Research Article

A retrospective study of *Borrelia burgdorferi* antibodies in dogs in Minnesota

Aneela Z. DURRANI[1,*], Sagar M. GOYAL[2]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences,
Out Fall Road, Lahore - PAKISTAN

[2]College of Veterinary Medicine, University of Minnesota, Minnesota - USA

Abstract: In order to determine the seroprevalence of *Borrelia burgdorferi* antibodies in suspected dogs in Minnesota, the present study examines the database of the Veterinary Diagnostic Laboratory at the University of Minnesota over a 10 year period (2001-June 2010). A total of 1081 serum samples from dogs suspected of having borreliosis were submitted by 112 private veterinary clinics. The samples were tested using an indirect fluorescent antibody (IFA) test. Samples with titers of ≥1:320 were considered positive. The rate of seroprevalence of antibodies was 88%. The increase in the rate of yearly seroprevalence of *Borrelia burgdorferi* antibodies in dogs was comparable with that of human borreliosis in Minnesota.

Key words: Lyme disease, dogs, Lyme antibodies, *Borrelia burgdorferi*, serologic survey

Introduction

The first documented case of human borreliosis occurred in 1977 in Old Lyme, Connecticut, USA, and the first case of *B. burgdorferi* infection in dogs was reported in 1984 (1). Lyme borreliosis has also been reported in North America, Europe, Asia, Australia, South America, Africa, and Korea (2). Lyme disease is the most commonly reported tick-borne disease in the United States, with most of the human cases reported from 10 states, including Minnesota (3). Lyme borreliosis in dogs is more common in the northeast, upper midwest, and west coast states of the US (4).

The causal agent of borreliosis, *Borrelia burgdorferi*, is transmitted to a variety of mammals by

Ixodes spp. ticks. *Ixodes scapularis*, as the tick vector for borreliosis in dogs, was first reported in Maine in 1985 while *I. scapularis* was reported from Wisconsin in 1968 (5). The range of *Ixodes* species in Minnesota is expanding, (6) with *Ixodes scapularis* frequently found in eastern central Minnesota and occasionally in the southeastern part of the state (7). These rapid increases in tick populations are introducing *Borrelia* to new geographical areas and among primary reservoirs such as the white-footed mouse (*Peromyscus leucopus*), shrews (*Blarina brevicauda* and *Sorex cinereus*), and chipmunks (*Tamias striatus*) in Minnesota (8). The infected nymphs are the most important stage for *B. burgdorferi* transmission to humans (9) while dogs are considered to be infected primarily by adult ticks (10).

---

* E-mail: aneela_nadeem8@hotmail.com

In human borreliosis there is a development of a characteristic rash at the tick bite site. This rash is often accompanied by flu-like symptoms (11) and, if the infection is left untreated, the spirochetes can then cause additional complications, such as arthritis, carditis, or neurologic disease. By contrast, skin rash is not known to occur in dogs. Rather, the illness becomes apparent only after the spirochetes have disseminated and caused a combination of polyarthritis, fever, anorexia, and/or lymphadenopathy with glomerulonephritis syndrome, referred to as 'Lyme nephropathy' (12). A significant correlation between the distribution of canine seropositivity and human cases has been reported from both endemic and non-endemic areas in the northeastern United States (13), while in coastal Massachusetts the prevalence of *B. burgdorferi* was significantly greater in dogs (74%) than in people (approximately 30%) (14).

Previous studies have shown that indirect fluorescent antibody (IFA) tests detect antibodies as early as 7 days after exposure to *B. burgdorferi* and no cross-reactivity with antibodies against other infectious agents has been reported (15). Geographic seroprevalence in dogs may be useful in predicting increased risk of exposure to *Borrelia burgdorferi* in humans. This retrospective study was conducted to determine the serological prevalence of *B. burgdorferi* antibodies in dogs in Minnesota.

Serum samples of dogs submitted to the Veterinary Diagnostic Laboratory at University of Minnesota over a 10-year period (2001-2010) were included in this study. A total of 1229 serum samples of dogs were submitted by private veterinary clinics in Minnesota for the detection of *Borrelia burgdorferi* antibodies.

