Dogs as Sentinels for Human Lyme Borreliosis in The Netherlands

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Received 31 July 2000/Returned for modification 17 October 2000/Accepted 14 December 2000

Serum samples from hunters (n = 440), their hunting dogs (n = 448), and hunters without dog ownership (n = 53) were collected in The Netherlands at hunting dog trials and were tested for antibodies against Borrelia burgdorferi by a whole-cell enzyme-linked immunosorbent assay. Additionally, 75 healthy pet dogs were tested. The results of this study indicate that the seroprevalence among hunting dogs (18%) was of the same order as the seroprevalence among pet dogs (17%) and hunters (15%). The seropositivity of a hunting dog was not a significant indicator of increased risk of Lyme borreliosis for its owner. No significant rise in seroprevalence was found in dogs older than 24 months. This indicated that seropositivity after an infection with B. burgdorferi in dogs is rather short, approximately 1 year. In humans this is considerably longer but is also not lifelong. Therefore, the incidence of B. burgdorferi infections among dogs was greater than that among hunters, despite a similar prevalence of seropositivity among hunters and their hunting dogs. Because no positive correlation was observed between the seropositivity of a hunter and the seropositivity of the hunter’s dog, direct transfer of ticks between dog and hunter does not seem important and owning a dog should not be considered a risk factor for Lyme borreliosis.

Lyme borreliosis (LB) is a zoonotic disease caused by the spirochete Borrelia burgdorferi (5, 41). An animal reservoir of approximately 40 mammals and birds has been established (15) in Europe. The disease is transmitted primarily by ticks feeding on mammals and birds, with the most common vector in Europe being the tick Ixodes ricinus (1). In humans, LB in its early stages is characterized by influenza-like symptoms, followed in 60 to 80% of the cases by erythema migrans (40), a skin lesion that spreads outward from around the site of a tick bite. If untreated, the disease may proceed to a second or a third stage in which neurological disorders and arthritis are common symptoms (42). Much less is known about LB in animals than is known about the disease in humans. In most mammals, the symptomatology of LB in dogs is migratory arthritis (30) without divergent radiographic findings. Other than this, less common symptoms reported in dogs are carditis (25), glomerulonephritis (17), and neuritis (2; B. M. Feder, R. J. Joseph, S. D. Moroff, et al., Abstr. Proc. 9th ACVIM, p. 892, 1991). B. burgdorferi infections or serologic evidence of B. burgdorferi infections have been reported in dogs in the United States (3, 7, 26, 29, 30). In Europe, relatively few reports exist on LB in animals. In Sweden (13), Denmark (18), Germany (20, 21, 35, 45, 47), The Netherlands (19), the United Kingdom (32), Belgium (33), France (9, 11, 12, 14), Switzerland (37), Slovakia (43), Slovenia (34), and Spain (10), antibodies to B. burgdorferi and/or clinical symptoms of LB have been found in dogs. However, in Europe, the use of dogs as sentinel animals for the estimation of the risk of Lyme borreliosis for humans in that region has not been examined. Moreover, it has been suggested that in the United States pet ownership increases the risk of getting Lyme disease (K. L. Curran and D. Fish, Letter, N. Engl. J. Med. 320:183, 1989), yet in Europe the relationship of dog ownership and an increased risk of Lyme disease for the dog owners has not been studied.

People recreating or working in tick-infested areas like forests show an increased prevalence of antibodies to B. burgdorferi compared to that for controls (22, 23, 36). Parallel to the findings for people with high levels of outdoor activity, a higher seroprevalence of antibodies B. burgdorferi could be expected for hunting dogs compared to that for controls. As dogs could be an intermediary source for human tick infestation, the risk of human Lyme disease could be increased by dog ownership. The aims of the study described here were to evaluate if high levels of outdoor activity can be related to an increased prevalence of antibodies to B. burgdorferi in both hunter and hunting dog populations, to search if dogs in an area of endemcity for LB pose a risk factor for LB for their owners, and to investigate if in The Netherlands the risk for LB in humans can be deduced from the seroprevalence of antibodies against B. burgdorferi among the dog population in the same area.

