The Journal of Animal & Plant Sciences, 21(3): 2011, Page: 481-484
ISSN: 1018-7081

RETROSPECTIVE STUDY ON SEROPREVALENCE OF *BORRELIA BURGDORFERI* IN ELK AND MOOSE IN MINNESOTA

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ABSTRACT

The database of the Veterinary Diagnostic Laboratory, University of Minnesota, was searched over a ten year period (2001-2010) to determine the seroprevalence of *Borrelia burgdorferi* (*B. burgdorferi)* in elk (*Cervus elaphus)* and moose *(Alces americanus*) in Minnesota, A total of 597 serum samples (62 from elk and 535 from moose) were tested for antibodies against *B. burgdorferi* using an indirect fluorescent antibody (IFA) test. Samples with titers of $\geq$1:320 were considered positive. Out of 597 sera tested, 135 (27%) were positive with IFA titers ranging between 1:320 and 1:1280. The rate of sero-prevalence was 52in elk and 19% in moose. These results indicate exposure of both species of cervidae to *B. burgdorferi* in Minnesota.

Key words: Lyme disease, elk, moose, *Borrelia burgdorferi,* serologic survey.

INTRODUCTION

Lyme borreliosis was first described in Europe as 'erythema chronicum migrans' (ECM) or 'Bannwarth's syndrome (Thomas *et al.*, 2008). In the U.S., the disease was first reported in 1975 in Lyme, Connecticut (Main *et al.*, 1982). The detection of *Borrelia* DNA in tissues of a white-footed mouse captured in Massachusetts in 1894 indicates that Lyme disease may have existed in wildlife for over a century (Aguirree, 2009). Certain environmental factors have increased its prevalence and improved testing capabilities along with awareness have led to increased reporting of the disease. (Louis *et al.*, 1984).

Lyme disease is transmitted in animals by several closely related *Ixodid* ticks that are part of the *Ixodes ricinus* complex and include *I. scapularis* in the northeastern and midwestern United States The prevalence of *Borrelia* positive *Ixodes* ticks is highly variable in different geographical areas and 35% of ticks in Baraboo Hills in Wisconsin and 50% in New York were found infected (Thomas *et al.*, 2001). In contrast, only 2% of the ticks in California were infected.

In the northeastern and midwestern United States, the preferred host for the larval and nymphal stages of *I. scapularis* is the white-footed mouse, *Peromyscus leucopus* (Levine *et al.*, 1985) while that for adult tick is the white-tailed deer, *Odocoileus virginianus* (Wilson *et al.*, 1986). Although deer are not involved in the life cycle of the spirochete, they seem to be critical for survival of the ticks (Wilson *et al.*1988). Eighty percent of 317 deer sampled at three Minnesota locations with established *Ixodes scapularis* populations and only 5% of 150 deer from three non-tick locations were positive for lyme disease antibodies through ELIZA test (Gill *et al.,*(1994).

In Minnesota, Lyme-endemic areas are expanding (Anne *et al.*, 2009) because of abundance of recreational parks, campgrounds, and nature preserves. The average summer temperatures in Minnesota range from 19^0C to 22^0C (Ross, 2010), which may favor the development and transmission of *B. burgdorferi*. Pronounced regional differences in the sero-prevalence of *B. burgdorferi* are recorded in Minnesota. These variations in white-tailed deer have been demonstrated based on habitat suitability for *Ixodes* species (Gill *et al.*, 1994).

B. burgdorferi infection has been documented in several wildlife species including gray wolves (Kazmierczak *et al.*, 1988), deer (Lane *et al.*, 1991), red foxes (Heidrich *et al.*, 1999), squirrels (Salkeld *et al.*, 2008). In domestic animals, the disease has been documented in dog, cats (Mishra *et al.*, 2007) and cattle and horses (Bhide *et al.*, 2005). There are no published reports on the detection of antibody against *B. burgdorferi* in elk and moose which are predominantly found in Minnesota. The present review was undertaken to determine the serologic prevalence of *B. burgdorferi* antibodies in elk and moose populations in Minnesota.

MATERIALS AND METHODS

Case Selection: The database of the Veterinary Diagnostic Laboratory at University of Minnesota was searched over a ten year period (2001 to April 2010). Over this period, 62 samples from elk and 535 from moose were received from private farms and Minnesota

Department of Natural Resources for the detection of Lyme disease antibodies.

