Canine Monocytic Ehrlichiosis – From Pathology to Clinical Manifestations

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ABSTRACT
Canine monocytic ehrlichiosis (CME) caused by Ehrlichia canis is a multisystemic tick-borne disease. The ubiquity of the rickettsia throughout the body organs leads to its involvement in the pathology of organs. The induced profound lymphoplasmacytosis of all parenchymal organs and the bleeding tendencies in all body systems, results in a great variety of clinical signs. This review sets out to present the pathological changes associated with CME, and in so doing attempts to correlate them with clinical manifestations and broaden their scope for the awareness and benefit of the practicing veterinarian. Understanding of this concept may result in an increased inclusion of CME in the differential diagnoses of many organ related problems. This approach may result in the improvement of the diagnosis of CME and consequently the initiation of a specific treatment at an early stage with an improved prospect of cure.

Key Words: Ehrlichia canis, Multisystemic, Pathology, Clinical signs, Diagnosis.

INTRODUCTION
Canine monocytic ehrlichiosis (CME), caused by Ehrlichia canis, is an important disease of dogs and other canids worldwide (1). It is transmitted by the brown dog-tick Rhipicephalus sanguineus, a tick with an expanding global distribution (2). The disease is manifested by a wide variety of clinical and hematological signs and three phases have been recognized: acute, subclinical and chronic (3). The non-specific clinical signs of the acute disease are characterized by a high fever accompanied by depression, lethargy and, anorexia (1). Physical examination typically reveals lymphadenomegaly, splenomegaly and hemorrhagic tendencies which are usually exhibited by dermal petechiae and ecchymoses as well as epistaxis. Thrombocytopenia is the most common hematological finding (4, 5). Non-regenerative anemia and a decline in leukocyte count may also occur in this phase. During the subclinical phase, which is characterized by persistent rickettsemia (6), there are no overt clinical signs and the hematological parameters usually fall into the normal range although the platelet counts may be in the lower normal range (7). The chronic form is characterized by pancytopenia due to suppression or destruction of the bone marrow (8). Clinically the dogs are lethargic, weak and anorexic. In this stage dogs may succumb to fatal secondary bacterial infections and/or bleeding. Therefore, the prognosis of the chronic form of the disease is grave (9). Whereas clinicians tend to concentrate on clinical and hematological aspects of the disease, they may be unaware of a broad range of pathological events and their subsequent clinical implications which may take place as a result of infection with E. canis. Current knowledge indicates that CME is in fact a multisystemic disease. This review sets out to present the pathological changes associated with CME, and in so doing attempts to correlate pathological findings with clinical manifestations and broaden their scope for the awareness and benefit of the practicing veterinarian.
ANATOMICAL AND CLINICAL PATHOLOGICAL FINDINGS

The capability of *E. canis* to affect a variety of organs was well illustrated in a pathological study of 100 dogs, suffering from the chronic pancytopenic severe form of the disease in Southeast Asia (10). Histopathologic findings including lymphocytic, plasmacytic and monocytic infiltrations and perivascular cuffing were detected in numerous organs including the central nervous system, eyes, lymph nodes, spleen, liver, kidneys, urinary bladder, pancreas, prostate and testes (10). The molecular detection of chrllicial DNA in lymph nodes, spleen, liver and kidneys (11), and the wide range of organs which show histological evidence of lymphocytic, plasmacytic and monocytic infiltration supports the finding that *E. canis* is widespread throughout the different body systems of infected animal with the potential to cause a variety of clinical signs. The ubiquity of the rickettsial DNA in many organs is in contrast to the difficulty to microscopically detect the organism in the blood of infected dogs (12). Even in the bone marrow, where the effects of the chronic form are so severe, it is difficult to microscopically visualize the etiological agent in spite of the presence of its DNA (8).

