EHRLICHIA CANIS-INDUCED HEPATITIS

Contributors

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Cytology

Giemsa-stained liver imprint smears (Figures 1 and 2): In a slightly hemorrhagic background, several round-to-oval and occasionally polyhedral hepatocytes were seen distributed as single cells or clusters. They had an eccentrically placed round nucleus (occasional cells were binucleated) and slightly basophilic cytoplasm. Dark green intracytoplasmic granules consistent with bile and solid green black bile casts between contiguous hepatocytes were also observed. Dispersed among hepatocytes, numerous mature-appearing lymphocytes, with fewer plasma cells, macrophages, and non degenerate neutrophils were visualized. Several dark-blue coloured cytoplasmic inclusions consistent with *Ehrlichia* sp. morulae were observed in lymphocytes and macrophages. Mild extramedullary hematopoiesis was also noticed in the form of occasional nucleated red blood cells, metamyelocytes and megakaryocytes.

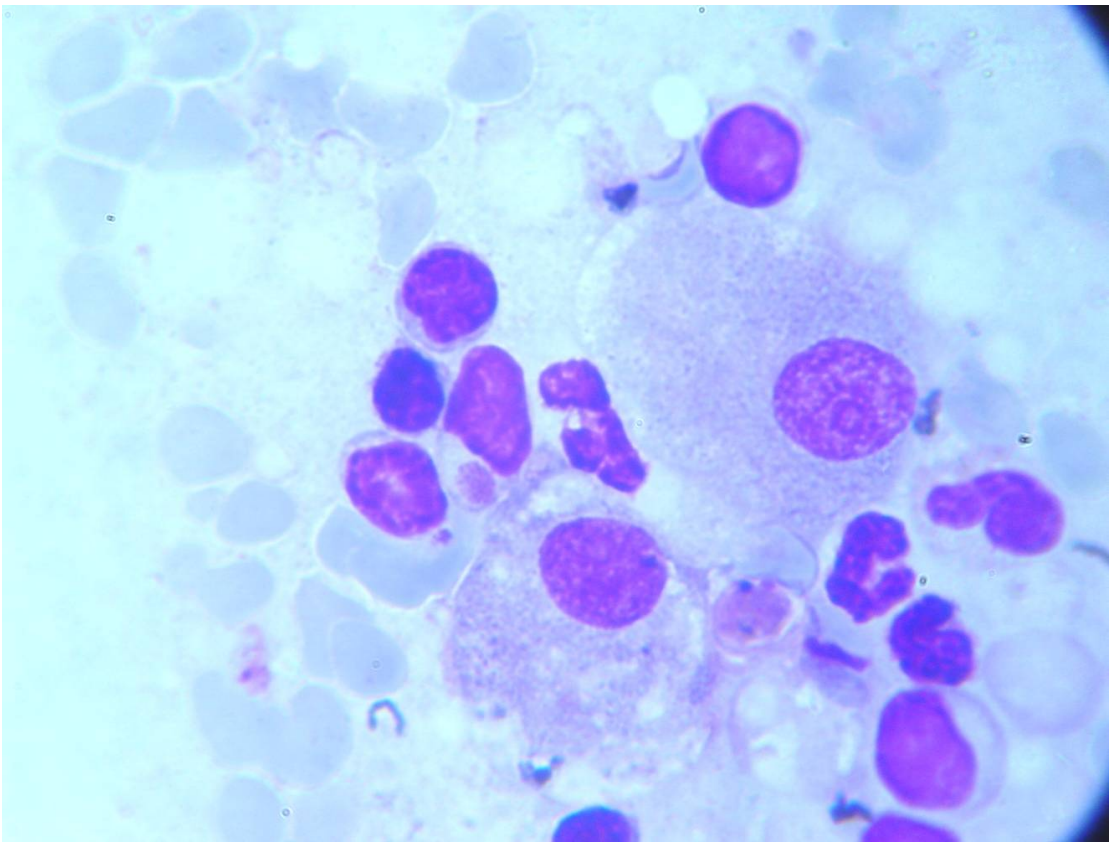

Fig. 1

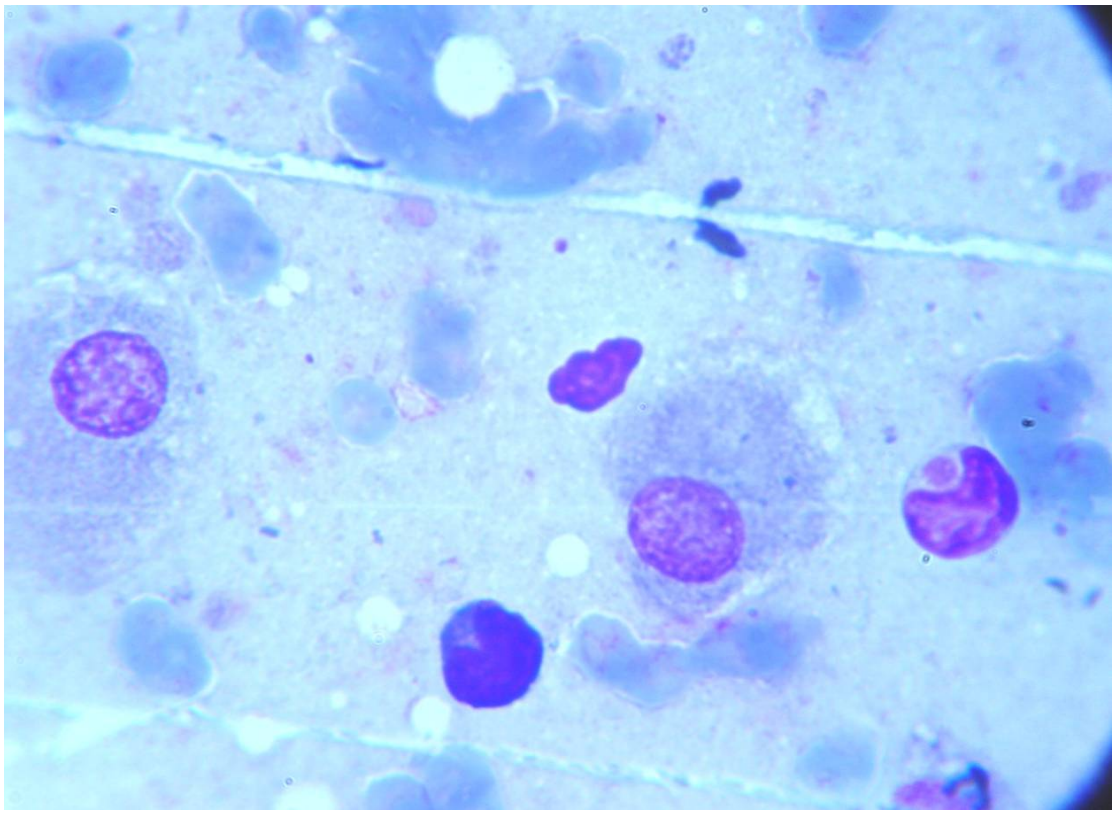

Fig. 2

Giemsa-stained bone marrow aspiration smears: Normocellular marrow with normally represented hematopoietic lineages. Mild plasmacytosis. No evidence of infectious agents.

Follow up buffy coat smear review (prior to institution of doxycycline therapy): No evidence of *Ehrlichia* sp. morulae following review of 2,000 OIF.

Additional serology

MAT for *Leptospira* sp.: negative.

ELISA/IFA for *Ehrlichia canis* (2 and 4 weeks after the first negative result, respectively): negative.

IFA for *Ehrlichia canis* (6 weeks after the first negative result): 1/800.

Liver histopathology and immunohistochemistry

A moderate portal fibrosis with bile duct proliferation, dilation of thin-walled vessels and subtle portal to portal bridging fibrosis was noticed. There was also a mild mixed

inflammatory infiltrate associated with the portal areas that included lymphocytes, plasma cells, macrophages, neutrophils and eosinophils. Some portal hemorrhage and occasional hemosiderophages were seen. The hepatocytes showed mild diffuse microvesicular vacuolation of the cytoplasm and there was generalized congestion. Overall, the findings were indicative of portal hepatitis. Immunohistochemistry on the liver tissue was performed by using *E. canis* monospecific polyclonal antibodies to several major *E. canis* glycoproteins. Several cells, presumably monocytes and lymphocytes, were found to be infected.

