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Co-infection with theileriosis and anaplasmosis in a Holstein Friesian crossbred cattle and its therapeutic management

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Abstract
A 5-year-old HF crossbred cattle was examined with history of anorexia, fever, nasal discharge and inability to stand. On clinical examination, the cattle revealed pyrexia (rectal temperature 104.2 °F), papery white conjunctival and vaginal mucous membranes, swollen lymph nodes and mild tick infestation. The cow was unable to stand due to severe weakness. History and clinical signs suggested for haemoprotozoan infection. The blood was collected from jugular vein for haemato-biochemical analysis and preparation of blood smear. The blood smear examination revealed the presence of intra-erythrocytic stages of *Theileria annulata* and *Anaplasma marginale* in blood smear. Haemato-biochemical analysis showed marked anemia (Hb 7.2 gm/dl; PCV 21% and TEC 3.8 $X10^6$/µl), hypoproteinemia, hypoalbuminemia and hyperbilirubinemia. The cattle was treated with single IM injection of buparvaquone @ 2.5 mg /Kg BW followed by IV administration of oxytetracycline @10mg/Kg BW in normal saline for 5 days along with supportive therapy as B-Complex injection @ 10ml IM daily for 5 days and hematinic orally for ten days. The IV administration of hydroxyethyl starch (Vetplasma^R) @ 10 ml/Kg as blood volume expander was also given in divided dose for two days. The affected cattle showed gradual clinical improvement from second day of treatment and onwards, and almost complete clinical recovery was observed after two week post treatment.

Keywords: Theileria sp., *Anaplasma marginale*, haemato-biochemical changes, treatment

1. Introduction
In cattle, tick-borne diseases (TBDs) are a major concern for livestock farmers, and they are frequently infected with more than one pathogen transmitted by ticks (Kumar *et al.*, 2021) [1]. They cause significant morbidity and mortality in cattle and buffaloes. *Babesia spp*., *Theileria* spp. and *Anaplasma* spp are the major hemoprotozoan parasites affecting cattle and buffalo. Clinically, *Theileria annulata*, produces lympho proliferative disease characterized by fever, swelling of lymph nodes and anemia (Sivakumar *et al.*, 2014) [3]. Anaplasmosis has been clinically characterized by anemia, icterus, high fever, weakness, weight loss and sometimes the death of the affected animals (Ashuma *et al*., 2013) [2]. Both *Theileria* spp. and *Anaplasma* spp. are responsible for causing anemia in cattle. In Bihar co-infection of theileria and anaplasma in crossbred cattle is available (Kala and Deo, 2018 and Prabhakaran *et al.*, 2021) [4, 5]. The majority of infections are often diagnosed using conventional approaches that rely on microscopic evidence of infective stages of various parasites in blood or tissue fluids (Terkawi *et al.*, 2011; Bharti *et al*., 2022) [14, 15]. These infections cannot be diagnosed symptomatically as the symptoms are overlapping and difficult to define. Early diagnosis and implementation of effective treatment are necessary to prevent death and associated production losses. Hence, the present case report describes successful therapeutic management of co-infection with Theileriosis and Anaplasmosis in a HF crossbred cow.

2. Case history and clinical examination
A 5-year-old HF crossbred cattle was examined on request of owner at village- Narma, Block - Dulhinbazar, District-Patna, Bihar with history of anorexia, fever, nasal discharge and inability to stand. On routine clinical examination, it was revealed that the animal was dull and

depressed with rectal temperature of 104.2 °F. The cow was on sternal recumbency and unable to stand due to severe weakness and, also exhibited papery white conjunctival and vaginal mucous membranes (Fig. 1, 2), swollen lymph nodes and mild tick infestation. Clinical symptoms and history pointed to hemoprotozoan infection. Jugular vein blood was drawn for haemato-biochemical investigation and preparation of blood smear. Giemsa stained smears of blood was examined for diagnosis of any haemoprotozoan infection. The haemato-biochemical examinations i.e. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total protein, albumins, alanine transaminase (ALT) and bilirubin were carried out as per standard procedure.

