Review Article

Combined Simultaneous In-Clinic, Serology and Molecular Analysis for the Diagnosis of Canine Monocytic Ehrlichiosis (*Ehrlichia canis*)

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ABSTRACT

This article intends to assist the veterinary clinician in the diagnosis of Canine Monocytic Ehrichiois (CME) caused by *Ehrlichia canis* by combining serological and molecular diagnostic test results. Based on suggestive historical, clinical and hematological findings Point-of-Care diagnostic kits can be used for serological tests for anti-*E. canis* IgG antibodies and molecular polymerase chain reaction (PCR) tests to verify the genetic identification for the presence of *Ehrlichia canis* DNA. The different phases of CME are reviewed and discussed in relation to results obtained from these two diagnostic methods. After assessment of the anamnesis, the clinical picture and hematological results and followed by preliminary suspicion of CME, a number of scenarios are presented which may be considered using a combination of serological and PCR tests in order to achieve an accurate diagnosis. Further circumstances which are recommended for the simultaneous use of serology and PCR such as thrombocytopenia, well-differentiated lymphocytosis and in the course of monitoring the treatment for CME, are discussed.

Key words: *Ehrlichia canis*; IgG; PCR; Serology.

INTRODUCTION

Point-of-Care diagnostic testing is now available for several tick-borne diseases of dogs, including *Ehrlichia canis* infection, the causative agent of canine monocytic ehrlichiosis (CME) (1, 2). The in-clinic Point-of-Care (POC) diagnostic methods available for CME, include serology tests for anti-*E. canis* IgG antibodies and molecular polymerase chain reaction (PCR) tests to verify the genetic identification for the presence of *Ehrlichia canis* DNA in whole blood samples (2,3). These commercial test kits have been tested and have been proposed to be useful when combined with patient physical examination, anamnesis and haematological and biochemical tests, assisting the clinician to reach a more exact diagnosis (2). With minimal training, and easy to operate compact equipment, these tests can be performed in a clinic with the added value that the results are available within a short period of time, without the necessity to send samples to an external laboratory.

Although POC tests are reliable and simple to use, the practicing veterinarian must be equipped with the knowledge of the pros and cons of each test in his locale of practice. Serology and molecular tests have very different windows of opportunity and very different interpretations. While in the case of Ehrlichiosis, blood PCR will determine if the pathogen is present and whether the disease is active. Antibody tests for CME will determine whether the patient has been exposed to the pathogen, however a positive result cannot be directly related to an active current state of Ehrlichiosis in the dog. In addition, antibody titres are known to remain high for long periods of time and only evidence of increasing titre will allow for assuming a recent infection. By combining serology testing concurrently with PCR, it may be possible to assist making a more thorough assessment of the stage, treatment and prognosis of *E. canis* infections in dogs.

This review is intended to assist clinicians in decision making regarding the advantages of combining serological

and molecular diagnostic tests in dogs suspected with *E. canis* infection, in relation to the phase of the disease.

Concurrent utilization of serological and molecular tests should follow a presumptive diagnosis of CME, based on suggestive historical, clinical and hematological findings (1).

OVERVIEW

CME caused by *Ehrlichia canis,* is a tick-borne disease transmitted by the brown dog tick *Rhipicephalus sanguineus*, manifesting as a multisystemic disorder, and may be acute, subclinical or chronic (4).

Clinical signs of acute CME include pyrexia, depression, lethargy, anorexia, lymphadenomegaly, splenomegaly, mucous membrane petechiae and ecchymoses and occasionally, epistaxis (1). Moderate to severe thrombocytopenia is the most consistent and indicative hematological abnormality in the acute phase of CME (1), while mild neutropenic leukopenia and normocytic, normochromic, non-regenerative anemia also occurs. Microscopic detection intra-monocytic *E. canis* morulae confirms the diagnosis of CME, but its detection has been reported in only 4% of infected dogs, and hence is an insensitive marker of CME (1, 4). An immunofluorescence antibody assay (IFA) titer (IgG) against *E. canis* > 1:40 is indicative of previous exposure to the rickettsia, but cannot be used to confirm the current disease. Acute active infection is gauged by a 4-fold antibody titer increase over a 1-2 week period. Therefore, using quantitative or semi-quantitative serological assays is considered more useful compared to purely qualitative serological assays.

During the subclinical phase no clinical signs are evident (4), however platelet counts may be subnormal (5, 6). Dogs in this phase may remain persistent carriers of *E. canis* for months and even years (6). For reasons still unclear, certain dogs, will progress to the chronic severe pancytopenic form of CME, which bears a poor prognosis (1).