Ehrlichial antigenemia in the plasma of *E. canis*-artificially infected dogs was detected using ELISA (13). Ehrlichial soluble antigen was present starting 15–20 days post-infection, after the development of clinical signs and detection of antibodies against *E. canis*. In addition, circulating immune complexes were detected both in naturally and artificially infected dogs (14). Studying clinical and clinical pathological findings in the latter dogs has suggested the possible association between the presence of circulating immune complexes and the severity of disease (14). Whether the pathological changes are caused by the rickettsia itself, its soluble antigens, due to immune reaction to the presence of immune-complexes with the rickettsial antigens, or due to other aberrant immunopathological processes is not clear.

Cutaneous and mucosal petechiae and ecchymoses are typically seen in infected animals. However, macroscopic and microscopic hemorrhages were found to be present in almost every organ system examined (10). These surface bleedings are the outcome of the thrombocytopenia and platelet dysfunction, both typical findings in CME (15). Hemorrhages in any organ system may result in pathological changes which might generally be of a non-specific nature depending on the location of the hemorrhage. This may further complicate the clinical picture and the diagnosis.

LIVER

Histopathological examination of livers of dogs dying from CME in South East Asia demonstrated that 18% had occasional cuffs of plasma cells around the centrilobular veins and in the portal triads (10).

Mild to moderate increases in the activity of the hepatic enzymes, alanine aminotransferase and alkaline phosphatase are often encountered in cases of *E. canis* infection (1). Liver pathology associated with experimental *E. canis* infection, without overt clinical disease, has been documented as a portal infiltration of lymphocytes, plasma cells and macrophages resulting in pronounced distortion of the surrounding acinar architecture (16). In another experimental study, centrilobular fatty degeneration and mild to moderate perivascular and periportal mononuclear cell infiltration were described (17). Centrilobular degeneration or necrosis and portal plasmacytosis have been seen in naturally infected chronic cases of CME from Greece (8).

A recent case report has described a dog with severe hepatitis manifested by anorexia, intermittent vomiting, and diarrhea of 5 days duration which was associated with acute CME (18). In this dog, portal hepatitis was diagnosed with the presence of *Ehrlichia*-infected monocytes and lymphocytes in the hepatic tissue. *E. canis* morulae were demonstrated by immunohistochemical methods using *E. canis* monospecific gp36 polyclonal antibody. *E. canis* 16S rDNA amplified by PCR from a whole blood sample and from the liver was 100% similar to the Greek strain. Treatment with doxycycline was effective in reducing the fever and resolving all clinical signs (18). *Ehrlichia chaffeensis*, a rickettisia genetically closely related to *E. canis*, has also been documented in a number of cases to cause an acute hepatitis in humans (19–21). Both in the dog and the human cases, seroconversion occurred in a late stage of the disease. Due to the disproportionate hepatic response in the face of only a few to no *E. chaffeensis* morulae in the liver parenchyma, the suspicion of an overzealous immune response was suggested (21).

Spleen

Splenomegaly is a prominent pathological and clinical finding in both the acute and chronic stages of the disease (10,
In the acute phase the splenomegaly is non-congestive and caused by a diffuse proliferation of lymphocytes and plasma cells in the white and red pulps. The spleen was shown to be a major reservoir of ehrlichial organisms probably due to the abundance of hosting macrophages in this organ. Some studies suggest also that it is the last organ to contain the rickettsia before its elimination (6, 22). The spleen has proven to be a rich source of ehrlichial organisms for diagnostic purposes and is considered therefore as the organ of choice for molecular diagnosis during the different phases of the disease (1, 6, 22). In a study of dogs showing thrombocytopenia and clinical signs of CME, the examination of spleen aspirates was fruitful in detecting ehrlichial morulae in 48.5% of dogs, whereas \textit{E. canis} DNA was detected in 72.5% of the aspirates (23).

