Predominance of Ehrlichia ewingii in Missouri dogs

Allison M. Liddell
Washington University School of Medicine in St. Louis

Steven L. Stockham
University of Missouri

Michael A. Scott
University of Missouri

John W. Summer
Centers for Disease Control

Christopher D. Paddock
Centers for Disease Control

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation
https://digitalcommons.wustl.edu/open_access_pubs/2618
Authors
Allison M. Liddell, Steven L. Stockham, Michael A. Scott, John W. Summer, Christopher D. Paddock, Monique Gaudreault-Keener, Max Q. Arens, and Gregory A. Storch
Predominance of *Ehrlichia ewingii* in Missouri Dogs

Allison M. Liddell, Steven L. Stockham, Michael A. Scott, John W. Sumner, Christopher D. Paddock, Monique Gaudreault-Keener, Max Q. Arens and Gregory A. Storch


Updated information and services can be found at: http://jcm.asm.org/content/41/10/4617

**REFERENCES**

This article cites 32 articles, 18 of which can be accessed free at: http://jcm.asm.org/content/41/10/4617#ref-list-1

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»
Predominance of Ehrlichia ewingii in Missouri Dogs

A. Allison M. Liddle,† Steven L. Stockham,‡ M. Michael A. Scott,§ John W. Sumner, Christopher D. Paddock, M. onique Gaudreault-Keener, Max Q. Arrens, and Gregory A. Storch

Departments of Internal Medicine and Pediatrics,† Washington University School of Medicine, and Diagnostic Virology Laboratory,‡ St. Louis Children’s Hospital,§ St. Louis, and Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri–Columbia, Columbia, Missouri, and Viral and Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Received 1 May 2003; returned for modification 1 July 2003; accepted 27 July 2003.

To investigate the species distribution of Ehrlichia present in Missouri dogs, we tested 78 dogs suspected of having acute ehrlichiosis and 10 healthy dogs. Blood from each dog was screened with a broad-range 16S rRNA gene PCR assay that detects known pathogenic species of Ehrlichia and Anaplasma. The species was determined by using species-specific PCR assays and nucleotide sequencing. Ehrlichia antibody testing was performed by using an indirect immunofluorescence assay with Ehrlichia chaffeensis as the antigenic substrate. The broad-range assay detected Ehrlichia or Anaplasma DNA in 20 (26%) of the asymptomatic dogs and 2 (20%) of the asymptomatic atopic dogs. E. ewingii accounted for 20 (91%), and E. chaffeensis accounted for 1 (5%) of the positives.

Anaplasma phagocytophilum DNA was detected in one dog, and the sequences of regions of the 16S rRNA gene and the groESL operon amplified from the blood of this dog matched the published sequences of this organism. Antibodies reactive with E. chaffeensis were detected in 14 (67%) of the 21 PCR-positive dogs and in 12 (19%) of the 64 PCR-negative dogs. Combining the results of PCR and serology indicated that 33 (39%) of 85 evaluable dogs had evidence of past or current ehrlichiosis. We conclude that E. ewingii is the predominant ehrlichiae agent of canine ehrlichiosis in the areas of Missouri included in this study. E. canis, a widely recognized agent of canine ehrlichiosis, was not detected in any animal. The finding of E. ewingii in asymptomatic atopic dogs suggests that dogs could be a reservoir for this Ehrlichia species.

Ehrlichiosis is an important emerging infection of dogs and humans. The first species recognized, Ehrlichia canis, causes monocytic ehrlichiosis in dogs. A closely related species, E. chaffeensis, was subsequently identified as the cause of an monocytic ehrlichiosis in dogs. A closely related species, E. chaffeensis, was subsequently recognized as the cause of granulocytic ehrlichiosis in dogs (15). E. chaffeensis has also been detected in dogs (12), coyotes (21), goats (13), and deer (3, 10). A closely related species, E. ewingii, was initially recognized as the cause of granulocytic ehrlichiosis in dogs (15) and was recently found to cause canine granulocytic ehrlichiosis in humans (7). Most cases of human granulocytic ehrlichiosis are caused by a species referred to as the agent of human granulocytic ehrlichiosis (4). This bacterium has also been detected in dogs (19), deer (5), horses (20), and rodents (31). The name Anaplasma phagocytophilum has recently been proposed to include this bacterium, in addition to the species previously known as E. phagocytophilum and E. equi (14), and this proposed name is used in the present study.

