

*Short Communication*

## HAEMATOLOGICAL AND CYTOPATHOLOGICAL ALTERATIONS IN *THEILERIA ANNULATA* INDUCED BOVINE LYMPHADENOPATHY

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**ABSTRACT:** The tick-borne intra-cellular parasite, *Theileria annulata*, causes mortality in bovines. Schizont of the parasite in lymphocytes divides continuously stimulating the clonal expansion of lymphoid cells in lymph nodes leading to the neoplastic-like lymphoid cell proliferation and sometimes may also cause lymphoma, especially in chronic conditions. Two cattle, with a history of fever and respiratory distress, were presented to the Large Animal Unit, Teaching Veterinary Clinical Complex, GADVASU. On clinical examination, the animals had a high fever (106-107°F), anemia, icteric mucus membranes, and prescapular lymph node enlargement. Whole blood and fine needle aspirate from the lymph node were collected. A blood smear examination revealed anemia, leucopenia with relative lymphocytosis, and thrombocytopenia. Further, there was the presence of schizonts (Koch blue bodies) in the lymphocytes and piroplasms of *T. annulata* in the red blood cells. Lymph node aspirate examination revealed the feature that mimics lymphoma by the presence of a monomorphic population of lymphoblasts and with all stages of mitotic figures along with lymphoglandular bodies. Numerous schizonts were also recorded in the lymphoblasts during the examination of lymph node aspirate.

**Key words:** Bovine, Koch blue bodies, Lymphadenopathy, *Theileria annulata*.

Theileriosis is an important tick-borne apicomplexan parasitic infection of cattle, which can induce lymphoproliferative disorders like lymphadenopathy and neoplastic alterations such as lymphoid cell proliferation. Exotics are more susceptible than indigenous breeds (Mohamad *et al.* 2018). A prevalence study was conducted for the period of 2011-2019, in that they recorded an increasing trend in incidence when compared to the past study (1984-2011). They further did the state-wise analysis, among the central zone (24%) recorded a higher incidence than other zones (Krishnamoorthy *et al.* 2021) The parasite has a biphasic life cycle, with the first phase occurring in the arthropod vector tick, *Hyalomma anatolicum*, and the second phase occurs in definitive hosts, *viz.* domesticated and wild ruminants (Mehlhorn and Schein 1984). Lethargy, fever, swollen lymph nodes, icteric mucus membranes, anemia, jaundice, pica, abortion, and stillbirths are the frequent clinical symptoms (Mohamad *et al.* 2018). Anemia is an important feature in the clinical pathology of the diseases especially when

the parasite rates are increased. Elevations in liver function enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) may be detected by serum biochemistry (Ayadi *et al.* 2017). The piroplasms in red blood cells and Koch's blue bodies in the lymphocytes of peripheral blood smears, as well as in fine needle aspiration cytology of lymph nodes, are used to make a clinical diagnosis of parasite infections (Onyinyechukwu *et al.* 2020). Theilerial schizonts alter the host cells into eternal lymphocytes, which gain the hallmarks of cancer like proliferative signaling, apoptosis resistance, and immune evasion (Tretina *et al.* 2015). These characteristics of infected lymphocytes cause host cell growth, resulting in the neo plastic transformation of parasitized cells and further lymphadenopathy (Woods *et al.* 2013).

### The study

A heifer and four-year-old adult cattle of Holstein Friesian female animals with a history of lethargy, in

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appetence, respiratory distress, and intermittent fever were brought to a large animal medicine unit, Teaching Veterinary Clinical Complex, GADVASU.

The heifer and four-year-old adult cattle exhibited high fevers of around 106-107°F and 103-104°F, respectively on clinical examination. Tachycardia, tachypnea, anemia, icteric conjunctival and vaginal mucus membranes, submandibular edema, and enlargement of bilateral prescapular lymph nodes were also observed during the clinical evaluation. To investigate the hematological and cytological changes, whole blood (about 2 mL) was collected from the jugular vein in the EDTA vial and fine needle aspiration cytology from the lymph nodes using a 24 gauze needle. Blood smear and lymph node cytology were then stained with Leishman's stain according to conventional staining procedures. The hematological parameters like hemoglobin, total erythrocyte count, total leucocyte count, and packed cell volume were analyzed by using the ADVIA 2120 Siemens automated hematology analyzer, and the differential leucocyte count was also screened in the blood smear. The biochemical parameters were analyzed in Vitros 350 system, an automated analyzer.

