The Journal of Animal & Plant Sciences, 21(1): 2011, Page: 8-11
ISSN: 1018-7081

SEROPREVALENCE OF *BORRELIA BURGDORFERI* IN HORSES IN MINNESOTA

A. Z. Durrani and S. M. Goyal*

Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan.
*College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota 55108, USA
Corresponding author (email:aneela_nadeem8@hotmail.com).

ABSTRACT

To determine the seroprevalence of *Borrelia burgdorferi* in horses in Minnesota, the database of the Veterinary Diagnostic Laboratory, University of Minnesota, was searched over a ten year period (2001- May 2010). A total of 1,260 equine serum samples submitted by 112 veterinary clinics were tested using an indirect fluorescent antibody test. Samples with titers of ≥1:320 were considered positive. The average rate of seroprevalence was 58.7% indicating high exposure of horses to *B. burgdorferi* in Minnesota. Our results indicate that Borreliosis should be considered as a differential in cases of horses with undiagnosed musculoskeletal or neurologic disease.

Key words: Lyme borreliosis, Horses, *Borrelia burgdorferi,* IFA test.

INTRODUCTION

Lyme disease is the most prevalent tick-borne zoonosis in Europe and America (Korenberg *et al*., 1993). It is a multisystemic disease caused by the spirochete *Borrelia burgdorferi* and has been reported in humans and animals including dogs, cattle, and horses (Blowey *et al*., 1994; Weaver, 1997). In horses, borreliosis was first reported in 1978 in South Africa and was serologically confirmed from the Baltics to the Far East by the beginning of 1992 (Masuzawa, 2004). The rate of seropositivity in horses in Europe ranges from very low to 68% (Goossens *et al*., 2001), which is higher than in Wisconsin (63%) (Salinas-Melendez, 2005) and Texas (0.2%) (Cohen *et al*., 1992) but lower than in Connecticut (84%) (Salinas-Melendez, 2005).

Minnesota provides a suitable habitat for several tick species particularly *Ixodes scapularis*, which is a competent vector for *B. burgdorferi* (Magnarelli *et al*., 1988). Urine from infected cattle (Burgess *et al*., 1986), horses (Tara *et al*., 1998) and white-footed mice (David *et al*., 1996) is considered as a source of infection for animals. Transmission of disease from a seropositive, subclinical horse to a human by means of a bite was reported in 1987 from Belgium (Malloy *et al*., 1990) but this route of transmission needs to be confirmed and further studied.

The counties in northcentral, eastcentral, southeastern and northwestern Minnesota are at a high risk for tick exposure due to the presence of woodlands and forests. The risk of borreliosis from these ticks is the highest during spring, early summer, and fall months. Larval ticks hatch from eggs and become infected with *B. burgdorferi* after feeding on infected rodents. The larval ticks then transmit the infection to new hosts during a subsequent blood meal (Wormser *et al*., 2006). Rodents, especially the white-footed mouse, serve as the natural wildlife host of the ticks.

Nymphs feed on a variety of wild and domestic species, including rodents, horses, cats, birds and even humans. Deer are the predominant wildlife host for adult ticks but domestic animals such as horses, cattle and even cats may serve as substitute hosts (Piesman and Eisen, 2008). Horses living in endemic regions may become infected from adult *Ixodes* ticks (Anthony *et al*., 1991).

Only 10% of the seropositive horses are reported to develop clinical signs (Bushmick, 1994) including lameness with or without joint swelling (Post, 1990). Less frequently, laminitis, uveitis, abortion, weight loss, encephalitis, foal mortality, neurological signs and blindness have been reported as a consequence of Lyme disease (Egenvall *et al*., 2001). Clinical diagnosis is particularly difficult in horses because lameness can also occur as a part of other musculoskeletal disorders (Madigan, 1993). This retrospective study was conducted to determine the serological prevalence of *B. burgdorferi* in Minnesota horses.

MATERIALS AND METHODS

Source of Samples: Serum samples from horses submitted to the Veterinary Diagnostic Laboratory at the University of Minnesota over a ten year period (2001-May 2010) were included in this study. During this period, a total of 1,260 equine samples from 112 veterinary clinics were obtained and tested.