Most studies of the prevalence of infection with Ehrlichia spp. in dogs have been based on serologic methods that often used antigens derived from E. canis. Because of serologic cross-reactions between E. canis and other Ehrlichia species, including E. chaffeensis and E. ewingii (25, 29), these studies do not provide identification of the species that elicits production of anti-Ehrlichia antibodies in the host animal. Four studies have used molecular techniques and/or cell culture methods to identify the Ehrlichia species infecting dogs. In these studies, carried out in North Carolina (6, 22), Virginia (11), and Oklahoma (25), 24 dogs were infected with E. chaffeensis, 21 were infected with E. canis, 19 were infected with E. ewingii, 10 were infected with E. phagocytophilum, and 1 was infected with A. phagocytophilum. A recent study described 15 dogs with E. ewingii infection, proven by PCR (18).

In our laboratory at Washington University Medical Center in St. Louis, Mo., we have detected nearly 200 cases of human ehrlichiosis in recent years by using PCR; 89% of these cases were caused by E. chaffeensis and 11% were caused by E. ewingii. To learn more about possible relationships between human and canine ehrlichiosis, we studied the occurrence and species distribution of Ehrlichia in pet dogs in Missouri. The focus of the study was on all dogs with clinical manifestations suggestive of ehrlichiosis, but we also studied a small number of asymptomatic atopic dogs.

MATERIALS AND METHODS

Canine subjects and blood samples. Participating Missouri veterinarians were recruited by the staff at the University of Missouri College of Veterinary Medicine. Participating veterinarians were asked to submit blood samples from dogs that they suspected of having ehrlichiosis on the basis of a distributed list of clinical manifestations of granulocytic or monocytic ehrlichiosis; these clinical manifestations included fever, evidence of a unilateral orbital disease, hepatomegaly, splenomegaly, reticulosis, mesenteric or mesenteric, cytopenia, hypergammaglobulinemia, and a distributed list of other symptoms. With the cooperation of these veterinarians, blood samples were collected from 78 dogs suspected of having ehrlichiosis and from 10 healthy control dogs.

...
RESULTS

A total of 88 pet dogs were included in the study, including 78 (89%) that were ill and 10 (11%) that were asymptomatic. The dogs included a wide variety of breeds, of which the most common were Labradors and Golden Retrievers (n 22, including 10 males). Fifty-five percent were female, and the mean age was 4.6 years (range, 1 to 13 years). Fever and musculoskeletal symptoms were common (Table 1), and 20 had anemia, 10 had thrombocytopenia, or anemia (Table 2). There were no significant differences between PCR-positive and PCR-negative dogs in gender, proportion female, mean age, or the presence of fever or musculoskeletal symptoms.

Routine laboratory test results were available for only a minority of the dogs and indicated that 19 dogs had thrombocytopenia (platelet count, 200,000/µL), 20 had anemia, 10 had leukopenia, and 4 had hyperglobulinemia. Gastrointestinal symptoms were observed on peripheral blood smears from two dogs that were later found to be PCR positive for E. ewingii.

The results of PCR testing of the 88 dogs are shown in Table 1. Ehrlichia or Anaplasma DNA was detected in the blood of 22 (25%) of the 88 dogs, including 20 (26%) of the ill dogs and 2 (20%) of the asymptomatic dogs. Species-specific PCR testing revealed 19 infections with E. ewingii, 1 with E. chaffeensis, and 1 with A. phagocytophilum. No additional dog was determined to be positive by the broad-range assay but negative by the species-specific assays. The species identity of this dog's infection was not determined.

