*Ehrlichia Canis*

**Steps to Successful in-Clinic Diagnosis**

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## INTRODUCTION

Canine monocytotropic ehrlichiosis (CME), caused by the obligate intracellular (rickettsia) organism *Ehrlichia canis*, is a canine disease with a worldwide distribution including Asia, Africa, Europe, and the Americas. Due to its different phases, co-infections with other vector borne disease pathogens (VBDP) and multiple clinical manifestations, diagnosis of the disease can be sometimes challenging. Infection occurs mainly during the warm season when the vector *Rhipicephalus sanguineus (*The Brown Dog tick) is active. Most dogs recover from the acute and subclinical phases when treated properly —dosage and time-wise— with doxycycline or other tetracyclines (Harrus et al., 1998, 2004). Unfortunately, some of the untreated or inadequately treated dogs will enter the chronic phase of the disease for which the prognosis is grave.

When a compatible history (living or visiting an endemic region; not receiving ticks preventive treatment; or having a previous tick exposure), clinical signs and typical hematological and biochemical findings are present, *CME* should be suspected.

Further valuable traditional diagnostic techniques include cytology, serology, isolation and PCR. With that being said, they should be considered for their accessibility as a point-of-care diagnostic tool, and/or for their relevance regarding the different phases of the disease.

## CLINICAL MANIFESTATIONS

*CME* is a multisystemic disease manifesting in different phases: acute, subclinical or chronic. Hematological changes are associated with inflammatory and immune processes triggered by the infection (such as a circulatory immune-complex mediated response.

Compared to other breeds, German shepherds appear to be the most susceptible to the disease and tend to suffer from the more severe chronic phase of the disease with higher morbidity and mortality rates than other breeds (Nyindo et al., 1980).

The acute phase of the disease is characterized by a high fever, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly, hemorrhagic tendencies, polyarthritis, ocular disorders and a wide range of neurological signs. During the subclinical phase dogs seem to be “clinically healthy” with no evidence of clinical

signs, however their bodies might still be harvesting the rickettsia (Waner et al., 1997).

For reasons that are still unclear, some dogs might progress to the chronic phase of the disease. During the chronic phase common physical findings may include significant weight loss, pale mucous membrane, weakness, and bleeding (Harrus et al., 1997). Depending on the affected organs infected dogs may also show other clinical signs such as splenomegaly, glomerulonephritis, renal failure, interstitial pneumonitis, anterior uveitis and a wide range of CNS signs. In general, clinical signs in the chronic phase can be similar to the signs of the acute phase but with much greater severity.

**LABORATORY FINDINGS**

A complete blood count (CBC) is essential in the diagnosis of *E. canis.*
During the acute phase a significant thrombocytopenia is expected between moderate to severe platelet counts (~20,000-52,000/µl) and it is accompanied by mild anemia and mildly decreased white blood cell counts.

During the subclinical phase in the absence of clinical signs, a mild thrombocytopenia may be present with counts as low as 140,000/ µl (Harrus et al., 1998; Waner et al., 1997). During this phase a reduction in WBC and RBC counts would be relatively mild and perhaps won't even be noticeable.

In the chronic phase, thrombocytopenia is usually severe and accompanied by significant anemia and leukopenia. Due to bone marrow hypoplasia marked pancytopenia is an indicator of the chronic severe phase.

When performing a biochemistry test, hypoalbuminemia, hyperglobulinemia and hypergammaglobulinemia (polyclonal>monoclonal) can be found. Mild rises in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are common in dogs during the acute phase.

Blood smears of infected dogs may contain reactive monocytes, erythrophagocytosis, thrombophagocytosis, phagocytosis of nuclear material and megaplatelets. However, red blood cell morphology is unremarkable in most cases (Harrus et al., 1997).
Blood smear demonstrations of *E. canis* morulae in monocytes strongly support the diagnosis but unfortunately the search for morulae is difficult and time consuming and has been estimated to be successful in only about 4% of the cases. Evaluating smears using X1000 magnification (oil immersion fields) of multiple buffy-coats and bone marrow would probably increase the sensitivity to 66% and 34%, respectively.

With that being said, the time required to screen 1000 oil immersion fields is very time consuming, ranging between 50 to 60 minutes (Mylonakis et al.,2003). Ehrlichial morula could be mistakenly identified due to artifacts in the blood smear such as platelets, lymphocytic azurophilic granules, phagocytosed nuclear material and other ehrlichia organisms.

**Currently available in-house point-of-care diagnostic tests include serology and PCR**. The indirect immunofluorescence antibody (IFA) test for anti-*E. canis* IgG antibodies is considered the serological 'gold standard'. Positive serology tests may reflect exposure to *E. canis*, rather than an active disease. When recovery occurs (w/o proper treatment) IgG antibodies titer begins to drop 6 - 9 months post exposure and could last up to 3-5 years. Therefore, during the acute phase, demonstration of a fourfold rise in titer is required over a 7-14-day interval. During the subclinical phase (before the drop in IgG when recovery occurs) and the chronic phase, the antibodies titer level is expected to be high, due to the longitudinal exposure time.

When compared to the yes/no result tests, ImmunoComb, which is a semi-quantitative antibody test kit, provides the possibility to demonstrate a fourfold rise in titer. This confirms an active disease during the acute phase and presents the expected high level of antibodies in the subclinical and chronic phases. Unlike the IgG, IgM is not considered a reliable indicator of *E. canis* exposure due to the inconsistent development of IgM antibodies in the course of the disease. That's why there is no available IgM kit in the market.

PCR – During the detection of *E. canis's* acute phase, DNA can be achieved as early as 4–10 days post-inoculation. During the subclinical and chronic phases, PCR performed on spleen samples - the last organ to harvest the rickettsia— is considered to be more sensitive for the evaluation of *E. canis* elimination when compared to blood and bone marrow samples. Splenic aspirates are not superior to blood samples for the acute phase detection of *E.canis* DNA by PCR.(Harrus et al., 2004, 1998).
PCRun®, a point-of-care diagnostic tool, can be performed in the clinic and aid in the diagnosis of all the *E. canis* phases. During the acute phase, *E. canis* can be demonstrated perhaps even before the rise of anti-*E. canis* IgG antibodies (false negative due to late response).

In conclusion, a combination of compatible findings (history, clinical signs and bloodwork), the aforementioned diagnostic tools and taking the suspected phase of the disease into consideration are all essential steps in making the most accurate diagnosis of *E.canis*.

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