Chronic pancytopenic CME develops only in some infected dogs. Factors that influence the development of the chronic disease are unclear, but the pathogen, host genetics and the interrelationship between them probably play a role (1). The presence of severe pancytopenia characterizes the severe chronic form of ehrlichiosis, and is a consequence of hypoplasia of all bone marrow cell lines. Clinical signs at this stage are usually severe and include lethargy, inappetence, bleeding tendencies, mucosal pallor, fever or hypothermia, weight loss, lymphadenomegaly, splenomegaly, dyspnea, anterior uveitis, retinal hemorrhage and edema. As a result of the effect on the bone marrow, secondary opportunistic infections such as protozoal infections, viral papillomatosis, and bacterial urinary tract infections can also develop.

Previous studies have shown that dogs may be naturally exposed to the *E. canis* without any signs of disease (1). In Israel and in Egypt, two unrelated studies demonstrated that about 30% of clinically healthy dogs demonstrated IgG antibodies to *E. canis* without a previous clinical history of CME (7, 8).

A result of the presence of *E. canis* IgG antibodies should not be considered as definitive for an *E. canis* exposure, because serological cross reactivity exists between *Ehrlichia canis*, *Ehrlichia. ewingii, Ehrlichia chaffeensis, Anaplasma phagocytophilum, Neorickettsia risticii* and *Neorickettsia helminthoeca* (9, 10).

Polymerase chain reaction (PCR) is a very sensitive and specific method for detection and identification *E. canis*-DNA in either blood, bone marrow or splenic aspirates (11, 12). With this in mind, the clinician should also be aware that a negative PCR result might be due to the fact that the concentration of DNA in the collected sample may be below the detection level of the test. Therefore, a negative PCR result does not necessarily signify that there was no *E. canis* DNA in the organ of the dog under examination. On the other hand, the presence of DNA in an organ does not necessary indicate that there are live or viable organisms.

After assessment of the anamnesis, clinical picture and hematological results, with a resultant preliminary diagnosis of CME, a number of scenarios may be considered when using a combination of serological and PCR tests. Generally serology for IgG anti-*E. canis* antibodies is the first of the tests to be undertaken and evaluated due to the simplicity of the test and its availability.

1. **Positive serology**: This is indicative that the dog has been exposed to the *E. canis* rickettsia (1). Depending on the clinical picture, the infection may be acute or possibly indicate a previous exposure to *E. canis* or an infection with a cross-reacting rickettsial infection (4). In the case of an acute infection, a dog presenting with a history of tick infestation, thrombocytopenia and with a positive and increasing antibody titer would be strong evidence for acute

CME (1). A positive blood PCR result at this stage would confirm an ongoing active specific infection with *E. canis* and authenticate the diagnosis (2).

In the case of negative blood PCR result, PCR testing of spleen biopsy samples should be considered, as this is regarded as more sensitive than PCR blood results.

2. **Negative serology**: In the case of an early infection of *E. canis* by ticks where antibodies may have not yet developed, a positive PCR result with or without a finding of thrombocytopenia would increase the possibility of an imminent acute disease with *E. canis*. In this case, detection of *E. canis* DNA may be useful as early as 3-10 days after infection by ticks, where the incubation period is 8-20 days and antibodies will only appear after 15 days (1, 2).
3. In the case of a dog with suggestive signs of CME, a negative PCR result together with negative serology is suggestive of a disease not associated with, *E. canis*.

It should be noted that a dogs might be co-infected with a number of tick borne diseases simultaneously. This may result in changes in the clinical presentation and may even cause the signs to be different and possibly more severe. The veterinarian should always undertake a though blood smear examination in order to eliminate other tick borne infections and possibly carry out further PCR tests specifically for other tick borne diseases (1).

DISCUSSION

After determining suspicion of a dog infected with *E. canis* based on anamnesis, clinical signs and hematological results, the use of a serological test will enhance the possibility of *E. canis* as the etiological agent. However, the recognized serological cross-reactivity of *Ehrlichia canis* with other rickettsial organisms demands the use of more definitive diagnostic tests for the determination of the exact nature of the infecting organisms (9, 10). In this regard, molecular PCR testing will provide a definite answer to the nature of the invading organism.

Regarding the dog infected with *E. canis*, during acute infection, the use of blood for testing both by serology and molecular characterization, is adequately accurate and informative for both a clinical diagnosis, strategy for therapy and recovery (2). Furthermore, a study evaluating the presence of *E. canis* in experimentally infected dogs has shown that that *E. canis* DNA could not be detected from the blood or spleen within 9 and 60 days, respectively, from the time of treatment initiation with doxycycline (13). Interpretation of these results may point towards testing dogs using *E. canis* PCR for up to 2 months following initiation of treatment.