Because there were no cases of ehrlichiosis caused by A. phagocytophilum in Missouri, we carried out nucleotide sequencing of portions of the 16S rRNA gene and the groEL operon of A. phagocytophilum and were thus considered to be positive only for E. ewingii. Infection with a multiple Ehrlichia species was not detected in any dogs in the present study.

Because A. phagocytophilum has been seen in Missouri (24), we carried out nucleotide sequencing of portions of the 16S rRNA gene and the groEL heat-shock operon amplified from the blood of the dog that was positive for A. phagocytophilum. Sequencing of the 16S rRNA gene segment was performed by using the sequencing primer pair ER-1R, which provides the sequence of a 126-bp segment that spans the highly variable region. The sequence determined matched the published sequence of A. phagocytophilum (GenBank accession no. U02521). The nucleotide sequence of the 16S rRNA gene amplified by PCR was very similar to or identical to sequences previously determined for A. phagocytophilum.

There were no significant differences between PCR-positive and PCR-negative dogs in gender, proportion female, mean age, or the presence of fever or musculoskeletal symptoms, thrombocytopenia, or anemia (Table 2). Elevated tick exposure (tick currently em bedded or recently removed) was reported in 75% of PCR-positive compared to 50% of PCR-negative dogs (P = 0.05 [chi-square]). As shown in Figure 1, m ost (81%) of the PCR-positive cases occurred during May through July. Figure 2 shows the distribution of PCR-positive and PCR-negative samples within the state of Missouri. Most positive cases were submitted from the southern portion of the state. Positive dogs were located throughout this region, with a cluster of positives in four counties (Jefferson, Washington, St. Francois, and St. Genevieve) located south of St. Louis and a smaller cluster in the southwest portion of the state.

Seems from 85 dogs, including 76 ill and 9 asymptomatic.
animals, were tested by IFA for antibodies reactive with E. chaffeensis. Table 3 shows the results compared to results of PCR testing. Of the 85 dogs, 26 (31%) had IgG antibodies reactive with E. chaffeensis at a titer of 64, including 14 (67%) of 21 that were PCR positive and 12 (19%) of 64 that were PCR negative (P < 0.001 [chi-square]). IFA was performed on samples from 19 dogs that were PCR positive for E. ewingii by PCR. Of these, 13 (68%) had titers of 64 (range, 32 to 2,048; geometric mean titer, 142). The single dog that was positive for E. chaffeensis by IFA had a reciprocal titer of 64, and the single dog that was positive for A. phagocytophilum by PCR was negative for antibodies reactive with E. chaffeensis. Of the 76 ill dogs tested, 24 (32%) were PCR positive compared to 2 (22%) of the asymptomatic dogs (P = 0.7 [Fisher exact test]).

In all, 33 (39%) of the 85 dogs tested by PCR and IFA had evidence of either past or current Ehrlichia exposure based on either a positive PCR or positive serology.

Although the most likely explanation for the finding of positive serology with a negative PCR in 12 dogs is that they had past infection, another possible explanation is the effect of antibiotic therapy given for the acute illness. Antibiotic prescribing information was available for 60 dogs at the time of sample collection. Nineteen had received antibiotics for at least 1 day before testing (range, 1 day to 7 months prior to sample collection); seven of these animals had received an antibiotic with significant anti-Ehrlichia activity (doxycycline or chloramphenicol). Of the seven was PCR positive and IFA negative after 6 days of chloramphenicol treatment, one was PCR negative but IFA positive after receiving 4 weeks of doxycycline, and the remaining five were PCR negative and IFA negative.

One possible explanation for the finding of seronegativity in seven PCR-positive dogs (six ill and one asymptomatic) could have been that blood samples were obtained early in the illness before a serologic response had occurred. Information on the day of onset of illness was available for three of the six ill dogs with this finding; in these dogs, the samples were obtained on days 3, 3, and 30 after onset of symptom.