MATERIALS AND METHODS

In the autumn of 1989 at trials for hunting dogs, blood samples were collected from hunters (n = 440) and their dogs (n = 448). Blood samples from an additional group of hunters who did not own a dog (n = 53) were also included. All participants in the study were asked to fill in a questionnaire about age, tick infestations, and clinical symptoms of LB for both the hunter and the dog. The ages of the 448 hunting dogs ranged from 4 to 20 months, with a mean age of 38 months, and the dogs were of various breeds. In the same year of the trial, blood samples were collected from 75 healthy dogs of various breeds that lived in the countryside, that had no clinical signs of Lyme disease, and that presented at veterinary clinics for their regular vaccinations. The ages of the 75 dogs ranged from 6 to 97 months, with a mean age of 35 months. None of the animals included in this study were vaccinated against LB. No vaccine against LB is available in The Netherlands. All sera were stored at −70°C until assayed. Serological testing was used to determine the prevalence of Lyme antibodies in the hunters, hunting dogs, and nonhunting dogs. To exclude differences related to test technology, both human and the dog sera were tested in an enzyme immunoassay (EIA) system by using the same antigen batch.
ELISA for human and canine sera. The sera of the hunters were tested for immunoglobulin G (IgG) antibodies to *B. burgdorferi*, and the results were compared to the previously reported results (36). For the in-house enzyme-linked immunosorbent assay (ELISA), the *B. burgdorferi* B31 strain (ATCC 35210) was used as antigen. The human and dog sera were tested as described by Craft et al. (8), with minor modifications. Briefly, the spirochetes were grown for 5 to 7 days at 35°C in BSK-II medium (modified Barbour-Stoenner-Kelly medium). The culture was centrifuged (10,000 × g, 30 min, 4°C), and the pellets were washed twice in phosphate-buffered saline (PBS; pH 7.2) with 0.005 M MgCl₂ (10,000 × g, 30 min, 4°C), resuspended in PBS, and sonicated 20 times for 15 s each time on ice water in a Branson sonicator-ultrasonic processor at the maximum microtip setting. The sonic extract was centrifuged (10,000 × g, 30 min, 4°C), and the protein content of the supernatant was determined by a protein assay (Bio-Rad Laboratories, Munich, Germany). The supernatant was divided into aliquots and was kept at −70°C. Immunoplates (Polysorp; Nunc, Roskilde, Denmark) were coated with 100 μl of sonicated antigen (2 μg/ml) that was diluted in 0.05 M carbonate buffer (pH 9.6) (15 h, 4°C). Non-specific binding was blocked with 200 μl of 1% fish gelatin for human and canine sera, respectively, and were tested in duplicate (100 μl per well). After 1 h of incubation at 37°C, 100-μl volumes of peroxidase-conjugated goat anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) and peroxidase-conjugated protein G (Sigma) were added to a dilution of 1/3,000 (in PBS-Tween 20) for human sera and a dilution of 1/10,000 (in PBS-Tween 20) for canine sera, respectively, and the solutions were incubated at 37°C for 30 min. Between all steps, the plates were washed in a Microplate washer (Flow Laboratories, Glasgow, Scotland) on a three-wash cycle with PBS–Tween 20 used as a washing buffer. As a substrate, 100 μl of ready-to-use tetramethylbenzidine (D-trek, Mons, Belgium) was used. The optical density (OD) at 405 nm was read in a Titertek Multiskan apparatus (ICN Pharmaceuticals Inc., Irvine, Calif.). The time of the substrate reaction was set to 15 min and was stopped with tetramethylbenzidine stop solution (D-trek).

