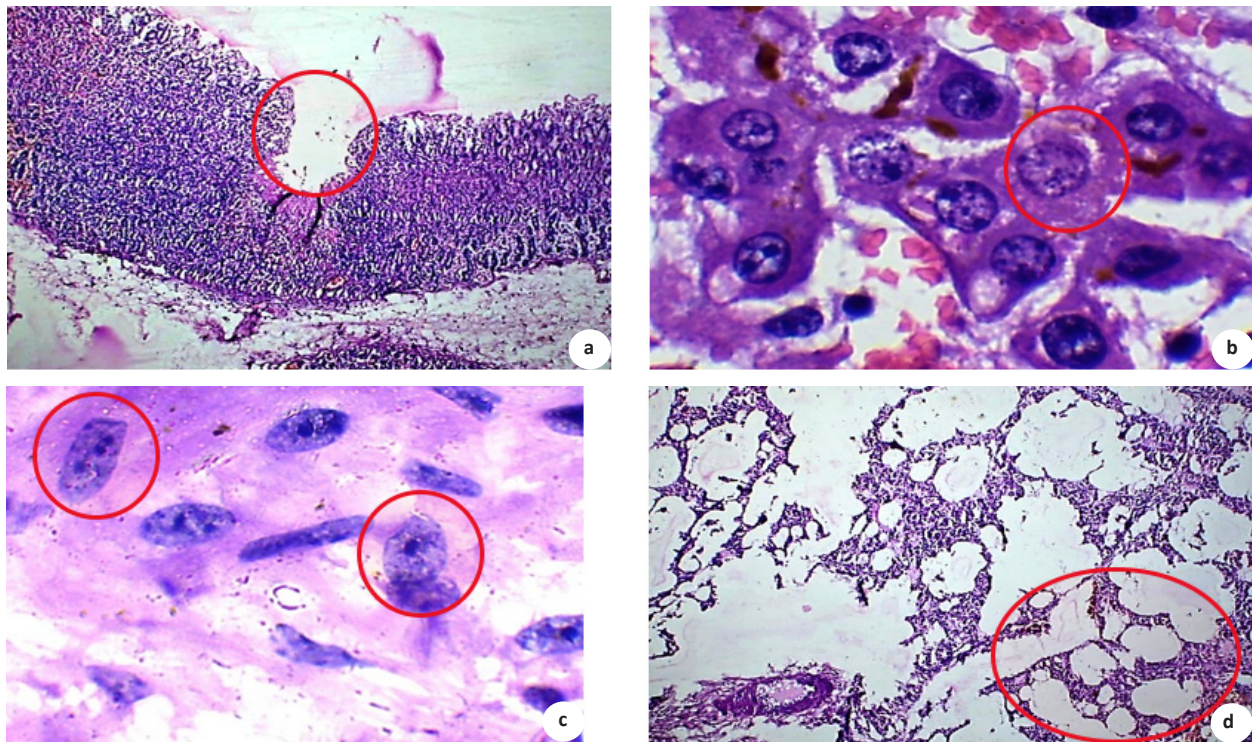


Image 3. a—Histological section of abomasum of Gaur (H&E, x100) demonstrates a punched out ulcerative lesion (circle) with surrounding chronic inflammation, epithelial necrosis and mucosal disruption | b—Histological section of Lymph node of Bison (H&E, x100) reveals lymphoid infiltration with macrophages containing Koch's blue bodies (circle) | c—Histological section of Liver of Bison (H&E, x 100) revealed moderate hepatocellular vacuolar degeneration with sinusoidal congestion, mononuclear inflammatory infiltration and prominent Kupffer cells (circle) | d—Histological section of Lungs of Bison (H&E, x 10) reveals diffuse interstitial pneumonia characterized by marked thickening of alveolar septa (circle) due to mononuclear inflammatory infiltration and vascular congestion. © Authors.

interstitial haemorrhage and lymphocytic infiltration while the kidneys exhibited mild interstitial nephritis with mononuclear cell infiltration, indicating multi-organ involvement.

PCR amplification targeting the 18S rRNA gene yielded a specific amplicon of approximately 591 bp (Image 4), confirming infection with *Theileria* spp. The use of 18S rRNA based PCR has been widely validated for detecting *Theileria annulata* and other *Theileria* spp. in bovines and field samples (Aktas et al. 2006; Prado et al. 2022). However, as the assay employed was genus-specific, species-level identification could not be achieved. Further confirmation using species-specific PCR or sequencing would be required to determine the exact *Theileria* species involved. These molecular findings support the gross and histopathological observations of theileriosis in the present case.

Outcome and Implications

No further mortality was observed during the 3-month follow-up period, indicating successful containment of the outbreak. These findings underscore

the susceptibility of captive wild bovids to theileriosis and highlight the critical need for early diagnosis, continuous health monitoring, and effective disease management in zoological settings. Control of theileriosis in captive wildlife relies on integrated tick management, timely therapeutic intervention with buparvaquone, regular acaricide application, and strict quarantine measures. Strategic implementation of tick control using topical synthetic pyrethroids and macrocyclic lactones is essential to prevent recurrence.

CONCLUSION

This study highlights fatal theileriosis in captive Indian gaur *Bos gaurus*, emphasizing the vulnerability of wild bovids to tick-borne haemoprotzoan infections under captive conditions. Early diagnosis and timely therapeutic intervention are crucial to prevent mortality and control disease outbreaks in zoological settings.

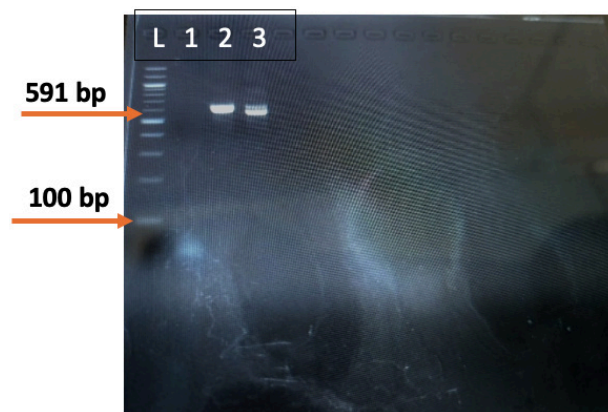


Image 4. PCR amplification showing 591 bp *Theileria* band: Lane 1–Negative control (nuclease free water), Lane 2–Positive control (*Theileria* spp. Positive DNA, 591 bp), Lane 3–Sample positive with 591 bp band. © Authors.

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