**DISCUSSION**

This study of the Ehrlichia species present in dogs in Missouri revealed several notable results. The first was the finding that more than 90% of dogs with molecular evidence of current Ehrlichia infection were infected with E. ewingii. Although E. ewingii had previously been demonstrated as a cause of ehrlichiosis in Missouri dogs (30), no study had yet documented its presence by molecular methods. The distribution of Ehrlichia species in Missouri dogs differs dramatically from that in humans with ehrlichiosis acquired in the state. In our laboratory, which receives canine specimens from a geographic region similar to the region from which dog samples were provided for the present study, E. chaffeensis has accounted for 89% of the cases, with E. ewingii accounting for the remaining 11%.

One possible explanation for this discrepancy may be differences in host pathogenicity; namely, E. chaffeensis may be more pathogenic for humans, and E. ewingii may be more pathogenic for dogs. Additional molecular studies of the prevalence of Ehrlichia in asymptomatic dogs would help clarify these results.

The absence of E. canis in the present study is also noteworthy. One other molecular study of canine ehrlichiosis, performed in Virginia, found only E. chaffeensis and E. ewingii, without any cases of E. canis infection (11). We do not think the absence of E. canis in the present study is the result of the failure of the PCR assay used to detect E. canis, since the PCR primers in the broad-range assay used for initial screening can...
FIG. 2. Geographical distribution of dogs included in the present study by county of residence. The results of Ehrlichia PCR assays are shown as designated in the key.
have been sampled very early in the course of their infection before an antibody response had occurred. Unfortunately, the interval between the day of onset of symptoms and the day when the blood sample was obtained was not available for all dogs. A further possible explanation may have been failure to make an antibody response to acute Ehrlichia infection in some of these dogs. Convalescent-phase samples were not available to test this hypothesis. It is also possible that in some of these dogs, the E. chaffeensis antigen used in the IFA may have failed to detect antibodies produced in response to infection with E. ewingii. This possibility is supported by the observation of inconsistent seroreactivity with E. canis antigen in serum from dogs found to be positive for E. ewingii DNA by PCR (16, 18).

There were no differences among the dogs with or without confirmed Ehrlichia infection in sex, age, breed, or fertility status. The larger overall representation of retrievers in the study may be explained by the popularity of these breeds as pets, but data on breed prevalence for the state were not available. Expected early summer peaks in both total suspected tick-borne illnesses and in actual PCR-positive cases of ehrlichiosis were noted. Prior studies have noted higher incidence, mortality rate, and chronicity among German shepherd dogs in South Africa with E. canis infection (32). However, no particular breed stood out in our study as having increased incidence.

We highlight here the potential relationships between human and canine ehrlichiosis. The finding that two of ten asymptomatic dogs were PCR positive for E. ewingii suggests that dogs might serve as a reservoir for E. ewingii. Goodin et al. (18) also recently showed evidence of asymptomatic dogs that were PCR positive for E. ewingii. The two asymptomatic PCR-positive dogs in the present study were sampled in March and April, neither of which is earlier in the year than those in which most cases of human ehrlichiosis occur in Missouri. This finding raises the possibility that chronic canine Ehrlichia infection could be a source for subsequent infections with Ehrlichia in humans residing in the same area. It is probably more likely that dogs and humans share similar exposures to infecting ticks, suggesting that cases of canine ehrlichiosis may serve as sentinels for human cases, as described for other tick-borne infections, including Rocky Mountain spotted fever (29). Most cases of asymptomatic canine ehrlichiosis do not currently undergo testing to reveal the etiologic agent. If confirmatory testing become more widely adopted, results could assist human public health officials in identifying environments where the risk of acquiring human ehrlichiosis is high.

ACKNOWLEDGMENTS

We are indebted to the veterinarians of Missouri who contributed samples for this study. We are grateful to Jim Struthers and Mark Arthur, School of Public Health, Washington University, for assistance in preparing the figures showing the location of study dogs throughout the state of Missouri and to Barbara Nash for assistance with preparation of the manuscript.

REFERENCES