Indirect Fluorescent Antibody Test: Serial dilutions of serum samples (1:40, 1:80, 1:160, 1:320, 1:640 and 1:1,280) were prepared in phosphate buffered saline (PBS) and 20µl of each dilution was transferred to Lyme antigen coated wells on a slide (Fuller Laboratories, Fullerton, CA). Both positive and negative controls were

included. The slides were incubated in a humid chamber for 30 min at 37^0C followed by soaking in PBS for 10 min. After rinsing in distilled water and drying, FITC labeled anti-equine IgG (H+L) antibody (KPL, Gaithersburg, MD) at a dilution of 1:40 was added and slides re-incubated for 30 min at 37^0C. After another wash, the slides were overlaid with one drop of glycerol-based fluorescent mounting medium (KPL Gaithersburg, MD). A cover slip was applied and the slides examined under epifluorescent illumination (Thieking *et al*., 1992; Marie *et al*., 2005). The highest dilution of serum showing fluorescing spirochetes' was considered to be the end point and samples with titers of $\geq$1:320 were considered positive.

RESULTS AND DISCUSSION

Of the 1,260 samples, 739 (58.7%) were positive for antibodies to *B. burgdorferi* at titers of $\geq$1:320 (Table 1). The highest prevalence (93.9%) was recorded in 2009 while the lowest (21%) was in 2001. From January to May of 2010, 92% of the samples were positive for *B. burgdorferi* antibodies. In general, the proportion of positive samples continued to increase each year. Similar trends have been reported in previous studies. For example, a serosurvey in northeastern USA showed 45.1% seropositive horses in 2001 (Magnarelli and Fikrig, 2005) as compared to 14-24% in earlier studies (Bushmick, 1994; Magnarelli *et al*., 1988).

The rate of seroprevalence reported in our study is higher than the 33% reported in France (Laurent *et al*., 2009), 6.8% in Sweden (Egenvall *et al*., 2001), 16% in Berlin (Käsbohrer and Schönberg, 1990), 34% in Mexico, and 6% in Turkey (Bhide *et al*., 2008). However, our seroprevalence rates are similar to those reported in Austria (60-90%; Müller *et al*., 2002); Wisconsin (63%) and Connecticut (84%; Salinas-Melendez, 2005); and New Jersey (60%; Cohen *et al*., 1986). It should be noted that samples in our study came from animals that had an underlying disease problem; hence higher rate of seroprevalence.

The risk of Lyme borreliosis in Minnesota may be attributed to the existence of favorable habitat for ticks. David *et al*. (1996) observed that natural foci of Lyme borreliosis existed in New England, Wisconsin, Minnesota, northern California and parts of the southeastern United States. The enzootic transmission cycles with different species of *Ixodid* ticks (I. *dammini, I. scapularis and I. pacificus*) and mammalian reservoirs show that the number and spread of ticks and infection with *B. burgdorferi* have increased in endemic areas of Lyme disease (David *et al*., 1996). These findings have supported the present study, which showed an increasing trend of seroprevalence (21% in 2001 compared with 93.9% in 2009).

During the present study, IFA test was used for the detection of *B. burgdorferi* antibodies. Serology by IFA test is a widely employed tool in diagnostics and epidemiological surveys (Littman *et al*, 2006) and has been used for the detection of *B. burgdorferi* antibodies in horses (Magnarelli *et al*., 1988 and 2005). The sensitivity of IFA has been reported to be 96% with 89% specificity (Egenvall *et al*., 2001). In the present study, titers of $\geq$1:320 were considered as positive. In previous studies, many different cut-off points have been used for this test. For example, IFA titers of 1:128 (Käsbohrer and Schönberg 1990), 1:120 (Post, 1990), 1:140 (Magnarelli *et al*., 1988), and 1:256 (Marcelis and Marneffee, 1987; Cohan *et al*, 1986) have been used to indicate positivity. Egenvall *et al*. (2001) reported average titers of 1:103 (range 1:80 to 1:320) in healthy horses as compared to 1:107 (range 1:80 to 1:1,280) in clinical cases. We used 1:320 as a conservative cut-off for indicating infection.