Dilutions of serum samples (1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280) were prepared in phosphate buffered saline and 20 mL of each dilution was transferred to Lyme antigen-coated wells on a slide purchased commercially (Fuller Laboratories, Fullerton, CA, USA). Both positive and negative control sera were included on each slide and 2 samples were tested on each slide. The slides were incubated in a humid chamber for 30 min at 37 °C followed by soaking in PBS for 10 min. The slides were then rinsed with distilled water and dried. Next,

FITC labeled anti-equine IgG (H+L) antibody (KPL, Gaithersburg, MD, USA) was added at a dilution of 1:40 and the slides were re-incubated for 30 min at 37 °C. After another wash, the slides were overlaid with 1 drop of glycerol-based fluorescent mounting medium (KPL, Gaithersburg, MD, USA). A cover slip was then applied and the slides were examined under fluorescent illumination (16,17). When no fluorescence was observed in the 1:40 well, the result was reported as <40. When fluorescence was observed in all dilutions to 1:280, the result was reported as >280. Samples having a titer of up to 1:80 were considered negative. Samples with a titer of 1:160 along with a report of symptoms were considered "suspected" while titers of 1:160 without reported symptoms were considered to be insignificant. Titers from 1:320-1:1280 were reported as positive. For the test to be considered valid, the positive control wells had to be positive at the expected value. The data available from the Minnesota Department of Health for the past 10 years were also analyzed.

Of the 1229 samples, 1081 were positive for antibodies to *B. burgdorferi* at titers of ≥1:320 (Table). The highest test positive rate (100%) was recorded from 2006 to 2008 while the lowest (62.5%) was in 2001. From January to June 2010, 92.3% of the samples were positive for *B. burgdorferi* antibodies. In general, the proportion of positive samples continued to increase each year.

During the present study, the yearly seroprevalence of antibodies recorded in Minnesota was higher than when compared with previous records of serological surveys from 1979 to 1988 in dogs from endemic areas of Minnesota (18.7%) (18), Massachusetts (20.3%) (19), Oklahoma (18%) (20), New York (49.2%) (21), Connecticut (66.5%) (22), and Wisconsin (53%) (23). Serological surveys in dogs from non-endemic areas had even lower seroprevalence. These rates included, for example, 0.6% in Michigan, 0.4% to 2.3% in North Carolina, and 2.3% in California (24).

The increase in the rate of serological prevalence of *Borrelia* antibodies in dogs during the past 10 years also suggests the spread of *Ixodes* ticks into different geographical areas of Minnesota. Walker et al. have also reported that the natural foci of Lyme borreliosis existed in New England, Wisconsin, Minnesota, northern California, and parts of the southeastern

Table. The seroprevalence rate of *Borrelia burgdorferi* antibodies in suspected dogs in Minnesota from 2001 to June 2010.

Year	Canine test positive			Confirmed human cases*	% Increase or decrease in human cases**
	Total	Positive	Positive percentage		
2001	272	170	62.5	461	Same as in year 2000
2002	199	198	99.5	900	88%↑***
2003	374	335	89.6	475	90%↓****
2004	104	104	100.0	1023	↑
2005	76	72	94.7	918	10%↓
2006	79	79	100.0	913	Same as in year 2005
2007	42	42	100.0	1239	36%↑
2008	43	43	100.0	1050	15%↓
2009	27	26	96.3		
2010	13	12	92.3		
Total	1229	1081	88.0		

*, ** reported by Minnesota Department of Health.
*** Increase
**** Decrease

United States. In 1985 and 1986, studies showed that 10% of the ticks in the eastern central part of the state of Minnesota were infected with *Borrelia* spp. (25).

In the present study, IFA tests were used for the detection of *B. burgdorferi* antibodies. −IFA tests have been widely employed in diagnostics and epidemiological surveys in humans as well as in wild and domestic animals, including dogs (26,27). In the present study, an IFA titer endpoint of ≥1:320 was considered to be positive, comparable to previously reported titer results for *B. burgdorferi* in dogs that ranged from 1:64 to 1:256 (27), 1:128 (28), 1:32 (29), 1:256 and 1:512 (21), 1:640 (30), ≥1:128 (31), and ≥1:64 (23). Rodgers et al. (32) reported on the consistent pattern of titers of antibodies of *Borrelia burgdorferi*.

Previous studies have suggested that dogs are quite sensitive indicators of the presence of ticks infected with *B. burgdorfeni*. In Minnesota, the endemic areas for ticks coincide with the primary endemic areas for Lyme borreliosis in humans. Similar reports have been made earlier; in the northeastern US, for example, 11.6% of dogs harbored specific antibodies to *B. burgdorferi* and prevalence was highest (>40%) in the areas where human illness was most common (33). The importance of serological surveillance of borreliosis in dogs can be highlighted by the results of previous studies, which have suggested that determining the risk of borreliosis in humans and dogs with serological surveillance is a more effective method when compared with the case reporting method. Serological testing from other host animals and the surveillance of vector ticks in areas of low tick density are other effective methods (34). In the present study, the data on human Lyme disease from the Minnesota Health Department are comparable with the rate of seroprevalence of *Borrelia burgdorferi* antibodies in dogs.

