Theileriosis (*Theileria annulata*) associated Lymphosarcoma in cattle

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Abstract

Bovine Tropical Theileriosis (BTT) is caused by *Theileria annulata,* an apicomplexan haemoprotozoan parasite transmitted by ticks. The disease in cattle is usually manifested with initial lympho-proliferation phase followed by destruction of lymphocytes. The current report describes a fatal case of theileriosis associated lymphosarcoma in a five and half year old female cross bred cattle presented to the clinics, College of Veterinary Science, Proddatur. The animal was brought with a history of anorexia, corneal opacity and sever tick infestation. Clinical examination revealed high temperature of 105^0 F, anemia with pale mucous membranes, enlarged lymph nodes with generalized lymphadenopathy, cachexic body, congested and cloudy eyes. The peripheral blood examination revealed presence of ring like piroplasms in red blood cells. Hematological parameters of the infected cattle suggested slight elevated levels of serum alanine transaminase (ALT) and aspartate transaminase (AST) and reduced levels of sodium, potassium and phosphorus. Lymph node biopsy demonstrated Koch blue bodies (KBBs) along with spherical lymphoblasts indicating maligancy. Based on clinical signs, lymph node biopsy and peripheral blood examination the case was diagnosed as theileriosis. Treatment with Inj.Buparvaquone (@2.5 mg/kg, Deep Intramuscular) along with symptomatic and supportive therapy turned in vain, as the animal succumbed to death due to complications with lymphosarcoma.

Keywords: Theileriosis; Lymphosarcoma; Anemia; Corneal Opacity; Buparavaquone

Introduction

Bovine tropical theileriosis (BTT) is caused by *Theileria annulata*, an apicomplexan haemoprotozoan parasite transmitted by ticks. It is one of the most significant vector-borne diseases of cattle in tropical and subtropical regions, particularly in Asia, the Middle East, North Africa, and southern Europe. The disease is transmitted by ixodid ticks of the genus Hyalomma, with *Hyalomma anatolicum* being the primary vector in many endemic areas. *Theileria annulata* is particularly noteworthy due to its broad geographical distribution and the presence of multiple strains with varying degrees of virulence (Farhang, 2017). On a global basis, the economic repercussions of theileriosis in the livestock sector are substantial, manifesting in reduced weight gain, decreased milk production, reproductive losses, and in severe cases, mortality (Denizhan et al., 2017). In India alone, the estimated annual economic loss due to BTT is approximately USD 1.295 billion (Narladkar, 2018). The impact is particularly severe in regions where high-yielding exotic or crossbred cattle are reared, as these breeds exhibit greater susceptibility compared to indigenous cattle, which have evolved varying levels of resistance (Muhanguzi et al., 2010).

The distribution of *T. annulata* is closely linked to the geographical range of its vector ticks. Climate change, altered land use, and global trade in livestock have contributed to shifts in tick distribution, leading to the expansion of endemic areas (Bouchard et al., 2019). The disease is enzootic in regions with warm temperatures and high humidity, which favor tick survival and reproduction. Infection occurs to a susceptible bovine host through the bite of *Hyalomma anatolicum*. Following invasion sporozoites most likely infect B lymphocytes and macrophages undergoing schizogony, leading to intracellular proliferation and host cell transformation. Moreover, the infection induces the transformation of bovine B lymphocytes and myeloid cells into cancer-like leukomas characterized by immortalization and hyperproliferation (Dobbelaere & Rottenberg, 2003). The parasite subsequently transitions to the erythrocytic stage, where it forms piroplasms, enabling further transmission to naive hosts via subsequent tick feeding cycles.

