Doxycycline treatment of asymptomatic dogs seropositive for *Ehrlichia canis*

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Abstract

In Grenada, West Indies dogs are at frequent exposure to the rickettsial pathogen, *Ehrlichia canis*, as demonstrated by high seroprevalence rates. However, many of these seropositive dogs are clinically normal. In this study we identified clinically normal, *E. canis* seropositive dogs and assigned half to an antibiotic treatment group and half to a no treatment group. All dogs were evaluated for the presence of *E. canis* DNA by PCR on whole blood before, during and after treatment. Only one seropositive dog was also PCR+ before treatment. Our results suggest that most clinically normal, *E. canis* seropositive dogs in a highly endemic geographic area are not concurrently infected and thus routine treatment of clinically normal, seropositive dogs is not warranted.

Key words: *Ehrlichia canis*, dogs, serology, PCR, treatment, Grenada

Introduction

Canine monocytic ehrlichiosis (CME) is a tick-borne disease of domestic dogs caused by the rickettsia *Ehrlichia canis*. Although *E. canis* has a worldwide distribution, high infection rates and disease in dogs are primarily observed in tropical and subtropical areas¹ ². Serologic surveys of dogs in Grenada in 2004 and 2006 documented *E. canis* seropositive rates above 40%, indicating continuous high exposure among dogs on this eastern Caribbean island¹.

Dogs infected with *E. canis* can present with acute disease approximately 10 days post-infection characterized by fever, anemia, thrombocytopenia, anorexia and depression. This may be followed by a long-term subclinical phase. A mild to severe chronic phase of CME can also occur, with recurrent pancytopenia, hemorrhage, monocytosis and weight loss¹ ² ³. Many dogs exposed to *E. canis* seroconvert, but never develop clinical disease. It is not known what percentage of these dogs clear the infection and what percentage remains chronically infected and for how long. However, it is known that dogs can remain seropositive long after clearance of the organism³.

Thus, serologic tests of exposure are not useful for determining current infection status or for assessing clearance of *E. canis* with treatment. One sensitive and specific test to determine infection status is PCR. If PCR is positive, the dog is actively infected. One limitation of PCR for *E. canis*, however, is that if the PCR is negative on blood, the usual sample tested, it is still possible that the organism is present in other tissues such as the spleen or the number of organisms circulating in the blood could be below the assay’s level of sensitivity⁶.

In endemic areas for *E. canis* many veterinarians routinely test healthy dogs for exposure using the IDEXX SNAP test for antibody⁷. The SNAP test for *E. canis* antibody is highly specific (100%) and sensitive (96.2%)⁷. Widespread use of this and other serologic tests for *E. canis* exposure has lead to treatment of clinically normal seropositive dogs and a controversy among veterinarians as to whether or not treatment is warranted⁸ ⁹ ¹⁰. Some state that testing and treating clinically normal dogs can prevent disease in these dogs and possibly reduce the reservoir for *E. canis*. Others point out that treatment of all seropositive dogs may increase the risk for development of doxycycline resistance. Some veterinarians recommend doing a complete blood count (CBC) on healthy SNAP+ dogs and only treating those with CBC values outside normal reference ranges⁹. One study done in collaboration with IDEXX laboratories evaluated 86 healthy dogs from various parts of the United States with *E. canis* SNAP+ results⁹. Fifty eight percent of the SNAP+ dogs were...
Doxycycline treatment of asymptomatic dogs seropositive for Ehrlichia canis

thrombocytopenic, and yet only 14% of the SNAP+ dogs were PCR positive. Thus, not only can SNAP+ healthy dogs with normal CBCs be PCR-negative, even healthy SNAP+ dogs with thrombocytopenia can be PCR-negative. One limitation of this study is that it did not evaluate dogs residing in highly endemic areas for E. canis exposure. Infection status of healthy SNAP+ dogs in highly endemic areas may differ significantly from the results of this study due to dogs being at continuous risk of exposure.

In the present study we evaluated clinically healthy dogs in Grenada for evidence of E. canis exposure and infection status followed by antibiotic treatment and monitoring of infection status. Samples were obtained from 89 dogs presenting for vaccination clinics conducted by the St. George’s University School of Veterinary Medicine in Grenada, West Indies during October 2008 and April, 2009. Of these 89 dogs, 40 tested positive for E. canis antibody using the IDEXX SNAP 4Dx test. Physical exams and CBCs were done on these 40 dogs and 24 met the following criteria for inclusion in the study: platelet levels > 150 X10^3/L; packed cell volume > 30%; body score ≥ 2.5 on a 5 point scale; no spontaneous bleeding; no evidence of lethargy and/or anorexia; CBC values within the normal reference range. The 24 dogs were divided into two groups with 12 dogs in each group. One group was treated for 21 days with 5-10 mg/kg doxycycline twice daily. The other group was not treated. Treatment was started on Day 0, the same day that the dogs were initially evaluated by physical examination, CBC and the SNAP 4Dx test. Although several dogs were lost to follow-up at various times during the study, subsequent blood samples were obtained from dogs remaining in the study on days 21, 28, and 45. CBCs were performed on all blood samples on the day of collection. PCR assays for the presence of Ehrlichia canis DNA were performed on all blood samples, including those from day 0, at the end of the 45 day study period. DNA was extracted from whole blood using DNeasy Mini Kit (Quiagen, GmbH, and Hilden, Germany) according to manufacturer’s protocol. A nested PCR reaction was used to detect the presence of E. canis as previously described with slight modification of the annealing temperature (50°C for 30 sec) in both primary and secondary PCR. The positive control was the plasmid encoding Ehrlichia Canis Jake 16S insert obtained, along with the primers, from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia.

Results and Discussion

On Day 0 all 24 dogs in the study were SNAP+, clinically healthy dogs with normal CBCs. All dogs remained clinically healthy with normal CBCs for the duration of the time that they were in the study. Importantly, only one of the initial 24 SNAP+ healthy dogs was positive for E. canis DNA by blood PCR on Day 0. This dog was in the doxycycline treatment group and was PCR-negative by day 21 post treatment. All other dogs tested remained PCR-negative. These results suggest that the vast majority of SNAP+ healthy dogs in a highly endemic area for E. canis exposure are not currently infected. Our data indicate that antibiotic treatment of these dogs is not warranted and is a poor use of valuable resources. Results on the one dog that was PCR+ prior to treatment confirm previous reports on the efficacy of this doxycycline treatment protocol for clearing E. canis infections in dogs. Others report, however, that this treatment protocol is not always affective.

One limitation of our study is that blood PCR may miss some Ehrlichia infections that have localized to other tissues. Because there is only one study of 4 experimentally infected dogs that demonstrates detection of Ehrlichia canis in other tissues concurrent with negative blood PCR results, we do not know how likely this is in general and, in particular, whether or not it is likely in naturally and continuously exposed dogs. We also do not know what percentage of healthy SNAP+ dogs that are also PCR+ go on to mount an effective immune response that clears the infection or how many dogs become carriers and/or later develop disease. Accurate detection of infection status and information on the ability of dogs to clear an active infection are needed in order to develop evidence-based treatment protocols for the large population of healthy, SNAP+ dogs in highly endemic areas such as Grenada.

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References