Standardization of the human and canine Lyme disease ELISA. For the human Lyme disease ELISA, 25 serum samples from patients with clinically defined late Lyme disease, 100 serum samples from blood donors, and 100 serum samples from patients with diseases that clinically mimic Lyme disease were used to determine the cutoff, which was 0.300 OD unit. For the canine Lyme disease ELISA, sera from 105 dogs from an experimental animal facility that had never been exposed to ticks (5 of which, however, had been hyperimmunized against leptospirosis) were used to determine the cutoff for a positive reaction. A mean OD and standard deviation (SD) of the mean were calculated for the 105 negative canine serum samples and were used to determine the cutoff value, which was 0.250 OD unit. In the *B. burgdorferi* ELISAs, commonly 2 times (8) or 3 times (28) the SD above the mean for a group of negative controls is used as a cutoff value (31). This might, however, vary between laboratories. To determine the cutoff levels for a positive canine Lyme disease test result, a mean OD and standard deviation (SD) of the mean were calculated for the 105 negative serum samples which had the same serum dilution as the test sera. To ensure the reproducibility of the test, all the sera included in the serumurvey were tested with the same batch of antigen. The best reproducibility was obtained when 3 SDs was used as the cutoff, because 98% of the canine serum samples were consistently either positive or negative by both tests. If, on the other hand, a 2-SD cutoff was used, only 86% of the canine serum samples were repeatedly positive or negative. Of the canine sera, which additionally became positive when the cutoff was lowered, 82% were borderline sera; i.e., when the same sera were tested repeatedly, they gave various results, either positive or negative. This led to a much poorer reproducibility of the test. On the basis of these results, the cutoff between a positive test result and a negative test result was set at 3 SDs above the mean OD for the negative canine control serum samples, and a seropositive animal was defined as one that had an OD ratio above this calculated cutoff value.

Control sera. For the human Lyme disease ELISA, sera with negative, cutoff, and positive values were tested in duplicate on each plate. For the canine Lyme disease ELISA, a pool of sera from three dogs hyperimmunized with sonicated *B. burgdorferi* ATCC 35210 antigen was used as a positive control. These dogs had been immunized subcutaneously with LB antigen in an adjuvant mixture of water in the mineral oil Specol (4) (ID-DLO, Zeist, The Netherlands) and were boosted after 4 weeks with *B. burgdorferi* antigen in PBS. The antibody response against LB was confirmed by Western blotting. One week after the last immunization, blood was collected from the animals and the serum was stored at −70°C until it was assayed. The immunized animals had an antibody titer of 51,200 (reciprocal dilution) in the ELISA and were pooled for use as positive control serum. No background reaction was observed for the positive control serum in control wells, which were blocked with 1% fish gelatin (Sigma) in PBS. Sera taken before and after immunization of these dogs were also tested for antibodies against *Leptospira interrogans* serovar hardjo (macroscopic agglutination test, internal house test) and *L. interrogans* serovar icterohaemorrhagiae (macroscopic agglutination test, internal house test) and for Treponema pallidum antibodies (T. pallidum distrofile, Tokyo, Japan); all sera were negative.

A pool of sera from 10 experimental dogs negative for *B. burgdorferi* antibodies (Vet Lyme Borreliosis ELISA; Genzyme-Virotech, Ruesselsheim, Germany) was used as a negative control.

To eliminate plate-to-plate variation, the ELISA result was expressed as an OD ratio, i.e., the ratio of the mean OD for a test serum sample to the mean OD for the serum sample with the cutoff value on the same plate. While testing the dog sera these positive and negative samples and a diluted positive control serum sample with the predefined cutoff value were tested on each plate. The mean OD value was calculated for each duplicate serum sample. Human or canine sera were retested if the OD values for the duplicates differed by more than 10% from the mean.

Statistical Analysis. Paired data were compared by McNemar’s test, assuming a binomial distribution of the data. Nonpaired data were compared by using the chi-square test.

RESULTS

The results of the testing of the hunters, as previously described by Nohlmans et al. (36), showed no significant differences compared to those of the current tests. Briefly, the prevalence of IgG antibodies against *B. burgdorferi* among owners of working hunting dogs (*n* = 440) was significantly higher (15%) than that among healthy blood donors (*n* = 1,052) matched for the same age (9%). In both groups the prevalence of seropositivity increased with age, but in hunters older than 40 years it remained relatively constant, as shown in Table 1. Only 3% of the hunting dog owners could recall having had symptoms most likely to be due to LB. Of the 68% seropositive hunters, 64 (94%) were asymptomatic. As shown in Table 1, the rate of seropositivity among the hunters increased with increasing age: from 7% for those <31 years of age to 20% for those >40 years of age (*P* < 0.005). Among the hunters older than 40 years, the seroprevalence no longer rose significantly and remained constant at approximately 20%.

