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Canine Monocytic Ehrlichiosis (CME) (13 Apr 2000)

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Introduction

The etiologic agent of canine monocytic ehrlichiosis (CME), the rickettsia *Ehrlichia canis* (*E. canis*), is a small pleomorphic gram-negative coccoid bacteria that parasitizes circulating monocytes intracytoplasmically in clusters of organisms called morulae. The disease is also known as canine rickettsiosis, canine hemorrhagic fever, tracker dog disease, canine tick typhus, Nairobi bleeding disorder and tropical canine pancytopenia, names representing different aspects of the same disease. The disease is acknowledged as an important and potentially fatal infectious disease of dogs and other members of the *Canidae* family.
E. canis was first recognized in Algeria in 1935. Historically the disease assumed great importance during the Vietnam War, causing the death of hundreds of military dogs. The disease has received further attention in 1987 when the closely related organism, *E. chaffeensis* was identified as the cause of human monocytic ehrlichiosis. Subsequently in 1996, *E. chaffeensis* was shown to cause disease manifestations in dogs indistinguishable from E. canis infection.

Pathogenesis

Ehrlichia canis is transmitted by the brown dog-tick *Rhipicephalus sanguineus*. Recently it has also been shown to be experimentally transmitted by the tick *Dermacentor variabilis*. Transmission in *Rhipicephalus sanguineus* tick occurs transstadially, but not transovarially. Larvae and nymphs have been shown to become infected while feeding on acutely ill dogs. The recent demonstration of ehrlichial DNA in the blood of persistently infected clinically healthy dogs 34 months after experimental infection also suggests that dogs in the subclinical stage may be a source of infection. In the tick, *ehrlichiae* are disseminated by hemocytes from the gut to the salivary gland. Throughout feeding, ticks inject *Ehrlichia canis*-contaminated salivary gland secretions into the feeding site. All three stages, i.e. larvae, nymphs and adults, are able to transmit the disease. It has been shown that ticks can survive 155 to 568 days as unfed adults and transmit infection for 155 days after becoming infected. This phenomenon allows ticks to over-winter and infect definitive hosts in the following spring. Ticks are more abundant during the warm seasons, and most acute cases of CME occur during this period. As ehrlichial transmission is mechanical and not biological, infected blood transfusions can also transmit the rickettsia.
Dogs in endemic regions and those traveling to and from endemic areas should be considered potential candidates for the disease. The distribution of CME is related to the distribution of the vector *Rhipicephalus sanguineus* and has been reported to occur in four continents including Asia, Africa, Europe and America. Serologically confirmed cases of CME have now been recorded in most states of the United States. Seroprevalence of *E. canis* antibodies in dogs in Zimbabwe, Egypt and Israel have been shown to be 42%, 33% and 30% respectively.
The pathogenesis of CME involves an incubation period of 8-20 days, followed by acute, subclinical and sometimes chronic phases. During the acute phase, the parasite enters the blood stream and lymphatics and localizes in macrophages of the reticuloendothelial system in the spleen, liver and lymph nodes, where it replicates by binary fission. From there, infected mononuclear cells disseminate the rickettsia to other organ

systems throughout the body.

The clinical signs of the acute phase vary in severity but usually resolve spontaneously, although some dogs may remain subclinically infected. Some dogs may recover the subclinical stage spontaneously, however others may remain persistent carriers for months or years. The demonstration of ehrlichial DNA in the spleen aspirates of 4 persistent carriers 34 months after experimental infection suggests that the spleen is the organ that harbors the rickettsia in subclinical cases. Immunocompetent dogs are believed to be able to eliminate the parasite during the subclinical phase. Some persistently infected dogs may subsequently develop the chronic severe form of the disease. Not all dogs develop the chronic phase of CME, and the conditions leading to the development of the chronic phase remain unclear. German shepherd dogs (GSD) tend to develop the severe chronic phase of the disease more often than other breeds, possibly due to a depressed cell-mediated immune response in these dogs. Death may occur in CME as a consequence of hemorrhage and/or secondary infections.

There is increasing evidence that immunological mechanisms are involved in the pathogenesis of the disease. These include positive Coombs' and autoagglutination tests in infected animals, and the demonstration of antiplatelet antibodies (APA) in dogs experimentally infected with *E. canis*. Both platelet bindable and platelet-bound APA have been demonstrated in the blood of infected dogs, and are proposed to play an important role in the pathogenesis of the thrombocytopenia and thrombocytopathia.

