

Original Research Article

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Co-Infection of *Babesia bigemina* and *Ehrlichia* sp. in a Holstein Friesian Bull – A Pathomorphological Report

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ABSTRACT

Keywords

Bull, Holstein Friesian, *Ehrlichia* sp., *Babesia bigemina*, Pathomorphology

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A pure bred imported Holstein Friesian bull aged 4.5 years was brought for necropsy with a history of treatment for suspected anaplasmosis. Gross examination revealed a dehydrated carcass with pale mucous membranes. Pericardial sac contained about 75 mL of serosanguineous fluid. Myocardial and subendocardial ecchymotic haemorrhages were seen in the heart. Lungs revealed patchy lobulated pattern and thickening of interlobular septa and emphysematous areas. Hepatic parenchyma was firm and tan yellow brown. Gall bladder contained about 25 mL of dark yellow green bile. Cortex and medulla of kidneys were dark red brown. Urinary bladder contained about 1.5 litres of coffee coloured urine suggesting haemoglobinuria. Peripheral and heart blood smears revealed *Babesia bigemina* in the erythrocytes and morula of *Ehrlichia* sp. in the monocytes with anaemic changes. Spleen imprints revealed macrophages with numerous *Ehrlichia* sp. Based on the above findings, the case was diagnosed as Babesiosis and Ehrlichiosis.

Introduction

Bovine Babesiosis and Ehrlichiosis are worldwide in distribution in cattle population transmitted by arthropod vectors (Mosqueda *et al.*, 2012). It causes serious economic losses in cattle industry. *Babesia* sp. is intra erythrocytic obligate apicomplexan parasite highly prevalent in tropical and subtropical countries. Ixodid ticks mainly transmit the *Babesia* sp. (Allred, 2003; Purnell *et al.*, 1976; Taylor *et al.*, 1986; Homer *et al.*, 2000). Ehrlichiosis in bovine is caused by Gram negative obligate intracellular bacteria belonging to the genus *Anaplasma*. Initially it

was named as *Ehrlichia bovis* and later renamed as *Anaplasma bovis* (Prasath *et al.*, 2016; Sreekumar *et al.*, 1999). It causes serious disease in tropical countries, cross bred cattle and newly imported cattle. They occur in cytoplasm of the monocytes. It is mainly transmitted through the tick species of *Amblyomma*, *Hyalomma* and *Rhipicephalus*. In India, *Ehrlichia bovis* is highly prevalent in Indian states (Ramesh *et al.*, 2008; Chirayath *et al.*, 2012; Nair *et al.*, 2013).

Microscopical detection of the parasites are still the most cheapest and economic method in the detection of the parasite. Other

immunological and serological methods can be made use of for further confirmation (Bock *et al.*, 2006). The main objective of the present study is to discuss describe the co-infection, its microscopical detection, gross and histopathological findings in a bull.

Materials and Methods

Necropsy examination

A pure bred Holstein Friesian bull was presented to the Department of Veterinary Pathology, Madras Veterinary College with a history of sudden death. It was clinically suspected for anaplasmosis based on the signs. Preliminary history was obtained and necropsy examination was carried out.

Collection of samples

The tissue samples of 4mm thickness of each were collected from heart, kidney, liver and spleen in 10 per cent formalin in wide mouthed bottles. After fixation, the samples were processed for histopathology. Paraffin embedded tissue sections were cut into 4 µm thickness and stained with Haematoxylin and Eosin and it was viewed under light microscopy. Peripheral blood smears, heart blood smears and impression smear from spleen were collected in a clean grease free microscopic slide. It was air dried and stained with Leishman Giemsa stain solution for 30 minutes, air dried and viewed under oil immersion objective of light microscope.

Results and Discussion

Blood smear examination:

The presence of organisms are represented in the Fig. 9 and 10. The heart blood smear revealed a pair of *Babesia bigemina* parasite within the erythrocyte which were pyriform in shape and in acute angle and few were

pleomorphic in shape. The parasitic burden was seen in almost 40 percent of the erythrocytes. The heart blood smear as well as the touch impression smears from spleen revealed the presence of a medium sized homogenous spherical dark blue colored *Ehrlichia* sp. morula in the cytoplasm of the monocytes. More than 60 per cent of the monocytes revealed parasitic burden.