Bovine tropical theileriosis primarily manifests in three clinical forms in cattle: acute, sub-acute, and chronic stages. In endemic countries like India, the disease in indigenous cattle (Zebu) is mostly sub-acute with milder symptoms with gradual weight loss, intermittent fever, and moderate anemia. Animals usually recover and remain resistant to reinfection, but often remain carriers serving as reservoirs for disease transmission. Crossbred cattle and imported breeds (Holstein Friesian and Jersey) are more susceptible to acute form of infection and exhibit profound clinical signs like high fever (40–42°C), severe lymphadenopathy, anemia, icterus, respiratory distress, and weight loss. A hallmark of *T. annulata* pathogenesis is its ability to induce uncontrolled proliferation of infected leukocytes followed by marked anemia due to erythrophagocytosis (Devi et al., 2011). Accurate and timely diagnosis is critical for effective disease management and without clinical intervention, mortality rates can be high (Brown, 1990). Repeated exposures in animals exhibit chronic form of disease with low-grade anemia, emaciation, and reduced productivity.

In Indian Subcontinent, tropical theileriosis has an estimated overall prevalence of 21 per cent. The meta-analysis data from National Institute of Veterinary Epidemiology and Disease Informatics (Krishnamoorthy et al., 2021) estimated a high infection rate in cattle (22%) than in buffaloes (14%). Most cattle infections showed mild to moderate pathology with symptoms such as fever, discharge, diarrhea, abortion, anemia, and reduced production (Mehlhorn, 2008). Rare but documented cases include corneal opacity (Saini et al., 2023; Kaur et al., 2020), direct vertical transmission (Selim et al., 2021) and cerebral theileriosis (Agarwal et al.,2023) in crossbred cattle in India. However, reports associated with *T. annulata* induced malignant fatalities in crossbred cattle from

India are very rarely documented in routine diagnosis. The present communication demonstrates a fatal case of theileriosis associated with lymphosarcoma in female crossbred cattle.

Materials and Methods

Case history and clinical examination

A five-and-a-half-year-old crossbred Holstein Friesian cow was brought to the clinics exhibiting a seven-day history of anorexia, pale mucous membranes, anemia, labored breathing, and fever. Upon clinical examination, the cow displayed a significantly high rectal temperature of 105°F, widespread lymphadenopathy (Fig. 1), open-mouth breathing, cloudy corneas, excessive tear production, sunken eyeballs (enophthalmos, Fig. 2), and impaired vision. Unusual symptoms were also noted, such as small, raised skin nodules in the cervical area, nasal discharge, diarrhea, and coffee-colored urine. Lung auscultation revealed fluid-filled crackling sounds and rapid breathing, suggestive of pulmonary edema.

Sample collection and processing

Further examination uncovered a heavy tick infestation on the affected cattle and tick samples were collected in formalin tubes for identification. In addition, peripheral blood smears were prepared from the ear vein, stained with Giemsa stain (diluted 1:10) for 25 minutes following the protocol of Petithory et al. (2005), and examined under an oil immersion objective lens for the detection of parasitic organisms. Additionally, approximately 3 mL of heparinized blood was collected from the jugular vein for hematological and biochemical analysis. Fine-needle aspiration smears from lymph nodes were obtained, methanol-fixed, stained with Giemsa, and subsequently examined. During autopsy, pericardial fluid, impression smears from lymph nodes were taken and submitted for staining techniques.

Fig 1 Widespread lymphadenopathy seen with enlarged prescapular and prefemoral lymphnodes.

Fig 2. Eye of the affected animal showing cloudy corneas, excessive tears production and enophthalmos.

Results and discussion

Blood smear examination revealed presence of intra-erythrocyte merozoites in red blood cells that appeared like comma, rod and signet ring shaped (Fig. 3). Hematological analysis revealed a decline in the mean erythrocyte count (4.2 X 10^6), hemoglobin concentration (6.4 g/dl), and leukocyte count (6.8 X 10^3).Similarly, serum biochemical analysis demonstrated a decline in total protein, sodium, potassium, and phosphorus levels, while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a mild elevation. In contrast, total bilirubin and creatinine levels were notably increased (Table 1). Further, peripheral blood smears revealed schizont staged Koch blue bodies (KBBs) with spherical lymphoblasts (Fig 4). The schizont of *T. annulata* first leads to the proliferation of lymphoid tissue, followed by its destruction, since schizonts are few in the peripheral blood of severely ill animals, and finding them in blood smears suggests a negative outlook.