As listed in Table 2, antibodies against *B. burgdorferi* were detected in 18% (95% confidence interval, 14.4 to 21.4%) of the hunting dogs and 17% (95% confidence interval, 8.5 to 25.5%) of the pet dogs. Hunting dogs older than 24 months appeared to have a greater risk of being exposed (22%) than younger hunting dogs (9 to 11%) (*P* < 0.05), but the seroprevalence among hunting dogs remained stable at approximately 22% among animals over 24 months of age (Table 2). In a comparison of the age distribution of seropositive hunting dogs and the total hunting dog population, no significant differences were ob-
When we compared the seroprevalence of antibodies against *B. burgdorferi* for the hunting dog population with that for the nonhunting dog population, no significant differences were seen for all age groups listed. Eleven (14%) of the 80 seropositive hunting dogs showed signs of lameness and were older than 24 months, with a mean age of 46 months. Eight (72%) of the 11 hunting dogs that had been suffering from migratory lameness had a history of regular tick infestations, whereas ticks had seldom been spotted on the other three dogs by their owners. Ticks had regularly been removed from 53 (66%) of the 80 seropositive hunting dogs and 217 (59%) seronegative hunting dogs. Most of the seropositive hunting dogs (86%) had not shown any clinical symptoms that could be attributed to LB, and only 11 (14%) had recently suffered from intermittent lameness, a clinical symptom that could be attributable to LB. Of the 217 seronegative hunting dogs, 36 (17%) had recently shown signs of lameness. All seropositive pet dogs were healthy, without any clinical signs pointing to LB. As shown in Tables 1 and 2, the prevalences of seropositive hunters (15%; 95% confidence interval, 11.7 to 18.3) and hunting dogs (18%; 95% confidence interval, 14.4 to 21.4) were not significantly different. However, if the results of the EIAs were matched between the dog and the dog owner, the prevalence of seropositivity among the hunters was significantly different ($P < 0.001$) from the prevalence of seropositivity among their hunting dogs. In only 12% of the hunter–dog pairs was a match of seropositivity observed. The seroprevalence among hunters with or without dog ownership was not significantly different.

**DISCUSSION**

**Outdoor activity as a risk factor for Lyme disease.** The seroprevalence of antibodies against *B. burgdorferi* among hunting dogs and hunters in this study is higher than that among healthy blood donors (9%) in The Netherlands but is of the same order as the seroprevalence among Dutch forestry workers (20 to 24%), as described by Kuiper et al. (22). In contrast, a significantly lower seroprevalence in Dutch soldiers (0.9%) conducting predominantly outdoor activities in areas of endemicity for ticks has been reported (44). The most likely explanation for the lower seroprevalence among Dutch soldiers is the fact that soldiers must wear a special uniform during field training: long sleeves, long pants, and high boots. This uniform is different from and much more protective against tick infestations than the clothes worn by most other people involved in recreational or occupational outdoor activities, especially during warm weather conditions. However, despite the observed similar seroprevalence among dogs and hunters in this study, the hypothesis of Eng et al. (T. R. Eng, M. L. Wilson, A. Spielman, and C. C. Lastavica, Letter, J. Infect. Dis. **158**:1410–1411, 1988) that dogs have a greater risk of *B. burgdorferi* infection than people is endorsed by the results of this study, as dogs stay seropositive for a much shorter period of time after an infection with *B. burgdorferi*. Because seropositivity seemed to last for only approximately 1 year, the seroprevalence in dogs in fact is more or less identical to the yearly incidence of infections with *B. burgdorferi* in dogs. Surprisingly, although it was supposed that hunting dogs have an increased risk of tick infestation compared to the risk for other dogs, no significant differences in seroprevalences between the hunting dog and the pet dog populations were found. These findings are in contrast to the seroprevalence among hunting dogs (40%) in the Slovak Republic (43), which was significantly higher than the seroprevalence among service dogs (12%).