Gross pathology

The gross morphology is represented in the Fig. 1 to Fig. 8. Necropsy examination revealed a dehydrated carcass with pale mucous membranes. Pericardial sac contained about 75 mL of sero-sanguineous fluid. Heart revealed myocardial and subendocardial ecchymotic haemorrhages.

Lungs revealed patchy lobulated pattern and thickened interlobular septa with multifocal emphysematous areas. Liver was firm with tan yellow brown parenchyma. Gall bladder had about 25 mL of dark yellowish green colored bile. Spleen was mildly enlarged, subcapsular vessels were congested and on incision it had dark red black parenchyma. Kidneys had dark red brown cortex and medulla. Urinary bladder was distended with about 1.5 litres of coffee coloured urine with serosal congestion.

Histopathology

The histopathology of the respective organs is represented in Fig 11 to Fig. 14. Microscopical examination revealed mononuclear cell infiltration in the hepatic parenchyma. Spleen revealed severe congestion and haemorrhage. Kidney showed congestion, tubular epithelial cell degeneration with mild interstitial nephritis. Congestion with focal haemorrhages were seen in the lymphnode. Heart revealed severe endocardial and myocardial haemorrhages.

Fig.1 A 4.5 year old pure bred imported Holstein Friesian bull – dehydrated carcass; **Fig.2** Heart - severe epicardial petechial to ecchymotichemorrhages; **Fig.3** Heart – Severe diffuse endocardial hemorrhage; **Fig.4** Lungs - Patchy lobulated pattern and thickening of interlobular septa and emphysematous areas; **Fig.5** Liver -Firm with tan yellow brown parenchyma; **Fig.6** Gall bladder contained about 25 mL of dark yellow green jelly like bile; **Fig.7** Kidneys -Dark red brown cortex and medulla; **Fig.8** Urinary bladder - Distended and contained about 1.5 litres of coffee coloured urine with serosal congestion