### Results and discussion

The haemogram results showed anemic appearances in both cases, including decreased hemoglobin, total erythrocyte count, packed cell volume, total leucocyte count, platelet count, along with relative lymphocytosis. The erythrocytes were highly parasitized with signet ring/rod/comma-shaped piroplasms of *Theileria annulata*, as indicated by microscopic inspection of the blood smear. Multiple piroplasms were seen in single RBCs in the blood smears (Fig. 1), with the severity of the infection differing only slightly among the cases. The schizonts (Koch blue bodies) were also seen in the lymphocytes of both cases (Fig. 2). The blood picture of the animals revealed normocytic normochromic anemia. The serum biochemical profiles exhibited a little rise in ALP, and AST with a slight decrease in total protein but no effect on ALT. The hematological and biochemical investigations were in congruence as per Tehrani *et al.* (2013) and Hala and Sara (2019).

The cytological findings of the aspirates of enlarged pre-scapular lymph nodes revealed the presence of encapsulated schizonts of *Theileria annulata* (Fig. 3) in the cytoplasm of lymphocytes and lymphoblasts. Inside the lymphoblasts, around 50-100 numbers of small, round

to oval blue granular bodies called Koch blue bodies, were observed (Onyinyechukwu *et al.* 2020). The cytology revealed multiple presences of schizonts in many lymphoblasts with a monoclonal population of lymphoid cells, bizarre lymphoblasts; hand mirrored activated T-cells, and also all stages of mitotic figures including prophase, anaphase, and telophase (Fig. 4 to Fig. 8).

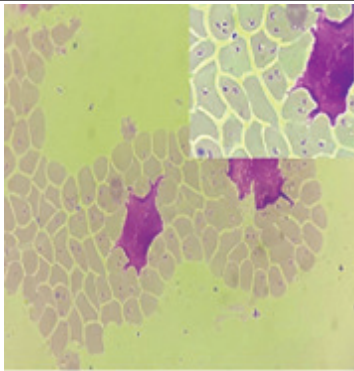
Due to the sheer repeated multiplication of schizonts in the cytoplasm of lymphocytes, the cytology indicated neoplastic-like lymphoid proliferation, grossly resulting in lymphadenopathy. During host cell mitosis, the schizonts bind to the mitotic spindle, allowing it to proliferate into the next generation of cells (Branco *et al.* 2010). These parasites stimulate prolyl isomerase, which helps the host in clonal cell proliferation and further appears as neoplastic transformation. Among the cancer hallmarks, immune evasion and apoptotic resistance are important for immortalized schizont infected cells. The activation of NF-KB-dependent proliferative signaling, induction of anti-apoptotic protein, cFLIP, and cytoplasmic p53 gene sequestration aid in the continuous proliferation of parasite-infected cells (Mohamad *et al.* 2018).

The animals were treated with Buparvaquone at a dose rate of 2.5mg/kg bwt intramuscularly and advised to repeat after a week. Anti-inflammatory meloxicam was given at a dose rate of 0.3mg/kg I/M for consecutive three days. Owners were advised to give the topical application of flumethrin 1% pour-on preparation over the spinal column. After seven days, the animals showed a reduction in fever, lymph node enlargement, and remarkable improvement in feeding and milk production (Verma and Singh 2016).

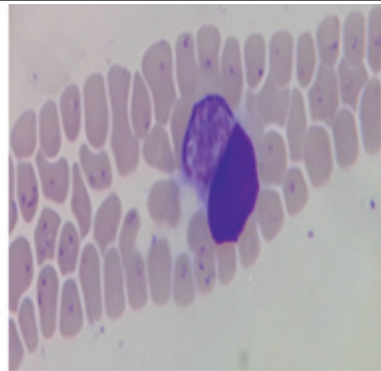
The bovine tropical theileriosis occurrence is mostly increased in recent periods in bovines. The preliminary diagnosis of parasitic stages of *T. annulata* in blood smear prompts the treatment and prevents the overt consequences like loss in production and also the animals. In this paper, the authors described the anemic features due to infection of *T. annulata* in cross-bred cattle which induced neoplastic changes like lymphoid growth and cytological transformation of the lympho- proliferative disorders.