3. Diagnosis and treatment

Based on microscopic examination, *Theileria* spp. and *Anaplasma marginale* intra-erythrocytic stages were found in the blood smear (Fig. 3). The estimation of haematological parameters revealed low level of Hb, PCV and TEC, indicating anaemia, and serum biochemical analysis revealed hypoproteinemia, hypoalbuminemia, and hyperbilirubinemia (Table 1). The cattle was treated with a single intramuscular injection of buparvaquone at a dose of 2.5 mg/kg BW, followed by five days of intravenous oxytetracycline at a dose of 10 mg/kg BW in normal saline, as well as supportive care in the form of B-Complex injections at a dose rate of 10 ml daily IM for five days and hematinic orally @ 50 gm for ten days. Additionally, divided IV doses of hydroxyethyl starch (VetplasmaR) @ 10 ml/Kg were administered as a blood volume expander. The affected cattle showed gradual clinical improvement from second day of treatment and onwards, and almost complete clinical recovery was observed after two week post treatment (Fig. 4).

Fig 1: Cattle showing on sternal recumbency with lateral kink of head

Fig 2: Pale white vaginal mucous membrane

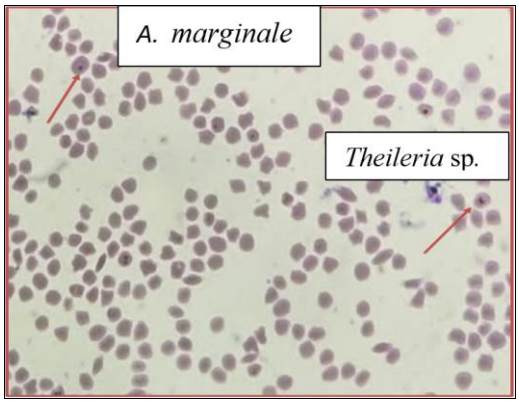

Fig 3: *A. marginale* and *Theileria* sp. in Giemsa stained blood smear (100x), (arrow mark)

Fig 4: Cattle showing complete recovery after treatment

Table 1: Haemato-biochemical changes in cattle co-infected with infected *Theileria* sp. and *A. marginale*

Parameters	Results	Reference*
Haemoglobin (Hb, g/dl)	7.2	8.0-15.0
Packed cell volume (PCV, %)	21	24-46
Total erythrocyte count (TEC, x 10^6/µl)	3.8	5.0-10.0
Total Protein (TP, g/dl	4.2	5.7-8.1
Albumin (g/dl)	1.9	2.1-3.6
ALT (IU/l)	48	11-40
Bilirubin (mg/dl)	0.98	.01-0.5

(*Radostitis *et al.*, 2006) [13]

4. Discussion

Tick-borne haemoparasitic diseases account for substantial losses in terms of decreased working capacity, growth and productivity of cattle. These haemoparasites have a negative impact on haematological parameters. Anaemia, jaundice, anorexia, weight loss, and infertility have all been associated with hemoparasites (Debbarma *et al.*, 2017) [11]. In this study, the fall in haematological values was indicative of anaemia (Table 1), and mainly attributed to persistent loss of blood caused by permanent blood sucking ticks (Durani *et al*., 2008) [12] and destruction of erythrocytes by macrophages in lymph nodes, spleen and other organs of reticuloendothelial system (Kaur *et al.*, 2020) [6]. The hypoproteinaemia and hypoalboumineamia is possibly due to the harmful effect of toxic metabolites of *Theileria* spp.(Al-Emarah *et al.*, 2012) [8] and also owing to liver damage in *Anaplasma marginale* (Ganguly *et al.*, 2022) infection [9] The increased ALT value might be due to severe anaemia which leads to hypoxic condition and liver damage (Ganguly *et al.*, 2022) [9] and total serum bilirubin might resulted from the destruction of parasitized erythrocytes by erythrophagocytosis in the spleen,

lymph nodes and other organs of the reticuloendothelial system (Khan *et al.*, 2011) [7]. Hydroxy ethyl starch (Vetplasma) can be used to maintain plasma oncotic pressure and hypoproteinemia/hypoalbuminemia (Hepworth-Warren, 2020) [10]. Buparvaquone is the most effective antitheilerial drug against the clinical form of Theileriosis in cattle (Saravanan *et al.*, 2017) [16]; however Oxytetracycline has an excellent results on recovery in most of the clinical cases of Anaplasmosis (Kumar *et al.*, 2015) [17]. Additionally, Oxytetracycline is also found to be effective against schizont stage of *Theileria*.

5. Conclusion

Concurrent infection with Theileriosis and Anaplasmosis can be successfully treated with Buparvaquone and Oxytetracycline along with supportive therapy by using Hematinic and Plasma extender to combat the effect of anemia and hypoproteinemia respectively.

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