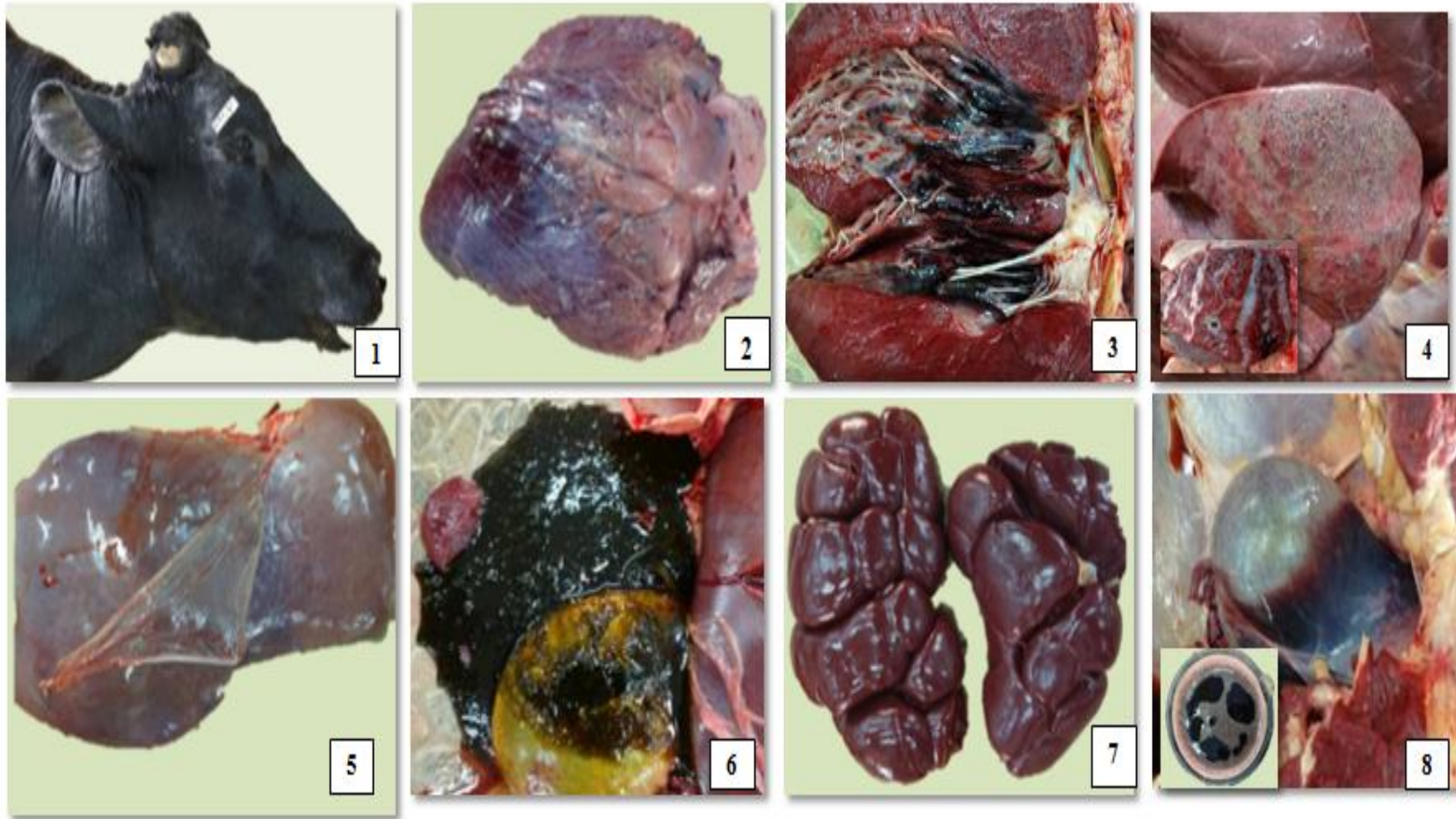
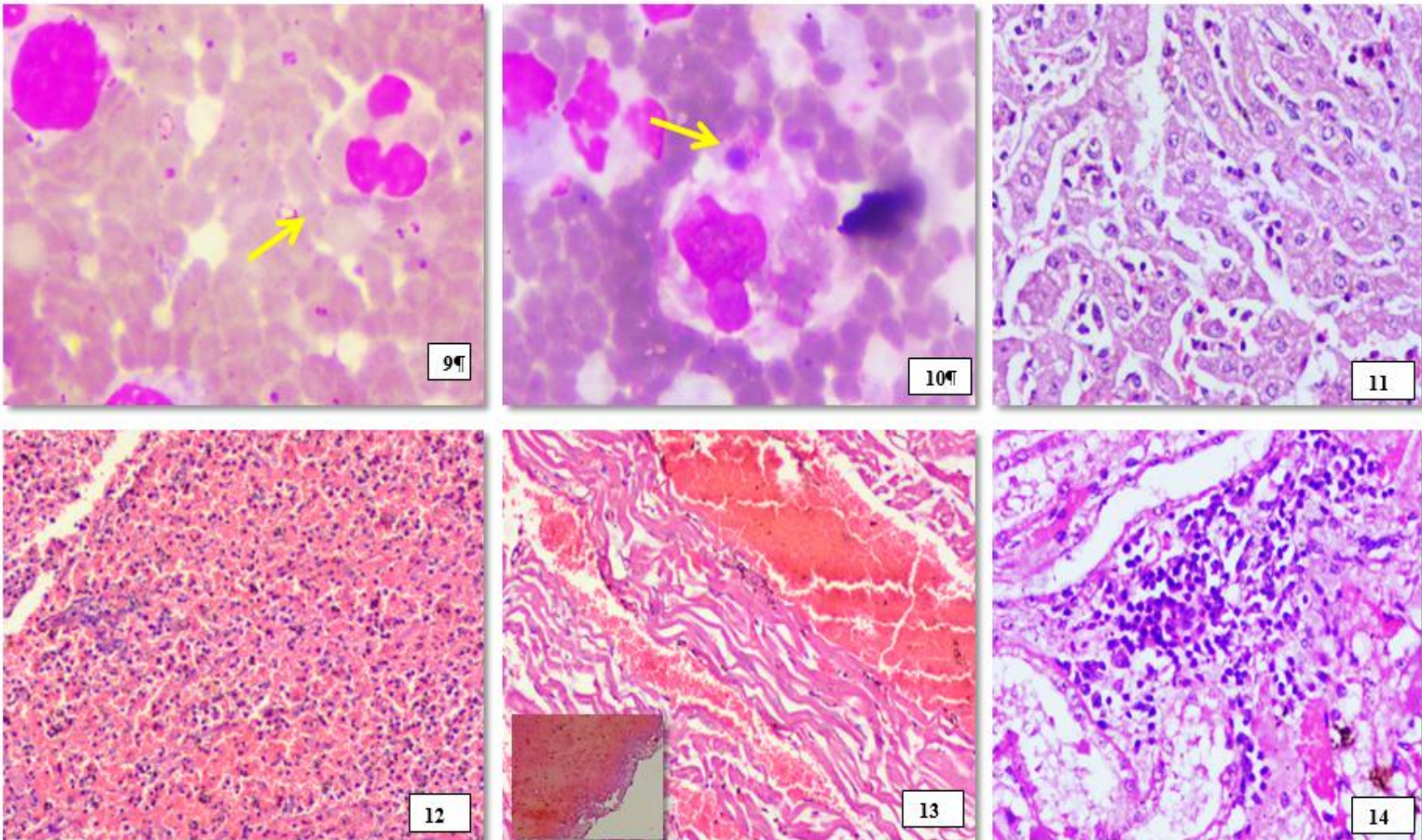