Serodiagnosis through Indirect Fluorescent Antibody Test: Serum samples were screened at dilutions of 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280. The dilutions were made in PBS and 20µl of each dilution was transferred on individual Lyme antigen coated wells on glass slides (Fuller Laboratories, Fullerton, CA). Both positive and negative controls were included with each set of slides. The slides were incubated in a humid chamber for 30 min at 37^0C followed by soaking in PBS for 10 min. After rinsing with distilled water, the slides were dried. Fluorescein isothiocyanate labeled anti-deer IgG (H+L) antibody (KPL, Gaithersburg, MD) at 1:40 dilution was then added to all wells and slides re-incubated for 30 min at 37^0C. The slides were then washed and overlaid with one drop of glycerol-based fluorescent mounting medium (KPL Gaithersburg, MD). After application of a cover glass, the slides were immediately examined under epifluorescent illumination (Thieking *et al.*, 1992). The highest dilution of serum showing fluorescing spirochetes was considered to be the end point. Samples with titers of ≥1:320 were considered positive for Lyme antibody (Maria *et al.*, 2005).

RESULTS AND DISCUSSION

Of the 597 samples, 135 were found to be positive for antibodies to *B. burgdorferi* at IFA titers of ≥1:320 (Table 1). Of the 62 elk sera, 31 (50%) showed titers of ≥1:320 while 104 of 535 (19%) moose were positive at these titers. For elks, the test positive rate was the highest in 2003 and 2007 (100%) and lowest in 2008(33%) while in 2009 and 2010 the test positive rates were 51 and 55 % respectively. In moose, the highest test positive rate was 96% in 2010 (and lowest in 2008 (2%) while in 2003, 2007 and 2009 it was 22, 20 and 17% respectively. No samples were received from 2004 to 2006.

Since no study is available in the literature on the prevalence of Lyme disease in elk and moose, we cannot compare our results with previous studies. However, it is evident that the rate of Borrelia seroprevalence in Minnesota elk approaches that of white-tailed deer (Gill *et al.*, 1994). Recent reports show an increase in moose mortality in the Arrowhead Region of Minnesota throughout the year. Blood samples from moose captured during the study showed that these animals tested positive to Lyme disease antibody and not to any other agent including the brain worms (Shawn, 2008).

Wild *Cervidae* contribute to the risk of Lyme disease mainly by supporting the population of vector ticks (Louis *et al.*, 1984). The proliferation of cervids, the preferred host of the adult tick, is a major factor in the emergence of epidemic Lyme disease and also considered a contributory factor for epedemics in Minnesota (Piesman *et al.*, 1979). Thieking *et al.* (1992) reported 29% prevalence of *B. burgdorferi* in white tailed deer during 2000-03 in Wisconsin and Minnesota. Close correlation between the distribution of infected ticks and the presence of seropositive dogs, deer, rabbits, and squirrels has been reported in south-central and eastern Connecticut (Magnarelli *et al.*, 1988).

The role of domestic animals and larger species of wildlife such as bear, coyote, lynx, bobcat, moose, elk and fox as reservoirs of *B. burgdorferi* has not been evaluated carefully so far (Lane *et al.*, 1991). In Norway, a marked rise in the density of tick population and in the incidence of Lyme borreliosis was cited to be due to distribution and population densities of host animals such as the roe deer and the European elk (Nygard *et al.*, 2005). The data under study shows an average seropositivity of 52% for *Borrelia* antibody in elk and 19% in moose. These results indicate that both elk and moose are susceptible to infection and may be a source of transmitting the spirochete to ticks (Kathleen *et al.*, 2003). However, this observation needs further confirmation.

Table 1: Prevalence of *Borrelia burgdorferi* antibody in elk and moose sera from 2003- April 2010

Year	ELK			Moose		
	Number of samples examined	Number positive at 1:320 -1:1280	Per cent positive at >1:320	Number of samples examined	Number Positive at 1:320 -1:1280	Per cent positive at >1:320
2003	1	0	100	115	23	20
2004	0	0	0	0	0	0
2005	0	0	0	0	0	0
2006	0	0	0	0	0	0
2007	1	1	100	112	25	22
2008	3	1	33	113	2	2
2009	37	19	51	168	28	17
2010	20	11	55	27	26	96
Total	62	32	52	535	104	19

In gray wolves, IFA titers of $\geq$1:100 are generally considered positive (Thieking *et al.*, 1992). We considered IFA titers of $\geq$1:320 as positive in this study. Further studies on the prevalence of *B. burgdorferi* in wild and domestic animal populations will help define the pattern of spread of Lyme disease including information on the range where *Ixodes* complex ticks are most commonly found and the animals on which they feed and breed. For the *Ixodes* complex ticks to be maintained within an area and to spread to new areas, they need large free roaming animals as hosts (Lane *et al.*, 1991). The presence of elk and moose in an area assures a new generation of ticks and more chances for spread of Lyme borreliosis. Our results indicate that the contribution of elk and moose to Lyme borreliosis epidemiology should be recognized and evaluated.

Acknowledgement: The paper was a part of post doctoral fellowship of first author there fore we thank the Higher Education Commission of Pakistan for providing funding for the fellowship.

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