The spleen appears to play an important role in the pathogenesis and severity of CME (24). Evidence for the deleterious role of the immune system in the pathogenesis of the disease is seen in a study comparing splenectomized with intact dogs artificially infected with \textit{E. canis} (24). Although splenectomized dogs developed antibodies to \textit{E. canis} at the same time interval and titer as intact dogs, they displayed a milder clinical form of the disease. During the acute stage, food consumption was significantly lower in the intact group compared to the splenectomized group and significant higher body temperatures were measured in the intact group. Hematological parameters, hematocrit, hemoglobin concentration and platelet counts were significantly less adversely affected as compared to intact dogs further indicating the involvement of the spleen in the pathogenesis of the disease.

**LYMPH NODES**

Lymphadenomegaly is a common clinical finding during the acute phase of the disease (1). The increase in size of the lymph nodes is in part due to hyperplastic activity of both B- and T- lymphocytes in response to stimulation by ehrlichial antigens (16). A histological characteristic of CME infection is seen in the altered architecture of lymphopoietic tissue with plasmacytosis and a generalized perivascular lymphoid and plasma cell accumulation. B cells, under appropriate stimuli differentiate into plasma cells which secrete \(\gamma\)-globulins. The intense plasmacytosis during CME is consistent with the hypergammaglobulinemia, a common finding in dogs in the acute disease (10). The cytomorphic abnormalities have been found to occur more frequently in dogs in the chronic phase of CME compared to those in the acute phase (25).

The exact nature and reason for the excessive presence of plasma cells in dogs with CME is unknown, however the response to \textit{E. canis} infection by the immune system of the dog appears as an exaggerated B cell response. It has been proposed that this hyperimmune response may be associated with the pathogenesis and pathological lesions seen in the disease (26).

**BONE MARROW**

The changes in the bone marrow closely reflect the course of the hematological findings in dogs with CME (27). Early in the course of the disease the bone marrow is hyperplastic in part, reflecting the abundance of plasma cells, however, few fat cells are present (28). There is an increase in megakaryocyte numbers in the bone marrow during the acute disease as a response to the peripheral thrombocytopenia, indicating active thrombopoiesis (16). In the chronic phase, all dogs examined at necropsy in the study carried out by Hildebrandt \textit{et al.} in South East Asia exhibited a marked decrease in both myeloid and erythroid cell populations with the presence of abundant plasma cells with the rare presence of megakaryocytes (10). Similar findings were present in biopsy samples taken from the bone marrow of dogs in the chronic pancytopenic phase of the disease for diagnostic aspiration cytology (29).

Bone marrow aplasia is a typical pathological finding of the chronic phase of the disease which may be the result of suppression and/or necrosis of the bone marrow. It results in severe peripheral pancytopenia, a typical hematological feature of the chronic phase (29, 30). The pancytopenia can lead to weakness due to anemia, an increased susceptibility of infections due to leukopenia, and widespread hemorrhages throughout the body due to thrombocytopenia and platelet dysfunction (15).

**CENTRAL NERVOUS SYSTEM**

Histological examination of infected dogs has shown that 96% of dogs suffering from chronic CME presented with plasmacytosis of the meninges. It was found that the majority of infiltrating cells were plasma cells with a few lymphocytes. Large immature reticular endothelial cells were also present.
In the more chronic cases, mononuclear cells formed focal aggregates while the more acute cases presented with more diffuse cell accumulations around blood vessels. The sites of predilection were the brainstem, midbrain and the cerebral cortex. In 13% of the cases hemorrhages were seen in the brain (10). Neurologic clinical manifestations as a result of meningitis and/or meningeal bleeding should be expected and may vary in CME (31, 32). In an experimental infection, all 14 dogs infected with *E. canis* developed meningitis (33). Signs of meningitis included seizures, stupor, ataxia, vestibular dysfunction (central or peripheral), anisocoria, cerebellar dysfunction, intention tremors and generalized or localized hyperesthesia (33). In contrast, meningitis was not observed in any of the *E. ewingii*, *E. chaffeensis* or *A. phagocytophilum* experimentally infected dogs (33). The meningitis was described as a lymphoplasmacytic leptomeningitis and was often accompanied by mild neuroparenchymal vascular cuffing and gliosis. In another experimental infection of German shepherd dogs, mononuclear cell meningitis with a broad range of mononuclear cells infiltrating the cerebral cortex, cerebellum and medulla was observed (17).