Table 1: Seroprevalence of Lyme borreliosis in horses in Minnesota from 2001- May 2010.

Year	Number of serum samples examined	Number positive	Per cent positive
2001	167	35	21.0
2002	52	43	82.7
2003	88	69	78.4
2004	138	89	64.5
2005	386	167	43.3
2006	121	98	81.0
2007	116	67	57.8
2008	87	73	83.9
2009	66	62	93.9
2010	39	36	92.3
Total	1260	739	58.7

It was suggested by Dzierzecka and Kita (2002) that interpretation of signs and serologic test results should be made with consideration of the regional prevalence as well as the animal's opportunity for exposure to infected *Ixodes* spp. We conclude that horses in Minnesota are exposed to *B. burgdorferi* and this exposure has increased over the years. Although this is a serological study, which may or may not indicate active infection, it is prudent for veterinarians to be cognizant of the increased spread of this disease along with dispersal to a variety of domestic animals including horses. It is also advisable to include Lyme disease as a differential diagnosis in cases of undiagnosed musculoskeletal or neurologic disease. With the current status of borreliosis in horses, it is clear that maintaining epidemiological data is necessary for effective control and prevention of *B. burgdorferi* in horses in Minnesota.

Acknowledgement: We thank the Higher Education Commission of Pakistan for providing a post doctoral fellowship to principal author.

REFERENCES

Anthony, C., T. Michael, P. Murtaugh, F., Russell, and L.L.Keith (1991). Ilo-kilodalton recombinant protein which is immunoreactive with sera from humans, dogs, and horses with Lyme borreliosis. J. Clin. Microbiol. 29: 2418-2423.

Bhide, M., M. Zeki, G., Esin, T., Serhat, and M. Ivan (2008). Seroprevalence of anti-*Borrelia burgdorferi* antibodies in dogs and horses in Turkey. Ann. Agric. Environ. Med. 15: 85–90.

Blowey, R., S. D. Carter, A. G. White and A. Barne (1994). *Borrelia burgdorferi* infection in UK cattle, a possible association with digital dermatitis. Vet. Rec. 135: 577-578.

Burges, E. C., D.Gillette and J. P. Pickett (1986). Arthritis and panuveitis as manifestations of *Borrelia burgdorferi* infection in a Wisconsin pony. J. Am. Vet. Med. Assoc. 189: 1340-1342.

Buschmich, S. L. (1994). Lyme borreliosis in domestic animals. J. Spirochet. Tick-borne Dis. 1: 24-28.

Cohen, N. D., C. Edward, M. Bosler, E. Robert and L. S. Terry (1986). Lyme disease in horses. Annals New York Acad. Sci. 45: 245-257.

Cohen, N. D., F. C. Heck, B. Heim, D. M. Flad and E. M. Bosler (1992). Seroprevalence of antibodies to *Borrelia burgdorferi* in a population of horses in central Texas. J. Am. Vet. Med. Assoc. 201: 1030-1034.

David, H. W., A. G. Barbour, H. O. James, S. L. Robert, D. Stephen, T. D. David, H. P. David, F. A. Abdu and M. S. Edward (1996). Emerging bacterial zoonotic and vector-borne diseases ecological and epidemiological factors. J. Am. Med. Assoc. 275:463-469.

Dzierzecka, M., and J. Kita (2002). The use of chosen serological diagnostic methods in Lyme disease in horses. Part II. Western blot. Pol. J. Vet. Sci. 5: 79-84.

Egenvall, A., P. Franzen, A. Gunnarsson, E. O. Engvall, I. Vagsholm, U. B. Wikstrom and K. Artursson (2001). Cross-sectional study of the seroprevalence to *Borrelia burgdorferi* sensu lato and granulocytic Ehrlichia spp., demographic, clinical and tick-exposure factors in Swedish horses. Prev. Vet. Med. 49: 191-208.

