

## A serological survey of *Ehrlichia canis*, *Ehrlichia equi*, *Rickettsia rickettsii*, and *Borrelia burgdorferi* in dogs in Oklahoma

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**Abstract.** Serum samples from 259 dogs were tested for antibodies to *Ehrlichia canis*, *Ehrlichia equi*, *Rickettsia rickettsii*, and *Borrelia burgdorferi* using the indirect fluorescent antibody test. The sera were obtained from submissions to the Oklahoma Animal Disease Diagnostic Laboratory during a 14-month period from June 1986 through July 1987. The rate for positive antibody titers to *E. canis* was 53%, to *E. equi* was 33%, to *R. rickettsii* was 38%, and to *B. burgdorferi* was 18%. Higher percentages of sera serologically positive to *E. canis* were found in the spring through the fall months, but there were no seasonal variations for *E. equi*, *R. rickettsii*, and *B. burgdorferi*. There was no consistent pattern of titers to the 4 antigens when age-groups of the dogs were compared. Forty-four different breeds were tested.

*Ehrlichia canis*, *Rickettsia rickettsii*, and *Borrelia burgdorferi* are gaining recognition as infectious agents that cause significant clinical disease in dogs. Because these agents are tick-borne, they are limited in their geographical and host distribution. *Ehrlichia canis* infection has been reported in humans<sup>8,29</sup> as a cause of serious illness characterized by rigors, myalgia, headache, bradycardia, and abnormal liver function.<sup>8,29</sup> Infections due to *R. rickettsii* have been documented in humans.<sup>33,44</sup> Oklahoma has the highest rate of human infection for *R. rickettsii*, with 106 confirmed cases in 1986 and 84 confirmed cases in 1987.<sup>33,37</sup> There have been 3 confirmed cases of *B. burgdorferi* infections in humans in Oklahoma, 1 in 1985 and 2 in 1987 (Barbara Gildon, personal communication, 1988). The incidence of human infection with *B. burgdorferi* has been directly related to its prevalence in dogs.<sup>1,31,39</sup> It is important to establish the geographic and host limits of these agents not only to alert veterinarians to disease potential in their locale, but also to correlate the etiologic agents with their vectors.

*Ehrlichia canis* is the causative agent of canine ehrlichiosis (tropical canine pancytopenia).<sup>9,42,46</sup> *Ehrlichia canis*, a rickettsial agent, was first recognized in 1935 by Donatien and Lestogard from tick-infested dogs in Algeria? The first reported case in the United States was in Oklahoma in 1963.<sup>20,34</sup> *Ehrlichia canis* was associated with an outbreak of a fatal hemorrhagic dis-

ease in military dogs in Southeast Asia, hence the name tropical canine pancytopenia.<sup>16,41,45</sup> The organism has been found worldwide with the exception of South America. *Ehrlichia canis* is found as cytoplasmic inclusions or morulae in canine leukocytes, with monocytes being the most frequently infected cells.<sup>6,41</sup> There is controversy as to whether the neutrophilic and eosinophilic strains reported in the literature are, in fact, *E. canis*.<sup>5,7,12,26,43</sup> The organism is carried by the brown dog tick, *Rhipicephalus sanguineus*.

The acute phase of canine ehrlichiosis usually lasts 2-4 weeks<sup>9,34</sup> and is characterized by thrombocytopenia, anorexia, dyspnea, lymphadenopathy, depression, fever, and ocular and nasal discharges.<sup>9,16,34,36,41,46</sup> The signs during this phase are usually mild to inapparent. The untreated dog may recover or may develop 1 of 2 clinical forms of the chronic phase.<sup>9,17</sup> In the mild chronic form there are vague clinical signs characterized by depression, weakness, and weight loss. This form is generally not fatal and responds to treatment.<sup>34,45</sup> The severe chronic form is frequently fatal if untreated. It is characterized by anemia, leukopenia, thrombocytopenia, depression, anorexia, emaciation, and bleeding disorders, and is most often found in German shepherds.<sup>9,22,34,46</sup> The most reliable laboratory test for diagnosis of canine ehrlichiosis is the indirect fluorescent antibody test.<sup>2,16,34,41,42,45,46</sup>

*Ehrlichia equi* is the etiologic agent of ehrlichiosis in horses.<sup>23,26-28</sup> It has been identified in the circulating neutrophils and eosinophils of horses, and it has been suggested that the neutrophilic form of *E. canis* may be *E. equi*.<sup>17,24,26,27</sup> Experimentally dogs are susceptible to infection with *E. equi*, but clinical signs have been mild.<sup>24</sup> Clinical signs described for *E. equi* in horses include fever, anorexia, depression, ataxia, leukopenia,