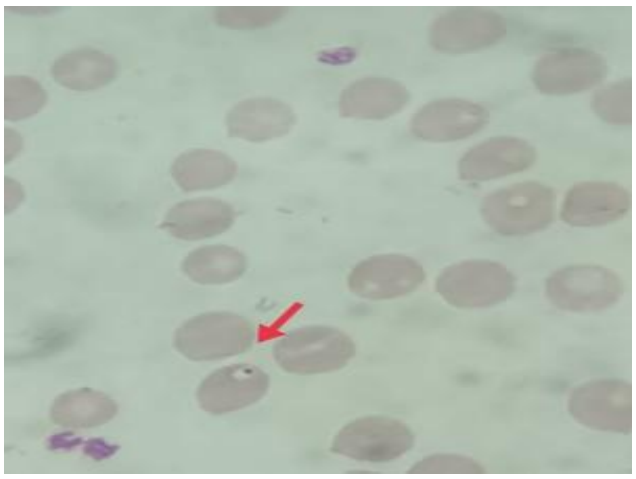

Fig :3 Piroplasm stage in red blood cells that appeared like signet ring shaped (1000X)

Table: 1. Comparison of serum biochemical parameters of Theileriosis infected cattle with healthy cattle values

Serum Biochemical Parameter	Theileriosis-Infected Cattle	Healthy Cattle/Normal range	Remarks
Sodium (Na^+) (meq/l)	128meq/l Decreased	132-152meq/l	Hyponatremia due to fluid imbalance and dehydration
Potassium (K^+) (meq/l)	3.4meq/l Decreased	3.9-5.8meq/l	Hypokalemia due to anorexia and metabolic disturbances
Phosphorus (P) (mg/dL)	5.0 Decreased	5.6-6.5	Possibly due to impaired renal function and hemolysis
Aspartate Aminotransferase (AST) (u/l)	137 Slightly Increased	78-132	Indicates hepatocellular damage or muscle injury
Alanine Aminotransferase (ALT) (u/l)	156 Slightly Increased	110-140	Reflects mild liver dysfunction
Total Protein (g/dL)	4.3 Decreased	6.0–7.9 g/dL	Due to malnutrition, protein loss, or hepatic dysfunction
Total Bilirubin (mg/dL)	0.3 Increased	0–0.1 mg/dL	Indicative of hemolysis and liver dysfunction
Creatinine (mg/dL)	2.37 Increased	0.5–1.7 mg/dL	Indicative of kidney dysfunction or muscle catabolism

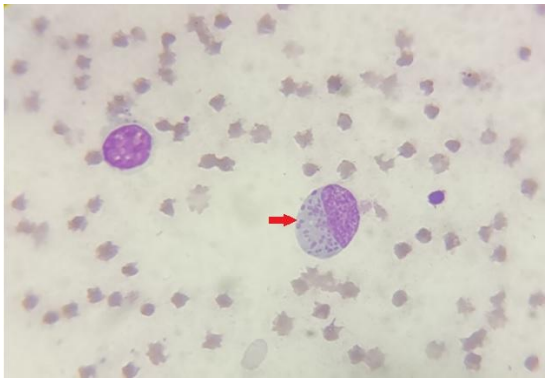

Fig 4. Peripheral blood smear revealed schizont staged Koch blue bodies (KBBs) in Lymphocyte (1000X)

