CLINICO PATHOLOGICAL ALTERATIONS INTO CONCURRENT INFECTION OF EHRLICHIA CANIS AND BABESIA GIBSONI IN A DOBERMAN DOG

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Present report discusses clinico-pathological alterations in a nine year old Doberman dog admitted to the teaching Veterinary College at Shirwal with history of severe anaemia, weakness, lateral recumbancy and tick infestation. Blood smear examination revealed intra-erythrocytic small piromplasms of B. gibsoni. Monocyte in the blood smear contained morulae of E. canis. Physical and hematology examination of the dog revealed fever, spleenic and hepatic enlargement, moderate normocytic regenerative anaemia with polychromasia and presence of immature nucleated erythrocytes, severe thrombocytopenia and higher level of enzymes alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT) with hypoproteinemia, hypoalbuminemia and hyperglobulinemia. Specific treatment with doxycycline and diminazene acetate along with appropriate supportive therapy was given. However, the dog died in 5 hours after treatment was initiated. Necropsy revealed pale mucous membranes, petechiae over serosal surfaces of visceral organs, generalized lymphadenopathy, hepatosplenomegaly, edema and ascites. Though babesiosis and ehrlichiosis can be managed indiually, the prognosis of mixed chronic infections is grave. This seems to be the first report of concurrent infection of mixed babesiosis and ehrlichiosis in dog from Maharashtra.

Key words: Babesia gibsoni, Clinical pathological, Dog, Ehrlichia canis.

Canine ehrlichiosis and canine babesiosis are two major tick-borne diseases of canines throughout the world. The concurrent infection with Ehrlichia canis and Babesia gibsoni both have been observed to occur in an endemic regions. Canine monocytic ehrlichiosis and canine babesiosis are most two important and prevalent diseases in India (Abd Rani et al., 2011). Individually, both the diseases have been frequently reported from various parts of the country. Concurrent infection with two or more protozoan parasite occurs infrequently, but may lead to grave prognosis (Kalaivanan et al., 2017). Furthermore, to the best of the author’s information, there is no report of concurrent infection of Canine babesiosis and Canine monocytic ehrlichiosis from the Maharashtra.

Case History and Observations

A nine year-old male Doberman dog was presented to the teaching Veterinary hospital of the College in the month of August2016 with the history of severe anaemia, weakness, and lateral recumbancy. Presented animal was having severe pale visible mucous membranes and tick infestation. Clinical examination revealed fever (104.20 F), tachycardia (heart rate- 120 beats/minute), irregular breathing. Palpation revealed hepatosplenomegaly and enlargement of popliteal lymph nodes. Blood was collected from the recurrent tarsal vein for hematology and serum biochemistry. Hematology revealed, severe anemia and pancytopenia. Leishman’s stained blood smear revealed moderate polychromasia and anisocytosis suggestive of a regenerative response. Small (1-2µm) signet ring-shaped B. gibsoni were seen individually within erythrocytes (Fig.-1). Morulae of E. canis in the cytoplasm of mononuclear cells were also observed in blood smear and buffy coat smears (Fig.-2). Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) were elevated. Total bilirubin, blood urea nitrogen (BUN) and creatinine were elevated with hypoproteinemia, hypoalbuminemia and hyperglobulinemia. On the basis of clinico-pathological findings, treatment was initiated with doxycycline and diminazene acetate along with appropriate supporting therapy. However, the dog died after 5 hours of treatment. Necropsy was performed and all the gross pathological alterations were noted.
and representative tissue samples were collected in the 10% buffered formalin solution for routine histopathology. Necropsy examination revealed pallor of visible mucus membranes. Ticks were identified as *Rhipicephalus sanguineus*. The thoracic cavity and pericardial cavity had a moderate amount of serous fluid. Generalized moderate lymphadenopathy along with severe diffuse enlargement of liver and spleen were noted in the present study (Fig.-3, Fig.-4). The mucous membrane of the urinary bladder was thickened with diffuse streaks of hemorrhages (Fig.- 5). Kidneys were moderately enlarged, renal cortex and sub capsular area had large focal patches of hemorrhages (Fig.-6).

Fig.-1. Showing Moderate polychromasia, anisocytosis and intraerythrocytic signet shaped inclusions of *B. gibsoni* (Leishman stain)

Fig.-2. Buffy coat smear showing morula of *E. canis* in monocyte (Leishman’s staining)

Fig.-3. Showing enlarged and mottled liver

Fig.-4. Showing enlarged spleen

Fig.-5. Showing diffuse streaks of haemorrhages at the urinary bladder mucosa

Fig.-6. Showing Hemorrhages at the cortical area with congestion corticomedullary junction

Histopathology of liver revealed multifocal areas of centrilobular to mid zonal necrosis and fatty changes with loss of hepatic architecture (Fig.-7). Spleen revealed presence of hyperemia and splenic architecture was displaced due to infiltration of inflammatory cells (Fig.-8). Lesion of moderate chronic multifocal plasmacytic interstitial nephritis, with tubular necrosis, atrophy and degeneration were observed in kidneys. There was moderate infiltration of mononuclear cells in interstitial tissues; along with the presence of multiple proteinaceous casts in the tubules (Fig.-9).
Discussion
The complete hematological and serum biochemical findings in the present study were also reported earlier by Alan et al. (1991) in either singly *Ehrlichia/Babesia* infected animals or from concurrently infected animals. Thrombocytopenia is the most common and consistent finding of canine monocytic ehrlichiosis as also reported by Dubie et al. (2014). Anaemia a common feature in babesiosis and ehrlichiosis was also observed in the present case. Anemia and thrombocytopenia during *E. canis* infection are observed because of endothelial injury, production of platelets migration inhibition factor, immune-mediated destruction of platelets and intravascular disseminated coagulopathies as also mentioned by Dubie et al. (2014). Anaemia in babesiosis is because of intravascular and extravascular lysis of erythrocytes and disseminated intravascular coagulopathies as also reported by Taboada and Lobetti (2006). The anemic response in the present study was a regenerative type which is similar to the findings of Alan et al., (1991). Increased serum level of ALP, AST, ALT, total bilirubin, BUN and creatinine in the present case are indicative of damage caused by the pathogens to various visceral organs as also mentioned by Matthewman et al. (1993); Waner and Harrus (2013). Hypoalbuminemia and hyperglobulinemia observed were similar to the findings of Harrus et al., (1996). Loss of albumin is because of increased vascular permeability, hemorrhages and hepatic dysfunction. Hyperglobulinemia may be due to increased production of nonspecific gamma globulin in the animal body as also reported by Dubie et al. (2014). Gross findings of co-infection of *B. gibsoni* and *E. canis* during the necropsy examination were in accordance with Assarasakorn and Niwetpathomwat (2007). Severe bleeding disorders, pancytopenia and
anemic appearance of the carcass may be due to intra and extra vascular hemolysis, haemorrhages, thrombocytopenia and platelet dysfunction because of the Babesia and Ehrlichia. Gross enlargement of lymph nodes, spleen and liver found in our study, has been also reported by Matthewman et al. (1993). Microscopic findings in the present case were characterized by infiltration by mononuclear cells and neutrophils in the various visceral organs like liver, kidney, spleen along with degenerative and necrotic lesions. These were in agreement with earlier findings of Assarasakorn and Niwetpathomwat (2007); Das and Konar (2013) and Waner and Harrus (2013).

Such concurrent infections of E. canis and B. gilsoni resulting in fulminating disease and require effective control measures to prevent transmission of tick-borne pathogens in domestic dogs in endemic areas.

References