In humans, *E. chaffeensis* has been documented to cause prominent neurological symptoms which included severe headache, meningismus and altered mental state. Histopathological findings in the brain and meninges demonstrated an infiltrate of atypical lymphoid cells in the leptomeninges and involvement of blood vessel walls, and extension into the Virchow-Robin spaces (34).

**OPHTHALMIC SYSTEM**

Ophthalmic pathologies have been extensively observed in dogs naturally and artificially infected with *E. canis* (33, 35). Ocular pathology was documented in 43% of dogs dying from *E. canis* infection at various installations of the American army in Southeast Asia (10). In one study, 50% of experimentally infected dogs developed ocular lesions (16), whereas in another study all dogs developed ocular lesions, mostly in the acute stage and a few in the subclinical phase (33). A retrospective study from Barcelona, Spain, reported a bilateral ocular disease in 37% of dogs with naturally confirmed infection with *E. canis* (36). In the opinion of the authors of the study, this incidence was an underestimate (36). In all cases, bilateral signs were present indicating the systemic nature of CME. The most marked pathological change observed in the eye was found to be related to plasma cell cuffing of the veins in the ganglion cell layer (43%). Cuffing of blood vessels in the sclera immediately posterior to the limbus was also noted (10). Ocular lesions have been reported to occur in almost every structure of the eye and in some cases were the sole presenting complaint (35). Anterior bilateral uveitis and retinal lesions are considered as the most frequent clinical findings among naturally infected dogs (35-37). Other ocular lesions such as corneal ulceration, necrotic scleritis, decreased tear production and orbital cellulitis have been diagnosed and are also common findings in CME (35).

Most dogs infected with *E. canis* develop hypergamma-globulinemia which in most cases is polyclonal. Some dogs however may develop paraproteinemia characterized by monoclonal gammopathy (38). The latter dogs may develop hyperviscosity with associated clinical signs. Cases of sudden blindness due to subretinal hemorrhage associated with hyperviscosity due to CME have been documented (38, 39).

In a study comparing the relative ability of *E. canis*, *E. chaffeensis*, *Ehrlichia ewingii* and *Anaplasma phagocytophilum* to induce inflammation in the canine eye and cerebral meninges, only *E. canis* infection consistently caused uveal inflammation and meningitis in all artificially infected dogs (33). The other ehrlichial organisms tested failed to cause ocular lesions in dogs.

**CARDIOVASCULAR SYSTEM**

Macroscopic and microscopic hemorrhages in the heart of 84% of CME cases were documented (10). Hemostatic alterations in the heart could possibly impair cardiac function. Furthermore, occasional mononuclear cell aggregates were found in the proximity of small myocardial blood vessels and in the pericardial fat tissue (10).

Recent evidence suggests an increased risk for myocardial injury in dogs acutely infected with *E. canis*. Electrocardiogram, echocardiogram, noninvasive blood pressure and serum cardiac troponin 1 (cTn1) were evaluated in 194 Brazilian dogs with clinical and laboratory abnormalities indicative of CME (40). Dogs infected with *E. canis* were found to have higher serum cTn1 concentrations compared to control naïve dogs. The authors concluded that acute infection with *E. canis* may be a risk factor for myocardial injury in naturally infected dogs. Furthermore, they pointed out that anemia and its severity might have contributed to
the pathophysiology of myocardial damage in these dogs. A single clinical description documenting apparent electrocardiographic (ECG) changes due to infection with *E. canis* has been reported. As the ECG changes resolved with doxycycline treatment the authors suggested their association with CME (41).