Further evidence for an immunopathological component in the pathogenesis of CME was demonstrated in experimental infection studies carried out on splenectomized dogs. Intact and splenectomized dogs were infected with *E. canis*. Serology, clinical signs and hematological parameters were followed during the course of the acute disease. Splenectomized dogs exhibited a milder form of the acute disease as compared to intact dogs. The results suggested the involvement of the spleen in the pathogenesis of CME. The typical lymphoplasmacytic splenitis with the resultant liberation of splenic inflammatory mediators and/or other splenic substances has been proposed to play a key role in the pathogenesis of the disease.

Clinical Presentation

Naturally occurring CME may be manifested by a wide variety of clinical signs. Large variations in clinical signs have been reported for different studies and have been proposed to be due to a number of factors, including differences in pathogenicity between strains of the ehrlichia, breed of dog, co-infections with other tick transmitted diseases, and the immune status of the dog. There is no predilection for age or sex in infection with *E. canis* and all breeds may be infected. However, the GSD seems to be more prone to develop clinical CME.

Ticks are commonly found on dogs during the acute stage. The clinical signs in the acute phase may be mild and non-specific, however some cases may present as severe and life threatening. The incubation period is 7 to 15 days after which the infected dog enters the acute phase of the disease which may last from 1 to 2 weeks. Signs may include depression, lethargy, anorexia, pyrexia, lymphadenomegaly, splenomegaly and mild weight loss. Dogs may present with bleeding tendencies, mainly petechiae and ecchymoses of the skin and mucous membranes, and occasional epistaxis.

Ocular signs are not uncommon and include anterior uveitis ± corneal opacity (edema and/or deposition of cellular precipitates), hyphema, tortuous retinal vessels and focal chorioretinal lesions consisting of central pigmented spots with surrounding areas of hyperreflectivity. Subretinal hemorrhages, resulting in retinal detachment and blindness may occur. Other clinical signs may include vomiting, serous to purulent oculonasal discharge, lameness, ataxia and dyspnea.

The common clinical signs of the chronic disease are weakness, depression, anorexia, chronic weight loss, pale mucous membranes, fever and peripheral edema, especially of the hind limbs and the scrotum. Platelet-related bleeding, such as petechiae and ecchymoses of the skin and mucous membranes and epistaxis are common findings. Secondary bacterial and protozoal infections, interstitial pneumonia, renal failure, and arthritis may occur during the chronic severe disease. Some reproductive disorders have also been associated with chronic CME including, prolonged bleedings during estrus, inability to conceive, abortions and neonatal deaths. Polymyositis has also been associated with CME. Neurological signs may occur during the acute and chronic disease. These include signs of meningoencephalitis, e.g.: arched back, severe neck or back pain, paraparesis or tetraparesis, ataxia, cranial nerve deficits and convulsions. Neurological signs may be attributed to hemorrhages, extensive plasma cell infiltration and perivascular cuffing of the meninges.

Hematology
Thrombocytopenia is the most common and consistent hematological finding in acute CME. A concurrent significant increase in the mean platelet volume is also usually seen reflecting active thrombopoiesis. Mild leukopenia and mild anemia (usually normocytic, normochromic, non-regenerative) commonly occur in the acute stage of the disease. Mild thrombocytopenia is a common finding in the subclinical stage of the disease. A decline in the neutrophil counts may occur. Erythrocyte parameters are not normally affected during this stage of the disease. Severe thrombocytopenia, leukopenia and anemia are most frequently seen during the chronic stage of CME. Severe pancytopenia is the hallmark of the severe chronic phase, occurring as a result of suppressed hypocellular bone marrow.

Biochemical Findings
Hypoalbuminemia, hyperglobulinemia and hypergammaglobulinemia are the principal biochemical abnormalities seen in dogs infected with CME. Serum protein electrophoresis usually reveals polyclonal gammopathy, however infected dogs on rare occasions may present with monoclonal gammopathy, which may be misdiagnosed as paraproteinemia. Pancytopenic dogs reveal significantly lower concentrations of total protein, total globulin and gammaglobulin concentrations as compared to non-pancytopenic dogs. The lower concentrations of the gammaglobulins coupled with the pancytopenia suggest that the immune state of the pancytopenic *E. canis* infected dogs is more compromised, and therefore secondary infections should be expected to occur more frequently in these dogs. Mild transient increase in alanine aminotransferase and alkaline phosphatase activities may be present.