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and thrombocytopenia.<sup>23,26</sup> The mode of transmission is unknown, but tick transmission is suspected.<sup>27</sup> Presently, the only method of antibody detection is the indirect fluorescent antibody test using *E. equi*-infected equine neutrophils.<sup>15,24,26</sup>

*Rickettsia rickettsii* is the etiologic agent of Rocky Mountain spotted fever (RMSF), a disease of canines as well as humans. Its principal vectors are *Dermacentor andersoni* (the wood tick) and *Dermacentor variabilis* (the American dog tick). Clinical signs in the dog vary from mild to severe and include depression, fever, anorexia, lymphadenopathy, neurological dysfunction, and petechial or ecchymotic hemorrhages. A serological diagnosis is based on a 4-fold or greater rise in antibody titers between acute and convalescent sera as demonstrated by the indirect fluorescent antibody test.<sup>11</sup>

*Borrelia burgdorferi* is the etiologic agent of borreliosis or Lyme disease, named after the town of Lyme, Connecticut, where a human epidemic occurred in 1975.<sup>21,40</sup> Since that time, the disease has been recognized in numerous states in the Northeast, Midwest, and Far West. The organism, a spirochete, is most commonly carried by the tick *Ixodes dammini*, but other ticks may also serve as vectors.<sup>13,18,19,21,25</sup> Arthritis is the major clinical sign seen in dogs; however, most canine infections are subclinical.<sup>18,21,25</sup> The indirect fluorescent antibody test is used to determine exposure to the spirochete.<sup>13,18,21,25,30,38,40,47</sup>

The purpose of this study was to determine the presence or absence of antibodies to these disease-producing organisms in Oklahoma dogs. *Ehrlichia equi* was included because it has been suggested that it may be the same agent that causes the neutrophilic form of ehrlichiosis described in dogs.<sup>26</sup>

### Materials and methods

**Clinical specimens.** A total of 259 dog sera were submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) from June 1986 through July 1987 for rickettsia and/or spirochete antibody testing. The sera were tested for the presence of antibody to *E. canis*, *E. equi*, *R. rickettsii*, and *B. burgdorferi* using the indirect fluorescent antibody test. Most of the sera submitted were from dogs that had clinical signs suggesting infections with either 1 or more of the 4 agents.

**Serologic determinations of antibody to *E. canis* and *E. equi*.** Commercial reagent test slides<sup>a</sup> were received frozen and stored at -70 C until used. The slides were thawed, air-dried, and fixed in acetone prior to use. Measurement of antibody to *E. canis* and *E. equi* in dog sera was done using an indirect fluorescent antibody test.<sup>36</sup> Serial 10-fold dilutions of each canine serum (1:10 to 1:1,000) were prepared in phosphate-buffered saline (PBS) (pH 7.4), and 20  $\mu$ l of each serum dilution was added to a well on a test slide. After incubation in a humid chamber at 37 C for 30 min, the slides

were washed twice in PBS and once in deionized water. The slides were air-dried, and 20  $\mu$ l of optimally diluted fluorescein-labeled F(ab)<sub>2</sub> goat anti-dog IgG<sup>b</sup> containing rhodamine-labeled bovine albumin<sup>c</sup> was added to each test well. The slides were incubated again in a humid chamber at 37 C for 30 min, and then washed and dried. Coverslips were mounted on the slides using glycerol medium (pH 9.0).

The detection of bright-green fluorescing morulae in the cells was used as the indicator for the presence of antibody specific for *E. canis* and *E. equi*. Sera with antibody titers of  $\geq 10$  were considered positive.

**Serologic determination of antibody to *R. rickettsii*.** Serial 2-fold dilutions (1:64 to 1:2,048) of each canine serum were prepared in PBS (pH 7.4). Preparation of reagent test slides<sup>d</sup> and sera testing were done as previously described.<sup>32</sup> The use of optimally diluted fluorescein-labeled F(ab)<sub>2</sub> goat anti-dog IgG<sup>b</sup> containing rhodamine-labeled bovine albumin<sup>c</sup> as a counterstain was substituted for the anti-human conjugate. The slides were washed, air-dried, mounted with coverslips using glycerol medium (pH 9.0), and examined.

The detection of bright-green fluorescing organisms was used as the indicator for the presence of antibody specific for *R. rickettsii*. Sera with antibody titers of  $\geq 64$  were considered positive.