In the case of dogs in the subclinical phase of the disease, the spleen has been shown to be the organ of choice, compared to blood or bone marrow for PCR detection when performed from fine needle aspirates of experimentally infected dogs (6). The length of treatment of subclinically infected dogs has not yet been defined, however from experimental study reports; it appears that an extended period of treatment may be necessary to clear dogs of infection.

A study of naturally infected dogs in the severe pancytopenic chronic phase revealed that DNA of *E. canis* could be detected in 68.4% of cases from bone biopsies. In this study no other organs were biopsied (14).

An interesting and novel application of the PCR and antibody tests is in the case of dogs incubating *E. canis* before the appearance of clinical signs and antibodies. This presentation may arise when a dog has been found to be infested with ticks by the owner, who then approaches the veterinarian for advice as to whether the dogs may be incubating CME. PCR testing in artificially infected dogs has demonstrated that *E. canis* DNA could be detected in dogs three days post-artificial infection, during the incubation period at which stage antibodies were still not present (2). This application should serve as a useful tool for the veterinarian, faced with the dilemma of a possible imminent acute *E. canis* infection. Although there are at present no clinical studies to verify this approach and the percentage of dogs that will prove positive for *E. canis* DNA, the idea maybe worthwhile studying in the future. In this case, PCR testing may reveal the presence of *E. canis* DNA in the blood, strongly signifying impending disease due to *E. canis*, even prior the appearance of anti-*E. canis* antibodies. Under these circumstances, the owner should be made aware of the possibility of development of CME and early treatment is strongly recommended.

Further recommendations for the simultaneous use of serology and PCR are as follows:

a. *E. canis* PCR can be used as an indicator of success of treatment: After confirmation of the presence of *E. canis* as the cause of the acute disease, a follow-up can be undertaken by testing blood and spleen for *Ehrlichia canis* PCR. Dogs recovering from the acute disease become PCR negative as clinical parameters improve. Antibody titers may take longer to decline over time. Even after recovery (15).

b. It should be considered to attempt to treat all dogs until they become PCR negative as experimental results indicate that *E. canis* may persist in clinically normal dogs in the subclinical phase, even after an extensive doxycycline treatment regimen. These results have important implications with regard to possible reactivation of ehrlichiosis due to persistent infections and signify that ehrlichiosis patients should be continually monitored even after a clinical response to antibiotic therapy. Splenic aspirate samples compared to blood samples are considered a better indication of success of treatment because the spleen is considered the last organ to harbor the organism. However, it must be pointed out that some dogs may remain PCR positive for extended periods, despite treatment.

c. Subclinical infection: It is recommend using PCR test of blood samples for dogs not presenting with symptoms of *E. canis,* beyond a mild thrombocytopenia, however with a persistent exceedingly high IgG antibody titers [IFA titers (≥ 1:3200)] and a history of exposure to ticks. Under these situations, dogs with positive *Ehrlichia canis* PCR should be treated with tetracyclines possibly for a longer period than dogs in the acute phase. The concern is that without confirmation that the *E. canis* has been eradicated; subclinically infected dogs may develop the pancytopenic form of the disease and in the meantime continue to harbor the parasite and pose a potential threat for the continued spread of the *E. canis* rickettsia.

d. Chronic pancytopenic phase: Dogs in the chronic pancytopenic phase typically have a high *E. canis* antibody titer. The sensitivity of PCR bone marrow biopsies for diagnosis of CME when performed in dogs with chronic ehrlichiosis has ranged from 25% to 68%. Convalescent antibody testing was found to be more sensitive than bone marrow PCR assays for chronic CME possibly due to the low concentration of the rickettsia at this stage. (11, 14).

e. All dogs presenting with thrombocytopenia should be tested using *Ehrlichia canis* PCR. Although thrombocytopenia has many etiologies, it should be borne in mind that one of the consistent hematological changes in CME is thrombocytopenia, which is in part due to the induction of circulating antiplatelet antibodies. If a positive *Ehrlichia canis* PCR result is found, treatment with tetracyclines should be initiated.

f. Dogs presenting with well-differentiated lymphocytosis should be tested for *E. canis* antibodies and *E. canis* DNA. This follows the finding that marked granular lymphocytosis and plasmacytosis, occasionally accompanied by monoclonal gammopathy may occur in all phases of CME. This precautionary testing may help exclude the diagnosis of lymphocytic leukemia or multiple myeloma (15).

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