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Buparvaquone mediated improvement in the health of Holstein Friesian infected with high parasitaemia of *Theileria annulata*

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Abstract
A clinically infected female Holstein Friesian cross-bred, age 5 years with the symptoms of anorexia, dullness and decline of milk yield, pale mucous membrane, swelling pre-scapular superficial lymph node and temperature 106 °F was diagnosed for acute theileriosis by microscopy and PCR. Microscopic examination recorded 85% of parasitaemia. The piroplasms in parasitized RBCs per field were counted and recorded 6, 5, 4, 3, 2, 1 piroplasms in 1, 2, 6, 10, 45, and 80 RBCs, respectively. Haematological values *viz.* haemoglobin (Hb gm/dl), packed cell volume (%) and total erythrocyte count (x10^6/ µl) were drastically reduced to 6.2 (gm/dl), 18% and 1.92 x 10^6/µl, respectively. Animal was treated with single dose of Buparvaquone (Butalex) @2.5 mg/kg.bwt i/m and hematinic (Ferritas) 10 ml i/m, and Dexamethasone 4ml s/c, Meloxicam 10 ml i/m for 3 days along with 5% dextrose as supportive therapy. Improvement on the health of animals was recorded after 3rd day post treatment.

Keywords: Tropical theileriosis, microscopy, Haematology, Buparvaquone

Introduction
Bovine tropical theileriosis, an important tick borne disease of cattle, is caused by an intracellular haemoprotozoa, *Theileria annulata*. The disease is endemic in tropical and sub-tropical regions of the world including India (Ulenberg 1981, Dolan 1989, Morrison and McKeever 2006) [16, 7, 13]. The parasite causes lymphoproliferation leads to high mortality and morbidity in cross bred cattle, resulting heavy economic losses to dairy industries (Uilenberg, 1995) [17]. Ixodid tick, *Hyalomma anatolicum* acts a potential vector for *T. annulata* (Bhattacharyulu *et al*., 1975) [2]. In endemic areas, sub-clinical form of theileriosis persists in indigenous animals and they serve as potential source of infection for ticks to be transmitted to the susceptible animals. The outcome of the disease is fatal in cross breed cattle (Jithendran 1997, Bishop *et al*., 2004) [11, 4]. In India, 39 million cross-bred cattle are at risk of *T. annulata* infection (Kolte *et al*., 2017) [12].
Generally, clinical form of theileriosis is diagnosed by clinical signs, tick infestation on the body, and finding of piroplasm and schizont stages in the Giemsa-stained blood and lymph node smears by microscopy (Gao *et al*., 2002) [8]. Molecular and serological tests are employed to detect carrier animals (Collins *et al*., 2002; OIE, 2004) [5, 15]. The control measure includes treatment using effective drugs and prevention of ticks using acaricides. This paper communicate the buparvaquone mediated restoration in the health of Holstein Frisian cow infected with 85% parasitaemia of *T. annulata*.

Case History and Observations
A female Holstein Friesian cross-bred, age 5 years was presented to Referral Veterinary Polyclinic Indian Veterinary Research Institute (IVRI) with the history of anorexia, dullness and decline of milk yield. Clinical symptoms observed were pale mucous membrane, swelling pre-scapular superficial lymph node and temperature 106°F. Blood was collected and examined by methanol fixed Giemsa stain thin blood smears. Haematological values *viz*. Hb (gm %), total erythrocyte count, packed cell volume and percent parasitaemia was estimated. Genomic DNA was extracted using QIAamp DNA kit, as per the standard protocol described by manufacture (Qiagen, Germany) and molecular confirmation was done using specific primer targeting cytochrome b gene of *T. annulata* (Bilgic *et al*., 2010) [3].

Result and Discussion

Microscopic examination revealed large number of infected red blood corpuscles (RBCs) with ring shaped piroplasms of *T. annulata*. The percentage of parasitemia was determined by counting of infected RBCs from the proportion of total RBCs in 15 to 20 microscopic fields and recorded 85% of the parasitized RBCs (Fig.1). The number of piroplasms in RBCs per field were counted and recorded 6, 5, 4, 3, 2, 1 piroplasms in 1, 2, 6, 10, 45, and 80 RBCs, respectively. In observed field, majority of the parasitized erythrocytes were infected with single piroplasm and maximum 6 piroplasms were recorded in one parasitized RBCs. Haematological values include, haemoglobin (Hb gm/dl), packed cell volume (%) and total erythrocyte count (x10^6/ µl) were drastically reduced to 6.2 (gm/dl), 18% and 1.92 x 10^6/µl, respectively. Piroplasm parasitemia is contributed by infective merozoites produced by intraerythrocytic merozoite and intralymphocytic shizogony (Conrad *et al*., 1985) [6]. The numerous piroplasms observed in parasitized RBCs could be due to intraerythrocytic multiplication of merozoite by schizogony (Conrad *et al*., 1985) [6]. The observed haematological values indicate anemia, which could be due to destructive or lytic effect on the RBCs by the developing intra-erythrocyte parasite (Nazifi *et al*., 2010) [14] or erythrophagocytosis of infected RBCs as a result of immune mechanism. Many authors observed similar trend of changes in haematological values in cattle infected by tropical theileriosis (Nazifi *et al*., 2010, Abubakar *et al*., 2019) [14, 1]. Molecular confirmation was done by analyzing PCR product in 1.4% agarose gel electrophoresis in Tris-acetate- EDTA (TAE) buffer and specific 312bp product size was visualized under UV light (Fig. 2).