**Dogs and their validity as sentinel animals.** The dog has been proposed for use as a sentinel animal for detection of the risk of *B. burgdorferi* infection in humans. Dogs exposed to infected ticks develop antibodies to the spirochete, and dogs are more likely than people to be exposed to infected ticks because their behavior brings them into direct and closer contact with tick habitats like brush. Moreover, ticks can easily hide in the hair coats of dogs and dogs are not protected

**TABLE 2. Prevalence of antibodies to *B. burgdorferi* among hunting and nonhunting dogs, by age**

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Hunting dogs</th>
<th></th>
<th></th>
<th></th>
<th>Nonhunting dogs</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Seropositive</td>
<td></td>
<td></td>
<td>Total</td>
<td>Seropositive</td>
<td></td>
</tr>
<tr>
<td>6–12</td>
<td>56</td>
<td>5 (9)</td>
<td>11</td>
<td>1 (9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–24</td>
<td>125</td>
<td>15 (11)</td>
<td>19</td>
<td>3 (16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–36</td>
<td>85</td>
<td>19 (22)</td>
<td>11</td>
<td>2 (18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37–48</td>
<td>73</td>
<td>16 (22)</td>
<td>15</td>
<td>3 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49–120</td>
<td>109</td>
<td>25 (23)</td>
<td>19</td>
<td>4 (21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>448</td>
<td>80 (18)</td>
<td>75</td>
<td>13 (17)</td>
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</tr>
</tbody>
</table>
against tick infestation by clothing like hunters are. Although it was expected that dogs were frequently bitten by ticks more often than hunters were, the seroprevalence of antibodies against *B. burgdorferi* in hunters and hunting dogs was of the same order in the present study. This indicated that estimates of seroprevalence among hunting dogs are predictive of the risk of LB in humans. This finding was supported not only by the overall seroprevalence but also by the fact that no significant variation was seen when the seroprevalences for hunters and hunting dogs from the same regions were compared (data not shown). The use of dog sera to detect and quantify the risk of Lyme disease for humans in a certain region is more sensitive than the use of reports of incident human clinical cases but is not more sensitive than the use of seroprevalence in humans. The use of dog sera, however, has the advantage that the seroprevalence among dogs is more likely to reflect the actual environmental risk of Lyme disease because of the short half-life of canine antibodies against *B. burgdorferi*. This study shows that the risk factors identified for dogs may directly or indirectly illuminate certain aspects of the epidemiology of human Lyme disease. Nonetheless, one should be aware that it is very difficult to standardize canine Lyme disease tests due to the lack of indisputable clinically defined cases of Lyme disease. Most studies use panels of canine sera reactive by other tests as a reference, but this is not a real “gold standard.” A serum sample can be regarded as a gold standard when clinically it is indisputably related to Lyme disease and when the presence of *B. burgdorferi* has been demonstrated. Therefore, to circumvent the problem of disputable reference sera, this study used the sera of dogs vaccinated with *B. burgdorferi* for the tuning of the linear response range of the test. For the cutoff determination, the mean OD for a dog population considered negative for Lyme disease and a population considered possibly cross-reactive to Lyme disease (hypermunized against leptospirosis) was used. Although this method is acceptable for seroepidemiological studies, this is not the ideal way and panels of an acceptable number of indisputably defined sera from dogs with Lyme disease should be made available for future test development. Parallel to the findings for human Lyme disease serology, that exclusion of diseases that seem to increase the risk of Lyme disease (Curran and Fish, 1981), the seropositivity of the hunting dogs was not an acceptable for seroepidemiological studies, this is not the ideal method capable of detecting changes in exposure to the pathogen. The use of dog sera to detect and quantify the risk of Borrelia burgdorferi infection in dogs in western France. Systematic serological survey of 806 hunting dogs and 88 military dogs in 14 departments. Rec. Med. Vet. 164:367–374.