Acknowledgements

References

1. Lindenmayer, J., Weber, M., Bryant, J., Marquez, E., Onderdonk, A.: Comparison of indirect immunofluorescent antibody assay, enzyme-linked immunosorbent assay, and western immunoblot for the diagnosis of Lyme disease in dogs. J. Clin. Microbiol., 1990; 28: 92-96.

2. Hartmann, K., Greene, C.: Diseases caused by systemic bacterial infections. In: Ettinger S.J., Feldman E.C., Eds. Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat. 6th edn., Elsevier, St. Louis. 2005; 619-625.

3. Hilton, E., DeVoti, J., Benach, J.L., Halluska, M.L., White, D.J., Paxton, H., Dumler, J.S.: Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the northeast United States. Am. J. Med., 1999; 106: 404-409.

4. Warner, R.D., Marsh, W.W.: Rocky Mountain spotted fever. J. Am. Vet. Med. Assoc., 2002; 221: 1413-1417.

5. Bacon, R.M., Kugeler, K.J., Mead, P.S.: Surveillance for Lyme disease – United States, 1992-2006. MMWR Surveill. Summ., 2008; 57: 1-9.

6. Davis, J.P., Schell, W.L., Amundson, T.E., Godsey, M.S., Spielman, A., Burgdorfer, W., Barbour, A., LaVenture, M., Kaslow, R.A.: Lyme disease in Wisconsin: Epidemiologic, clinical, serologic and entomologic findings. Yale J. Biol. Med., 1984; 57: 685-696.

7. Drew, M.L., Loken, K.I., Bey, R.F., Swiggum, R.D.: *Ixodes dammini*: Occurrence and prevalence of infection with *Borrelia* spp. in Minnesota. J. Wildlife Dis., 1988; 24: 708-710.

8. Paddock, C.D., Yabsley, M.J.: Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum* associated zoonoses in the United States. Curr. Top. Microbiol. Immunol., 2007; 315: 289-324.

9. Diuk-Wasser, M.A., Gatewood, A.G., Roberto Cortinas, M., Yaremych-Hamer, S., Tsao, J., Kitron, U., Hickling, G., Brownstein, J.S., Walker, E., Piesman, J., Fish, D.: Spatiotemporal patterns of host seeking *Ixodes scapularis* nymphs (Acari: Ixodidae) in the United States. J. Med. Entomol., 2006; 43: 166-176.

10. Brisson, D., Dykhuizen, D.E., Ostfeld, R.S.: Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic. Proc. R. Soc. Biol. Sci., 2008; 275: 227-235.

11. Levy, S.A., Lissman, B.A., Ficke, C.M.: Performance of a Borrelia burgdorferi bacterin in bon-eliosis-endemic areas. J. Am. Vet. Med. Assoc., 1993; 202: 1834-1838.

12. Littman, M.P., Goldstein, R.E., Labato, M.A., Lappin, M.R., Moore, G.E.: ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. J. Vet. Inten. Med., 2006; 20: 422-434.

13. Daniels, T.J., Fish, D., Levine, J.F., Greco, M.A., Eaton, A.T., Padgett, P.J., LaPointe, D.A.: Canine exposure to *Borrelia burgdorferi* and prevalence of *Ixodes dammini* (Acari: Ixodidae) on deer as a measure of Lyme disease risk in the northeastern United States. J. Med. Entomol., 1993; 30: 171-178.

14. Eng, T.R., Wilson, M.L., Spielman, A., Lastavica, C.C.: Greater risk of *Borrelia burgdorferi* infection in dogs than in people. J. Infect. Dis., 1988; 158: 1410-1411.

15. Chambers, M.A., Swango, L.J., Wright, J.C.: Novel indirect fluorescent antibody test for Lyme disease. J. Vet. Diag. Invest., 1996; 8: 196-201.

16. Hansen, M.G.B., Christoffersen, M.C., Thuesen, L.R., Petersen, M.R., Bojesen, A.M.: Seroprevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in Danish horses. Acta. Vet. Scand., 2010; 52: 321-327.

17. Thieking, A., Goyal, S.M., Bey, R.: Seroprevalence of Lyme disease in gray wolves from Minnesota and Wisconsin. J. Wildl. Dis., 1992; 28: 177-182.