Diagnosis
The majority of CME cases occur in endemic areas during the spring and summer months when the tick population is most active. Diagnosis of CME is based on anamnesis, clinical presentation, clinical pathological findings and confirmed by laboratory tests. Owners may report previous tick infestations or a recent visit to an endemic area. Diagnosis of CME is confirmed by visualization of the morulae in circulating monocytes (Fig. 1), detection of increased serum antibodies to *E. canis*, or by the demonstration of *E. canis* DNA by polymerase chain reaction (PCR).
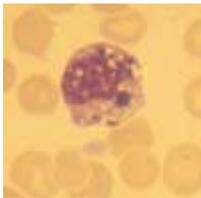

Figure 1.*Ehrlichia* morulae in circulating mononuclear cells of infected dog (Giemsa Stain). - To view this image in full size go to the IVIS website at www.ivis.org .

Presently, the indirect immunofluorescent antibody (IFA) test using *E. canis* antigen is the most acceptable serological test. The presence of anti-*E. canis* antibody titers at a dilution greater than 1:40 is considered evidence of exposure. In the acute stage of the disease when dogs are clinically ill, antibody titers increase rapidly. From experimental studies it appears that at the time of presentation, dogs clinically ill in the acute stage of the disease have substantial antibody titers.
Dot-ELISA tests have recently been developed for use in the clinic. These tests require the minimum of equipment and will make serologic diagnosis of CME available on a wider basis. This should prove to be an invaluable in-clinic aid in the serodiagnosis of CME.
When assessing IFA antibody titers to *E. canis* in dogs it is essential that the diagnostician take in account the range of cross-reactivities that may also confound the diagnosis. In areas endemic to other *Ehrlichia* species, cross reactivity between *E. canis* and *E. ewingii*, *E. equi* or *E. risticii* should be taken into consideration. A recent study has shown that dogs artificially infected with *E. canis* develop antibodies cross-reacting with *E. equi* about 4 months after infection. However, titers of *E. equi* antibodies were found to be considerably lower compared to those for *E. canis*. Cross-reactivity between *E. canis*, *Neorickettsia helminthoeca* (the etiologic agent of salmon poisoning disease) also has been documented. There is no serologic cross-reaction between *E. canis* and *E. platys*.
Due to the confusion caused by cross-reacting infections, it may be desirable under optimal conditions to test sera against a number of agents. Generally a fourfold difference between the titers of antibodies to the

different antigens is considered to infer etiology, where patients react to a number of antigens. The possibility of multiple tick-borne infections may confound the use of serological testing. Co-infection with *E. canis*, *E. chaffeensis* and *E. ewingii*, *E. equi*, *E. platys*, *Rickettsia* species, *Bartonella* species and *Babesia canis* has been documented in a kennel of heavily tick infested dogs.

Microscopic demonstration of typical intracytoplasmic *E. canis* morulae in monocytes is seen during the acute stage of the disease in about 4% of cases and is diagnostic of the disease. Therefore, blood and buffy-coat smears should be carefully evaluated. Other methods used mainly for research purposes are culturing the parasite, PCR and Western immunobloting. A study comparing PCR, culturing the parasite, IFA and Western immunobloting in the early detection of the parasite has shown that cell culture re-isolation method proved to be the most sensitive and definitive for early diagnosis of ehrlichiosis. However, it is not a convenient method as it requires 14 to 34 days to obtain positive results.

Diagnosis of the subclinical disease should be based on anamnesis, geographic location of the dog, persistent antibody titers to *E. canis*, mild thrombocytopenia and hypergammaglobulinemia. The diagnosis of the disease at this stage is a challenge to the practicing veterinarian. The importance of early diagnosis lies in the relatively good prognosis before some of the dogs enter the chronic phase, at which stage the prognosis is grave. The chronic disease is the end-stage of the disease process and the diagnosis is based on the anamnesis, the typical severe pancytopenia, presence of antibody titers to *E. canis*, serum hypergammaglobulinemia and lack of response to doxycycline therapy. This stage is usually easier to diagnose.