### ACKNOWLEDGEMENT

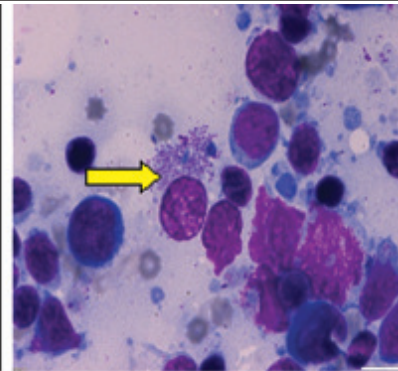
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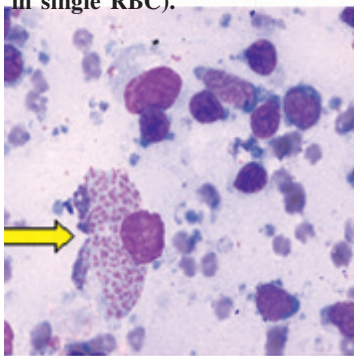
**Fig. 1. Heavy infection of piroplasms of *Theileria annulata* in erythrocytes (Inset: 2-3 piroplasms in single RBC).**



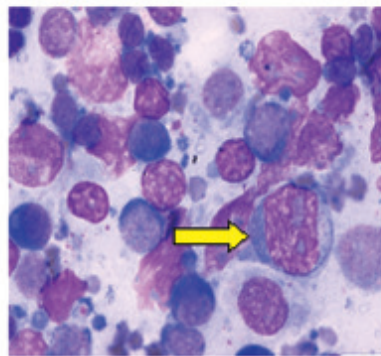
**Fig. 2. Koch Blue Body in Lymphocyte along with multiple piroplasms in RBCs.**



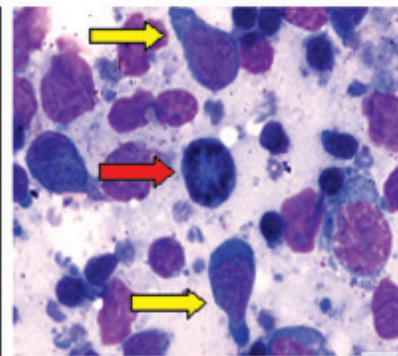
**Fig. 3. Microschizont in a lymphoblast.**



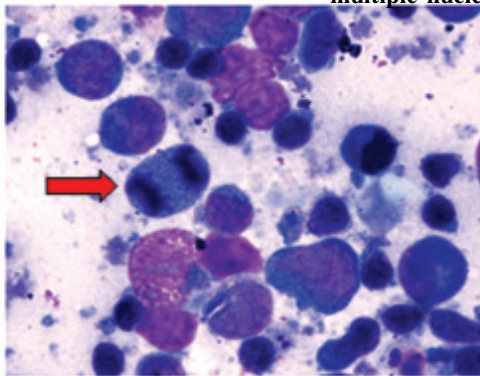
**Fig. 4. Macroschizont in a lymphoblast.**



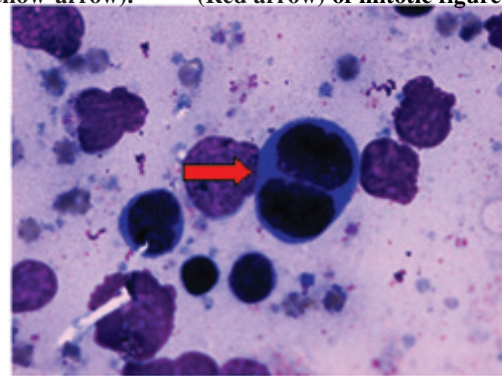
**Fig. 5. Neoplastic like lymphoid proliferation with prominent multiple nucleoli (Yellow arrow).**



**Fig. 6. Hand mirror shaped T Cells (Yellow arrow) with prophase stage of mitotic figure. (Red arrow).**



**Fig. 7. Anaphase stage (Red arrow).**



**Fig. 8. Telophase stage (Red arrow).**

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