**PULMONARY SYSTEM**
Artificial infection of dogs with *E. canis* resulted in an interstitial pneumonia during the acute phase of the disease (16). The alveolar septa in the lungs were found to be thickened by mononuclear cell and macrophage infiltrations. Focal accumulations of lymphocytes and macrophages within and beneath the vascular endothelium were present in small to medium sized arteries and veins (16). In another experimental study with German shepherd dogs, small foci of mononuclear cell infiltrations in the alveolar septa were evident (17).

Microscopic and electron microscopic studies from lungs of dogs experimentally infected with *E. canis* showed mononuclear cells containing morulae of *E. canis* adherent to the luminal surface of epithelial cells of arterioles or capillaries (42). Electron microscopy showed an intimate relationship between the parasitized mononuclear cells and the endothelium of the arterioles. The endothelium of the bifurcation of arterioles was the most common site at which the morulae containing monocytes were present. Although *E. canis* parasitized monocytes are renowned to be difficult to detect in stained blood smears, it was suggested that morulae may be more readily observed in stained mononuclear cells in impression smears taken from the lungs (28, 43).

In the chronic phase of CME, pulmonary changes consisting of mild thickening of the septal wall due to an infiltration of immature reticular endothelial cells and cuffing of medium and small sized blood vessels by plasma cells have been documented in affected dogs. Mononuclear cell infiltrate was also seen in large arteries of the lungs (28, 42).

**RENAL SYSTEM**
Ninety eight percent of dogs, naturally infected with *E. canis*, were histologically diagnosed with plasma cell infiltrate around the veins and arteries in the corticomedullary junction (10). Reardon and colleagues studying the sequential reaction to *E. canis* infection found an infiltration of plasma cells and histiocytes into the outer cortex of the kidney extending to the corticomedullary junction. Perivenular and periglomerular edema, focal necrosis with accompanying cell infiltrate were also documented (16).

Pathological changes have been demonstrated in both the renal tubular and glomerular elements of dogs artificially infected with *E. canis* (44, 45). Transient proteinuria was demonstrated during the acute phase after artificial infection with *E. canis*. Peak urine albumin loss was observed 2.5 to 3.5 weeks after infection. Histological examination during the acute phase revealed perivenular and lymphocytic and plasmacytic infiltrates especially in the renal cortex. Immunofluorescent staining showed moderate to marked deposition of anti-canine IgG and IgM antibodies in the glomerular tufts and mesangium. Electron microscopic studies revealed fusion of the podocyte processes during the period of peak proteinuria. The changes were interpreted as minimal-change glomerulopathy attributable to the cause of the transient proteinuria which might have contributed to the hypoalbuminemia seen during acute CME.

A clinical case report has documented an 8-year old, female, Old English Sheepdog diagnosed with CME based on recurrent fever, mild peripheral lymphadenopathy, mild thrombocytopenia, monoclonal hyperglobulinemia and positive *E. canis* antibody titer which developed proteinuria and renal failure. Immunohistochemical staining of renal biopsy specimens demonstrated amyloid A deposition and amyloidosis. Chronic ehrlichiosis was suspected by the authors to be the cause of the renal amyloidosis (43).

**CONCLUSION**
Pathological and molecular studies have indicated that *E. canis* is widely distributed throughout the body organs of infected dogs (11). The ubiquity of the organism may lead to its involvement in the pathology in a variety of organs. Pathology of numerous organs and associated clinical signs has in fact been encountered in many natural and experimental cases. The profound lymphoplasmacytosis affecting parenchymal organs and the general surface bleeding characterizing CME affect all body systems, resulting in a great variety of clinical signs. Clinicians should be aware that *E. canis* infection is a multisystemic disease which may cause a large variety of different clinical manifestations. Acknowledgement of this notion may result in an increase in the inclusion of CME in the differential diagnoses of many organ related...
problems and hence in an improved diagnosis. Identifying the etiology in early stages of the disease will allow a specific treatment with a greater chance of success.

CONFLICT OF INTEREST
The authors of this paper have no financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of this paper.

REFERENCES