Fig.9 Heart blood smear revealed *Babesiabigeminaw* within the erythrocytes (Leishman & Giemsa 100x); **Fig.10** Heart blood smear revealed *Ehrlichia* sp. morula within the cytoplasm of the monocytes (Leishman & Giemsa 100x); **Fig.11** Liver – Mononuclear cell infiltration (H&E 20x); **Fig.12** Spleen – Severe congestion and haemorrhage (H&E 20x); **Fig.13** Heart – Severe Myocardial and endocardial hemorrhages (H&E 20x); **Fig.14** Kidney - Mild interstitial nephritis with tubular epithelial cell degeneration (H&E 20x)



The occurrence of co-infection correlated with the previous reports of Ramesh *et al.*, (2008) who described only the clinical and treatment aspects of the case in a cow. Wherein, this case describes the complete clinico-pathological, post mortem and histopathological findings in a bull. Another earlier report by Andersson *et al.*, (2017) stated the co-infection of different strain of babesia and anaplasma (*Ehrlichia*) viz., *Babesia divergens* and *Anaplasma phagocytophilum* in cattle in Sweden.

The characteristic appearance of the organism in the blood smear was in agreement with the earlier reports (Sreekumar *et al.*, 1999; Homer *et al.*, 2000; Chirayath *et al.*, 2012; Mosqueda *et al.*, 2012 and Bock *et al.*, 2012; Nair *et al.*, 2013; Prasath *et al.*, 2016; Ramadevi *et al.*, 2017). However, Bock *et al.*, (2012) reported that thick blood smears are more sensitive in detection of the parasite. But, in the present case, since the parasitaemia was higher than the average quoted levels, the thin blood smears obtained from the heart were good enough in detecting the parasite.

The gross and histopathological findings are in agreement with the pathological descriptions in previous reports (de Vos *et al.*, 2004; Bock *et al.*, 2012). The presence of haemoglobinuria, excessive granular and thick bile, haemorrhages over the epicardium and endocardium were the salient findings in this case suggestive of babesiosis. However, they have not reported the salient pathomorphological features in cases of co-infection. Hence, this report describes in detail the presence of co-infection and its corresponding gross and histopathological findings.

Hence, the present study describes the co-infection of Babesiosis and Ehrlichiosis in a bull and its gross and histopathological manifestations.

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