Lymphnode aspirations recorded the presence of large lymphoblasts, accounting for more than than 50% of the total cell population (Fig 5). These lymphoblasts appeared discrete, maintaining distinct celluar boundaries with higher nuclear to cytoplasmic ratio (N:C) characteristic of lymphoproliferative malignancies. The cytoplasm seemed intensely basophilic and nuclei are large and demonstrated prominent nucleoli. The observation of lymphoglandular bodies (LGBs) (basophilic cytoplasmic fragments of large lymphoblasts) is a crucial cytological hallmark. Based on the cytological evaluation of lymph nodes demonstrated the features indicative of lymphosarcoma. Moreover, pericardial fluid collected during autopsy revealed hydropericardium and cytology showed presence of lymphoblasts with limited RBCs signifies a severe parasitic infection that compromises the immune system (Fig 6).

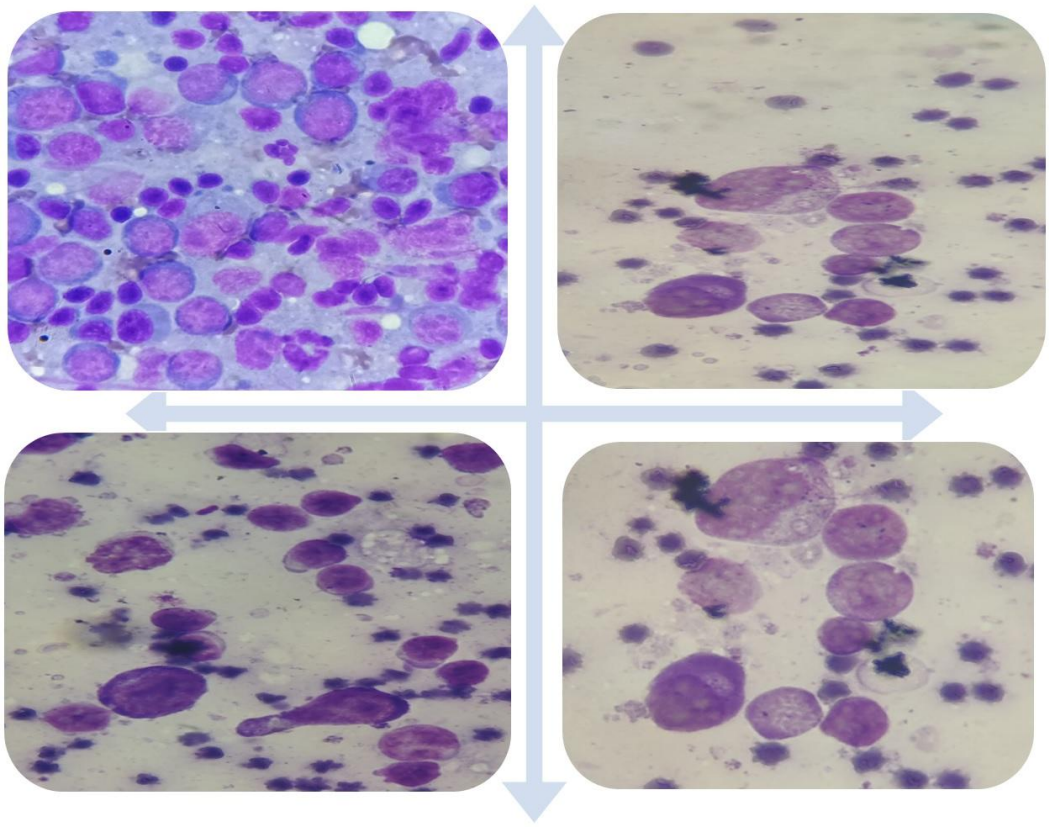

Fig 5. Lymphnode aspiration revealed lymphoblast cells which constitute greater than 50 percentage of cells in a highly cellular smear along with lymphoglandular bodies (1000x)