**Serologic determination of antibody to *B. burgdorferi*.** Reagent test slides were prepared by the application of fixed *B. burgdorferi* organisms<sup>e</sup> onto black ceramic background slides (toxoplasmosis slides)<sup>f</sup> using the procedure previously described.<sup>28,38,45</sup> Serial 2-fold dilutions (1:64 to 1:2,048) were prepared in PBS (pH 7.4). After reaction of the antigen with sera at 37 C for 30 min, the slides were washed and stained with fluorescein-labeled F(ab)<sub>2</sub> goat anti-dog IgG<sup>b</sup> that also contained rhodamine-labeled bovine albumin<sup>c</sup>. The slides were washed, air-dried, mounted with coverslips using glycerol medium (pH 9.0), and examined.

The detection of bright-green fluorescing organisms was used as the indicator of the presence of antibody specific for *B. burgdorferi*. Sera with antibody titers of  $\geq 64$  were considered positive.

**Microscopic examination.** All slides were examined for specific immunofluorescence using a microscope equipped with an ultraviolet light source<sup>g</sup>. The titer of each serum was expressed as the reciprocal of the highest serum dilution in which fluorescing morulae (*E. canis* and *E. equi*) or organisms were found.<sup>5</sup>

**Serologic determination of antibody to *Leptospira interrogans*.** Sera were tested for antibodies to the following serovars of *Leptospira interrogans* by the microscopic agglutination microtiter procedure:<sup>34</sup> *canicola*, *grippityphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona*.

### Results

Fifty-three percent of the serum samples tested had titers  $\geq 10$  to *E. canis*, 33% had titers  $\geq 10$  to *E. equi*, 38% had titers  $\geq 64$  to *R. rickettsii*, and 18% had titers  $\geq 64$  to *B. burgdorferi* (Table 1).

Twenty-four percent of the dogs tested for *E. canis* had low titers (10), 8% had moderate titers (100), and 21% had high titers ( $\geq 1,000$ ) (Table 2). Nineteen per-

**Table 1.** Number and percentage of canine sera positive to *E. canis*, *E. equi*, *R. rickettsii*, and *B. burgdorferi* during a 14-mo period.

Antigen	No. positive/ no. tested	Percent positive
<i>E. canis</i> *	138/259	53
<i>E. equi</i> *	85/257	33
<i>R. rickettsii</i> †	99/259	38
<i>B. burgdorferi</i> †	45/256	18

\* Antibody present at  $\geq 1:10$  dilution.† Antibody present at  $\geq 1:64$  dilution.

cent of the samples had low titers (10) to *E. equi*, 8% had moderate titers (100), and 6% had high titers ( $\geq 1,000$ ) (Table 2). Sixteen of 25 dogs (64%) that tested negative for antibody to *E. canis* were positive for *E. equi*, with titers ranging from 10 to  $\geq 1,000$ . Thirty of 138 (22%) dog sera positive for *E. canis* with titers of  $\geq 100$  were negative for antibody to *E. equi*.

Low antibody titers (64) were seen in 11% of the dog sera tested for *R. rickettsii*; 21% had moderate titers (128-512) and 6% had high titers ( $\geq 1,024$ ) (Table 3). Eleven percent of the dog sera tested for *B. burgdorferi* had low titers (64) and 7% had moderate titers (128-512). None of the sera had high antibody titers to *B. burgdorferi*.

A seasonal increase in the number of sera with antibodies to *E. canis* occurred in the late spring through summer months (Fig. 1). There was very little seasonal variation in the number of sera positive for *E. equi*, *R. rickettsii*, or *B. burgdorferi* (Fig. 1).

Antibody titers to the 4 antigens were evenly distributed among all ages of dogs (Table 4). However, no titers were detected to *E. equi* in serum from dogs less than 1 year of age.

A total of 259 dog sera representing 44 breeds were tested. The largest number of submissions were from the hound and shepherd breeds, which were comprised of 28 (11%) and 29 (11%) dogs, respectively. The hounds included black and tan, bluetick, coon, and Walker hounds. Ninety-six percent of the 27 hounds' sera were positive for *E. canis* as compared to 50% (14) for *E. equi*, 32% (9) for *R. rickettsii*, and 29% (8) for *B. burg-*

**Table 2.** Distribution of antibody titers in canine sera to *E. canis* and *E. equi* during a 14-mo period.

Titer*	No. positive (%)	
	<i>E. canis</i>	<i>E. equi</i>
<10	121 (47)	172(67)
10	62(24)	48(19)
100	21 (8)	21 (8)
$\geq 1,000$	55(21)	16 (6)
No. tested	259	257

\* Expressed as the reciprocal of the final serum dilution.