18. Goldstein, R.E., Cordner, A.P., Sandler, J.L., Bellohusen, B.A., Erb, H.N.: Macroalbuminuria and comparison of serologic testing for exposure to *Borrelia burgdorferi* in nonclinical Labrador and Golden Retrievers. J. Vet. Diag. Invest., 2007; 19: 294-297.

19. Lindenmayer, J.M., Marshall, D., Onderdonk, A.B.: Dogs as sentinels for Lyme disease in Massachusetts. Amer. J. Pub. Health, 1994; 81: 1430-1456.

20. Greene, C.E., Straubinger, R.K.: Borreliosis In: Greene, C.E., Ed. Infectious Diseases of the Dog and Cat, 3rd edn., Saunders Elsevier, Philadelphia. 2006; 417-436.

21. Purych, D.B., Perry, I.L., Bulawka, D.: Isolation of *Borrelia burgdorferi*, the Lyme disease spirochete, from rabbit ticks, *Haemaphysalis leporispalustris* — Alberta. J. Spiro. Tick-borne Dis., 1998; 2: 23-4.

22. Joppert, A.M., Hagiwara, M.K., Yoshinari, N.H.: *Borrelia burgdorferi* antibodies in dogs from Cotia County, São Paulo state, Brazil. Rev. Inst. Med. Treo. S. Paulo., 2001; 43: 251-255.

23. Burgess, E.C.: Natural exposure of Wisconsin dogs to the Lyme disease spirochete. Lab. Anim. Sci., 1998; 36: 288-290.

24. Hamer, S.A., Tsao, J.I., Walker, E.D., Mansfield, L.S., Foster, E.S., Hickling, G.J.: Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. Am. J. Vet. Res., 2003; 70: 49-56.

25. Walker, D.H., Barbour, A.G., Oliver, J.H., Lane, R.S., Dumler, J.S., Dennis, D.T., Persing, D.H., Azad, A.F., McSweegan, E.: Emerging bacterial zoonotic and vector-borne diseases ecological and epidemiological factors. J. Am. Med. Assoc., 1996; 275: 463-469.

26. Magnarelli, L.A., Meegan, J.M., Anderson, J.F., Chappell, W.A.: Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. J. Clini. Microbiol., 1984; 20: 181-184.

27. Wieler, L.H., Szattelberger, C., Weiss, R., Bauerfeind, R., Kutzer, P., Failing, K., Baljer, G.: Serum antibodies against particular antigens of *Borrelia burgdorferi* sensu stricto and their potential in the diagnosis of canine Lyme borreliosis. Berl .Munch. Tierarztl. Wochenschr., 1999; 112: 465-471.

28. Olson, P.E., Kallen, A.J., Bjorneby, J.M., Creek, J.G.: Canines as sentinels for Lyme disease in San Diego County, California. J. Vet. Diag. Invest., 2000; 12: 126-129.

29. Artsob, H., Barker, I.K., Fister, R., Sephton, G., Dick, D., Lynch, J.A., Key, D.: Serological studies on the infection of dogs in Ontario with *Borrelia burgdorferi*, the etiological agent of Lyme disease. Can. Vet. J., 1993; 34: 543-548.

30. Kybicova, K., Schanilec, P., Hulinska, D., Uherkova, L., Kurzova, Z., Spejchalova, S.: Detection of Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato in dogs in the Czech Republic. Vector Borne Zoon. Dis., 2009; 9: 456-468.

31. Cohen, N.D., Carter, C.N., Thomas, M.A., Angulo, A.B., Eugster, A.K.: Clinical and epizootiologic characteristics of dogs seropositive for *Borrelia burgdorferi* in Texas: 110 cases (1988). J. Am. Vet. Med. Assoc., 1988; 197: 893-898.

32. Rodgers, S.J., Morton, R.J., Baldwin, C.A.: A serological survey of *Ehrlichia canis*, *Ehrlichia equi*, *Rickettsia rickettsii*, and *Borrelia burgdorferi* in dogs in Oklahoma. J. Vet. Diag. Invest., 1989; 1: 154-159.

33. Bowman, D., Little, S.E., Lorentzen, L., Shields, J., Sullivan, M.P., Carlin, E.P.: Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. Vet. Parasitol., 2009; 160: 138-148.

34. Hamer, S.A., Tsao, J.I., Walker, E.D., Mansfield, L.S., Foster, E.S., Hickling, G.J.: Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. Am. J. Vet. Res., 2009; 70: 30-56.