Goossens, H. A. T., A. E. B. Van Den and M. K. E Nohlmans (2001). Dogs as sentinels for human Lyme borreliosis in the Netherlands. J. Clin. Microbiol. 39: 844-848.

Käsbohrer, A and A. Schönberg (1990). Serologic studies of the occurrence of *Borrelia burgdorferi* in domestic animals in Berlin (west). Berl. munch. Tierärztl. wochenschr. 103: 374–378.

Korenberg, E. I., V. N. Kryuchechnikov and Y. V. Kovalevsky (1993). Advances in investigations of Lyme borreliosis in the territory of the former USSR. Eur. J. Epidemiol. 9: 86-91.

Laurent, M., M. Jean-lou, A. Olivier, C. Cline, G. Slim, C. Daniel and D. Bernard (2010). Seroprevalence survey of equine lyme borreliosis in France and in sub-Saharan Africa. Vectorborne and Zoonotic Dis. 10: 535-537.

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

Madigan, J. E. (1993). Lyme disease (lyme borreliosis) in horses. Vet. Clin. N. Am.: Equine Pract. 9, 429-434.

Magnarelli, L. A., J. F. Anderson, E. Shaw, J. E. Pos, and F. C. Palka (1988). Borreliosis in equids in northeastern United States. Am. J. Vet. Res. 49: 359-362.

Magnarelli L. and E. Fikrig (2005). Detection of antibodies to *Borrelia burgdorferi* in naturally infected horses in the USA by enzyme-linked immunosorbent assay using whole-cell and recombinant antigens. Res. Vet. Sci. 79: 99-103.

Malloy, D. C., R. K. Nauman and H. Paxton (1990). Detection of *Borrelia burgdorferi* using the polymerase reaction. J. Clin. Microbiol. 28: 1089–1093.

Marcelis, P., and D. Marneffee (1987). Horse reservoir for *Borrelia burgdorferi*. Lancet l: 977.

Marie, G. B., M. Hansen, R. Line, M. Thuesen, R. Petersen and M.B. Anders (2005). Seroprevalence of *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in Danish horses. Acta Vet. Scand. 52: 321-327.

Masuzawa, T. (2004). Terrestrial distribution of the Lyme borreliosis agent *Borrelia burgdorferi* sensu lato in East Asia. Jpn. J. Infect. Dis. 57: 229-235.

Müller, I., G. Khanakah, M. Kundi and G. Stanek (2002). Horses and Borrelia: immunoblot patterns with five *Borrelia burgdorferi sensu lato* strains and sera from horses of various stud farms in Austria and from the Spanish Riding School in Vienna. Int. J. Med. Microbiol. 291: 80-87.

Piesman, J. and L. Eisen (2008). Prevention of tick-borne diseases. Annu. Rev. Entomol. 53: 171-172.

Post, J. E. (1990). Lyme disease in large animals. N. J. Med. 87: 575-577.

Salinas-Me´lendez, J. A., G. Galva´n de la, V. M. Riojas-Valde´s, A. Wong Gonza´lez and R. Avalos-Ramı´rez (2005). Antibody detection against *Borrelia burgdorferi* in horses located in the

suburban areas of Monterrey, Nuevo Leo´n. Rev. Latinoam. Microbiol. 43: 161–164.

Tara, B. M., I. K. Mazhar, D. James and L. B. Sandra (1998). Viable *Borrelia burgdorferi* in the urine of two clinically normal horses. J. Vet. Diagn. Invest. 10: 196–199.

Thieking, A., S. M. Goyal, R. F. Bey, K. L. Loken, D. L. Mech, R. P. Thiel, and T. P. O'Connor (1992). Seroprevalence of Lyme disease in gray wolves from Minnesota and Wisconsin. J. Wildl. Dis. 28: 177-182.

Weaver, A.D (1997). Joint conditions. In: Greenough PR (ed.): Lameness in Cattle: 162-180. 3rd ed. W.B. Saunders, Philadelphia.

Wormser, G. P., R. J. Dattwyler and E. D. Shapiro (2006). The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. Clin. Infec. Dis. 43: 1089-1134.