Polymerase chain reaction in blood, bone marrow and liver

E. canis 16S rDNA was amplified and sequenced from the blood, bone marrow and liver of the dog. The liver and BM were negative for *Bartonella* sp. DNA.

Treatment

Doxycycline at 5 mg/Kg BW PO BID x 4 weeks was administered. The alternative melarsomine adulticide protocol for *D. immitis* was also instituted after the end of doxycycline therapy.

Clinical and laboratory follow-up

Fever abated within 12 hours and complete clinical recovery was witnessed in 48 hours. Progressive but complete resolution of biochemical abnormalities was finally achieved after three weeks. The dog remains clinically healthy after 3.5 years.

Definitive diagnosis

Ehrlichia canis-induced hepatitis and dirofilariosis (stage one, amicrofilaremic)

Interpretation of laboratory findings and discussion

An *Ehrlichia canis*-associated severe hepatitis was documented in this dog, based on liver cytology, histopathology and immunohistochemistry, PCR amplification of *E. canis* DNA in the blood, BM and liver, along with a positive response to therapeutic

trial with doxycycline. Although mild-to-moderate elevations of liver enzymes and liver pathologic changes are frequently recognized in canine monocytic ehrlichiosis (CME), symptomatic *E. canis*-induced hepatopathy is a rare occurrence[1,2]. Absence of seroconversion in this dog along with the normocellular BM seem to be indicative of the acute CME. Severe *E. chaffeensis*-associated hepatitis, showing similar liver pathology has been reported even in acutely infected people[3]. Immunohistologic demonstration of *E. canis* offers a direct means of establishing the diagnosis of CME. The fact that no morulae were found in the hepatic cells, may indicate that the main pathogenic drive in *E. canis* hepatitis is immune-mediated, as has been hypothesized with *E. chaffeensis* infection in human[3]. Dirofilariosis could be considered an incidental finding, as no historical, clinical or radiographic evidence of an advanced disease was documented.

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