**Table 3.** Distribution of antibody titers in canine sera to *R. rickettsii* and *B. burgdorferi* during a 14-mo period.

Titer*	No. positive (%)	
	<i>R. rickettsii</i>	<i>B. burgdorferi</i>
<64	160 (62)	211 (82)
64	28 (11)	29 (11)
128	21 (8)	14 (6)
256	17 (7)	2 (1)
512	15 (6)	0
1,024	13 (5)	0
$\geq 2,048$	5 (1)	0
No. tested	259	256

\* Expressed as the reciprocal of the final serum dilution.

*dorferi*. In the shepherd breed, 66% (19) of the dog sera tested had detectable antibody levels to *E. canis*, 21% (6) to *E. equi*, 29% (9) to *R. rickettsii*, and 10% (3) to *B. burgdorferi*.

Of the 20 dogs tested for antibodies to the *L. interrogans* serovars (*canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona*), 13 (65%) were negative. Four of the sera with *Leptospira* antibody titers of 100-400 to the serovars *canicola*, *grippotyphosa*, and *icterohemorrhagiae* were negative for antibodies to *B. burgdorferi*. Three of the sera with antibody titers of 100 and 200 to the *Leptospira* serovars *icterohemorrhagiae* and *grippotyphosa* also had antibody titers of 64 and 256 to *B. burgdorferi*.

## Discussion

The majority of dog sera tested were from sick dogs rather than a random sampling of dogs. Thus, the numbers of positive sera are expected to be larger when compared to a normal population of dogs.

The data obtained support the opinion that *E. canis* infection is common in Oklahoma dogs. A large num-

**Table 4.** Age distribution of dogs in Oklahoma with antibodies to *E. canis*, *E. equi*, *R. rickettsii*, and *B. burgdorferi* during a 14-mo period.

Age (yr)	No. tested	No. of positive sera (%)			
		<i>E. canis</i> *	<i>E. equi</i> *	<i>R. rickettsii</i> †	<i>B. burgdorferi</i> †
<1	13	5 (39)	0 (0)	3 (23)	2 (15)
1	23	18 (78)	11 (48)	10 (44)	5 (22)
2	21	12 (57)	5 (24)	10 (48)	6 (29)
3	23	15 (65)	10 (44)	10 (44)	8 (35)
4	19	9 (47)	6 (32)	3 (16)	4 (21)
5	17	9 (53)	9 (56)	5 (29)	3 (18)
6	16	12 (75)	6 (38)	12 (75)	3 (13)
$\geq 7$	40	22 (55)	14 (35)	16 (40)	5 (13)
Unknown	87	36 (41)	21 (24)	29 (33)	8 (9)

\* Antibody present at  $\geq 1:10$  dilution.† Antibody present at  $\geq 1:64$  dilution.

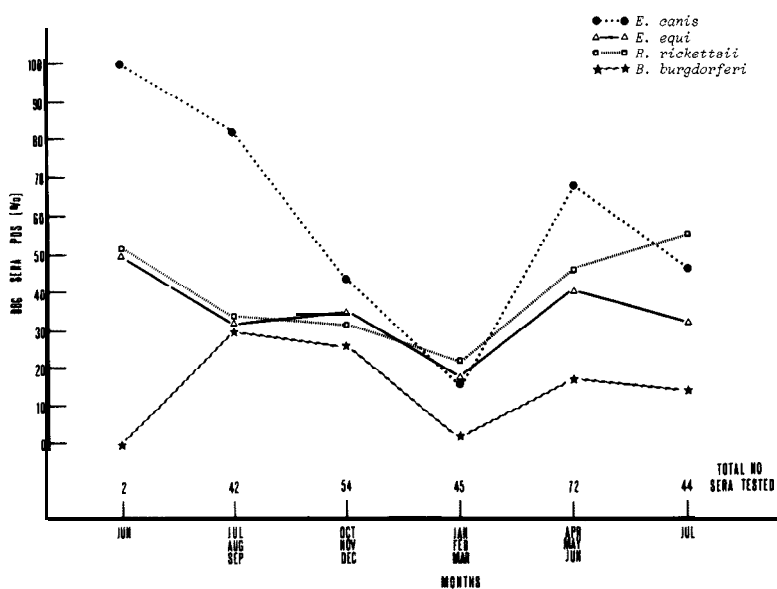


Figure 1. Distribution by months of antibodies to *E. canis*, *E. equi*, *R. rickettsii*, and *B. burgdorferi* during a 14-month period.