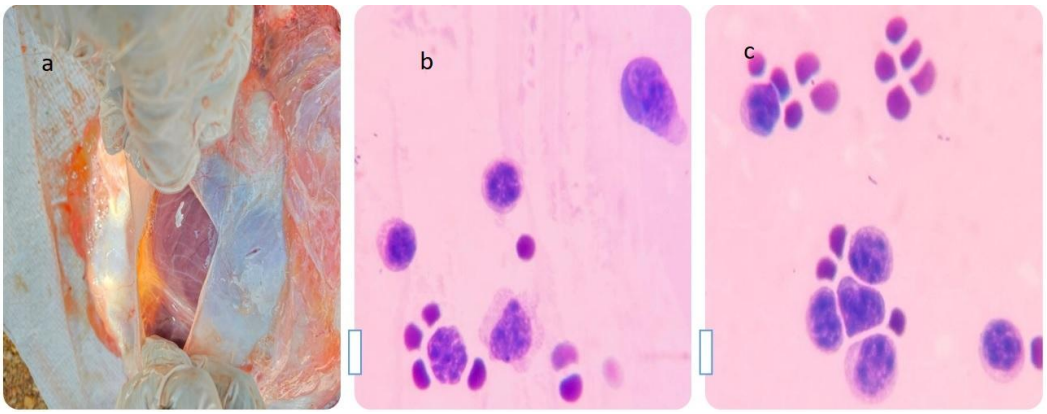

Fig 6 a. Hydropericardium during postmortem examination; b & c. Pericardial fluid cytology revealed lyphoblast cells with very few RBCs.

Following a comprehensive diagnostic evaluation through blood smear, lymph node cytology, and serum profiling, the case was confirmed as theileriosis concurrent with lymphosarcoma. The animal received Inj. Buparvaquone (2.5 mg/kg IM), haematinic (Liq. aRBCcRakhit -50ml BID for seven days) and hepatoprotective tonics (Livotas-50ml BID for fortnight), and antipyretic therapy (Melonex Plus-0.5mg/kg). Futher to relieve the respiratory distress the animal was administered Deriphyllin (bronchodilator-1mg/kg) for three days. Despite intensive treatment, the animal eventually succumbed to lymphosarcoma complications.

In the Indian subcontinent, the management of bovine tropical theileriosis has been successfully achieved through the use of Buparvaquone, given at a dosage of 2.5 mg/kg of body weight (Varma et al., 2023; Azhahianambi et al., 2021; Nampoothiri, 2021). Conversely, the antitheilerial drug Buparvaquone can convert transformed leukocytes back to a non-transformed state (Dobbelaere & Heussler, 1999). A defining characteristic of T. annulata pathogenesis is its capability to cause unchecked proliferation of infected leukocytes, a phenomenon prompted by the parasite's activation of host signaling pathways. The parasite influences host cell apoptosis, immune signaling, and inflammatory responses, resulting in widespread systemic pathology. Research conducted by Tajeri et al. (2021) provides additional evidence that the parasite triggers lethal leukoproliferative disorders, causing altered leukocytes to spread throughout the host, resulting in a condition resembling malignancy. An uncommon and fatal instance of lymphosarcoma associated with bovine theileriosis has been reported in a crossbred cow, which exhibited corneal opacity, fever, anemia, and generalized lymphadenopathy (Singh et al., 2015). Such occurrences are rarely noted in typical veterinary practice, underscoring the complexity and sometimes atypical manifestations of theileriosis. Theileriosis continues to pose a considerable veterinary and economic challenge, highlighting the need for ongoing research and effective management approaches to reduce its effects on the livestock sector.

Conclusion

The present case highlights a rare and fatal manifestation of bovine tropical theileriosis associated with lymphosarcoma in a crossbred cow. Despite specific and supportive therapy with Buparvaquone and adjunct medications, the animal succumbed due to malignant complications. The findings emphasize the aggressive course of theileriosis when linked with neoplastic transformation and the need for prompt diagnosis and integrated disease management. Continued research into host–parasite interactions and therapeutic strategies is essential to mitigate such complex infections in cattle.

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Conflict of interest: None

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