Animal was treated with single dose of Buparvaquone (Butalex) @2.5mg/kg.bwt i/m and hematinic (Ferritas) 10ml i/m, and Dexamethasone 4ml s/c, Meloxicam 10ml i/m for 3 days along with 5% dextrose as supportive therapy. Improvement on the health of animals was recorded after 3rd day post treatment. Buparvaquone is a promising therapeutic compound act on both schizont and piroplasm stages of *T. annulata* and therapeutically effective including patent phase. Dexamethasone (Glucocorticoides) restores the vascular integrity and antagonizes the effect of chemical mediator of inflammation, thereby prevents the formation of pulmonary oedema in theileriosis (Gwamaka *et al.*, 2004) [10]. Many authors reported successful treatment of this disease using Buparvaquone along with other supportive drugs Dexamethasone and Meloxicam (Gwamaka *et al.*, 2004, Gupta *et al.*, 2004, Verma and Singh, 2016) [10, 9, 18]. Dairy farmers require special attention to keep animal sheds free from ticks for economical and viable dairy farming, as cross-bred milk animals are highly susceptible to *T. annulata* infection.

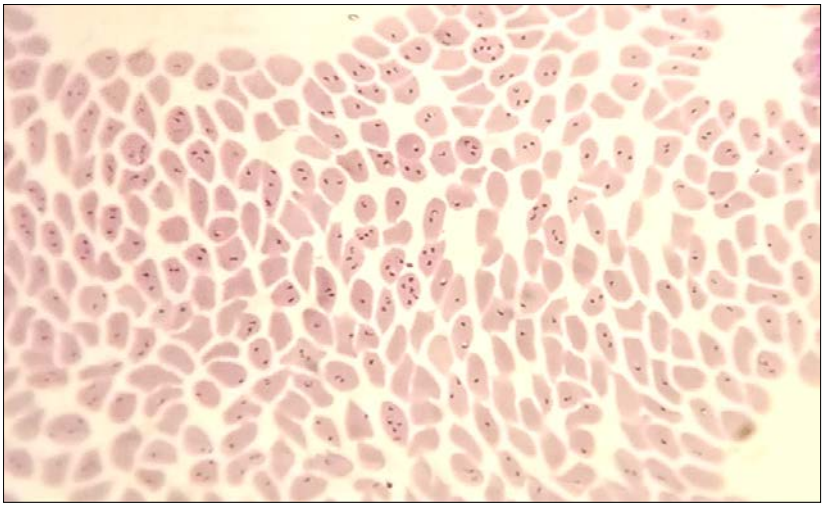

Fig 1: Giemsa stained blood smear showing infected RBCs with piroplasm of *T. annulata*

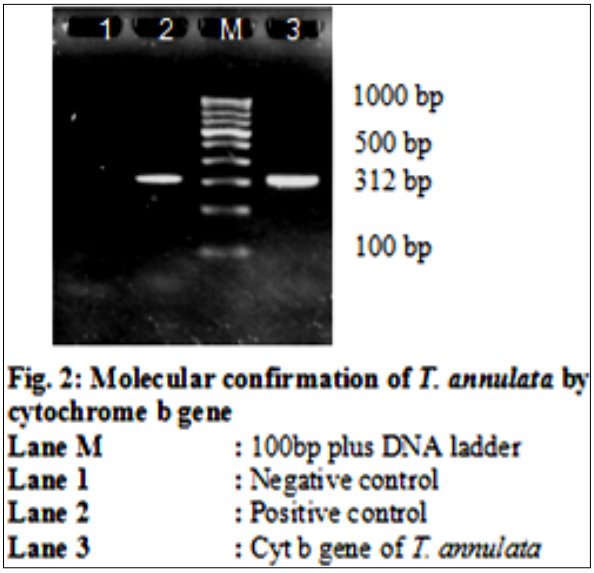

Fig. 2: Molecular confirmation of *T. annulata* by cytochrome b gene

Lane M	: 100bp plus DNA ladder
Lane 1	: Negative control
Lane 2	: Positive control
Lane 3	: Cyt b gene of *T. annulata*

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