Treatment
Doxycycline at a dose of 10mg/kg once daily (or 5 mg/kg twice daily) for a period of three weeks at least, is the treatment of choice for acute CME. Short term treatment with doxycycline (10 mg/kg, once daily, for 7 days) has been shown to result in failure, while 10 days doxycycline treatment has shown success. In our experience, 10 days treatment may not be sufficient for all acute cases. In most cases, dogs suffering the acute phase of CME respond to treatment and show clinical improvement within 24-72 hours. Dogs in the subclinical stage may need a prolonged treatment compared to dogs suffering the acute stage as demonstrated by persistent infection (by PCR) in one out of four subclinically infected dogs treated with doxycycline (10 mg/kg q 24 hrs) for 42 days.

It is likely that the mechanism by which ehrlichiae survive and multiply in the infected cell relies on their ability to inhibit phagosome-lysosome fusion. Doxycycline was previously shown to restore phagosome-lysosome fusion in cells infected with *E. risticii* and *E. sennetsu*. Imidocarb dipropionate (5 mg/kg, one or two injections at 14 day interval, IM) may be used in conjunction with doxycycline. Although previous studies have shown the in vivo efficacy of imidocarb in the treatment of, a recent in vitro study has indicated that it may not be effective. An additional advantage of treatment of dogs with imidocarb lies in the elimination of other tick-borne diseases, such as babesiosis, which may be concurrent with CME.

Other drugs with known efficacy against *E. canis* include tetracycline hydrochloride (22 mg/kg, q 8 hrs), oxytetracycline (25 mg/kg, q 8 hrs), minocycline (20 mg/kg, q 12 hrs) and chloramphenicol (50 mg/kg, q 8 hrs). In a recent report it has been shown that oral enrofloxacin (5 or 10 mg/kg q 12h for 21 days) was not effective in elimination of the rickettsia from experimentally infected dogs.

As alluded to earlier, there is increasing evidence that immune mechanisms are involved in the pathogenesis of the disease. Therefore, the use of immunosuppressive doses of glucocorticosteroids in the treatment of the acute stage of CME should be considered. However, as no clinical studies to prove the efficacy of steroids in the treatment of CME have been performed, therefore they should be used with caution.

When demonstrating other *Rhipicephalus*-borne parasites such as *Hepatozoon canis* or *Babesia canis*, in blood smears, co-infection with *E. canis* should always be considered, as these are common. Co-infections with *E. platys*, which is presumed to be transmitted by *Rhipicephalus sanguineus*, are also common. Concurrent infections of *E. canis* and *Borrelia burgdorferi* or *Leishmania donovani* have been documented, indicating the possibility of co-infections with other parasites, that are not transmitted by the brown dog tick.

Treatment of the chronic severe form of the disease is unrewarding and the prognosis of these pancytopenic dogs is grave. Only one report described a successful treatment of a dog with severe chronic CME, using a combination of haematopoietic growth factors (recombinant human granulocyte colony stimulating factor and recombinant human erythropoietin) low dose vincristine, doxycycline and a long course of glucocorticoid therapy. However, the use of growth factors in the treatment of chronic ehrlichiosis has not been proven effective and requires further investigation.

After treatment, anti-*E. canis* antibody titers may persist for months, and even for years. It has also been

shown that persistence of *E. canis* antibody titers post treatment was related to the initial titer at the time of treatment. The persistence of high antibody titers for extended periods after prolonged treatments may represent an aberrant immune response, or treatment failure. After successful treatment, sero-positive dogs are susceptible to rechallenge. A progressive decrease in the serum gammaglobulin concentrations has been found to be associated with elimination of the parasite.

Prophylaxis
To date, no effective *E. canis* vaccine has been developed and tick control remains the most effective preventive measure against infection. In endemic areas, low dose oxytetracycline treatment (6.6 mg/kg) once daily has been suggested as a prophylactic measure. Recently this method has been used with success by the French army on dogs in Senegal, Ivory Coast and in Djibouti, where dogs were treated prophylactically with 250 mg per os per day with oxytetracycline. The estimated failure rate of the treatment was found to be 0.9%. Despite the success of the treatment, the authors do not consider this practical due to the possibility of the future development of resistant strains of *E. canis*. This development would make treatment of dogs more complicated and as a result decrease the rate of successful treatment.

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