ber of positive sera to *E. canis*, both absolute and relative, occurred during the months of May through September. This might be related to increased exposure to the tick vector during the warmer months.<sup>14</sup> The highest percentage of dogs with detectable antibodies to *E. canis* was in the hound breeds. This is an expected result, as hounds are generally outdoors and would be expected to have higher exposure to ticks. However, antibody to *E. canis* was detected in more than 50% of all the breeds tested, indicating a broad range of breed susceptibility and exposure. No data were available to compare indoor versus outdoor dogs or rural versus urban dogs.

The *Ehrlichia equi* serology findings are significant in that they demonstrate the presence of the agent or a similar agent in Oklahoma. Although there is some cross-reaction between *E. canis* and *E. equi*, it is not a complete cross-reaction.<sup>15,16,35</sup> Sixty-four percent of the negative *E. canis* sera had antibodies to *E. equi*. Conversely, 22% of the positive *E. canis* sera were negative for *E. equi*. The neutrophilic strain of *E. canis* that is seen in dogs is thought by some to be *E. equi*.<sup>26</sup> This neutrophilic strain of *Ehrlichia* has been seen in Oklahoma dogs,<sup>5,12</sup> and these serology results support the hypothesis that the neutrophilic strain in dogs may be the same agent that causes ehrlichiosis in horses. Although *E. equi* is not known to cause severe disease in the dog, it can cause moderate to severe clinical signs in the horse.<sup>27,28</sup> Equine ehrlichiosis has been geographically limited, with no reported cases in Oklahoma. A survey on 100 horse sera conducted at OADDL during 1986 detected no antibody titers to *E. equi* (unpublished data). However, it appears that the

organism or a serologically related agent is present but may be limited to the canine population. The transmission of *E. equi* is unknown, although a tick vector is suspected. Titers were not seen in dogs less than 1 year of age, indicating minimal exposure during puppyhood. *Ehrlichia equi* was the only agent for which antibody was not detected in dogs less than 1 year of age.

*Rickettsia rickettsii* is prevalent in Oklahoma, as indicated by the large number of human cases of Rocky Mountain spotted fever reported.<sup>33,44</sup> During 1986 and 1987, a total of 190 cases of confirmed human infection were reported.<sup>33</sup> Our results indicate canine exposure to the agent. There was no age or breed correlation with positive titers, but there was a large increase in positive sera, both absolute and relative, in May that gradually declined during the summer and fall months to a low in March and April.

*Borrelia burgdorferi* infection has not been previously reported in Oklahoma in dogs. The positive serology results indicate that this agent is present in Oklahoma dogs. This is supported by the report of 3 confirmed cases in humans in Oklahoma since 1985. This reported low incidence in humans is consistent with finding a low number of infected dogs in Oklahoma. Investigations have shown minimal antigenic relatedness between *B. burgdorferi* and 20 *Leptospira* serovars.<sup>10,21,30,31</sup> Although only 20 samples were tested for antibodies to both *B. burgdorferi* and *Leptospira*, no correlation was observed, suggesting minimal or no cross-reactions between the 2 spirochetes. Sera positive for *B. burgdorferi* were evenly distributed among the ages and breeds of dogs.

This was the first serological survey of Oklahoma dogs for antibodies to *E. canis*, *E. equi*, *R. rickettsii*, and *B. burgdorferi*. The data serve as a baseline for future work and indicate the presence of all 4 agents in the Oklahoma canine population.

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### Sources and manufacturers

- a. ProtaTek International, Inc., St. Paul, MN.
- b. Cooper Biomedical, Malvem, PA.
- c. Becton, Dickinson and Co., Cockeysville, MD.
- d. *Rickettsia rickettsii* was kindly supplied by Jerry Kudlac, Public Health Laboratory Service, Oklahoma State Department of Health, Oklahoma City, OK.
- e. Live cultures of *B. burgdorferi* were kindly provided by Eric Shaw and Dr. John Post, Department of Pathobiology, University of Connecticut, Storrs, CT.
- f. Bello Biotechnology, Vineland, NJ.
- g. Leitz Orthoplan Microscope, xenon arc lamp (400 HBO), and exciter (KP500) and barrier (KP515) filters, E. Leitz, Inc., Rockleigh, NJ.

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