Journal of Animal and Veterinary Advances 10 (20): 2720-2723, 2011
ISSN: 1680-5593

Prevalence of Antibodies Against *Borrelia burgdorferi* in Dogs from Monterrey, Mexico

[1]J.A. Salinas-Melendez, [1]J.J. Zarate-Ramos, [1]R. Avalos-Ramirez, [1]J.J. Hernandez-Escareno, [1]G. Guzman-Acosta, [1]V.M. Riojas-Valdes and [2]J.C. Segura-Correa
[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Nuevo Leon, Francisco Villa Street, Ciencias Agropecuarias Campus, 66050 Escobedo, Nuevo Leon, Mexico
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Yucatan, Km 15.5 Carretera Merida-X'matkuil, Apdo, Postal 4-116 Itzimna, 97100 Merida, Yucatan, Mexico

Abstract: The goal of the present epidemiological research was the detection of anti-*Borrelia burgdorferi* antibodies in order to determine the presence and prevalence of Borreliosis in dogs from the city of Monterrey, Nuevo Leon, Mexico. A total of 391 animals were included. Detection of antibodies against Lyme disease was performed using a commercial kit. Observed prevalence was 1%. This result allowed the conclusion that seropositive animals for the bacteria *Borrelia burgdorferi* do exist in Monterrey. Serological evidence was found in animals at South of the city limits indicating the presence of the disease in the locality.

Key words: Borreliosis, lyme, dogs, monterrey, children, infection

INTRODUCTION

Lyme disease or borreliosis is one of the most common tick borne zoonosis (Appel, 1990; Guy and Stanek, 1991; Schnarr *et al*., 1998). The infection has been described in Europe >100 years ago and in America was first reported when a rheumatoid arthritis outbreak happen in children from the Lyme county at Connecticut, USA (Hubbard *et al*., 1998). Afterwards, it was determined that deer ticks (*Ixodes dammini*), infected with *Borrelia burgdorferi* were responsible of the disease (Bowman *et al*., 2009; Greene, 1990; Wodecka *et al*., 2009). The clinical signs in dogs were first diagnosed in 1984 (Greene, 1990; Kornblatt *et al*., 1985; Lissman *et al*., 1984). Spirochetes are classified into two families: Spirochataceae and Leptospiraceae. Spirochataceae family has four genuses and two of them, Borrelia and Treponema are known agents causing disease in animals and humans (Holt *et al*., 1993).

Borrelia species do not survive in the environment. They must be linked with hosts and are transmitted between vertebrate intermediate hosts and hematophage arthropod vectors (Bhide *et al*., 2008). In order to transmission ocurr, it is necessary for the tick to be on the host for at least 36-48 h, time in which the microorganism, already present in the ectoparasite, multiplies and goes through the intestinal epithelium towards the hemolimph. From there, it spreads to the salivary glands and infects the host by the saliva. It is probable for *Borrelia burgdorferi* to proliferate at the inoculation site during the whole time of infection (Greene, 2006).

The bacteria later diffuse by the blood and lymphatic routes towards the central and periphery nervous system, joints, hearth, spleen, liver and kidneys, generally producing inflammatory lesions (Gustafson *et al*., 1993). Not all infected animals after the tick bite show the clinical disease (Greene, 2006).

There are vertical or transplacenta transmission as well as iatrogenic by blood transfusions and contaminated hypodermic needles; the spirochete can be seen in some body fluids such as urine, blood and colostrums of infected animals (Greene, 2006; Gustafson *et al*., 1993).

Once inside the body, *B. burgorferi* acts as a persistent parasite. Clinical signs found in dogs can be summarized in three phases; acute form, lasting 7-21 days in which the animals shows fever (39.5-40.5°C), joint inflammation, lethargy, polyartritis, lameness and lymphadenomegalia; subclinical form with a duration of 1-3 years and the animals does not show clinical

Corresponding Author: J.A. Salinas-Melendez, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Nuevo Leon, Francisco Villa Street, Ciencias Agropecuarias Campus, 66050 Escobedo, Nuevo Leon, Mexico

signs and chronic form, in which serious symptoms appear such as non-erosive arthritis, general pain, progressive acute renal failure, neurological syndrome, cardiac arrhythmia, myocarditis and peripheral edema (Appel *et al*., 1993; Kaufman *et al*., 1993). *Borrelia burgdorferi* is hard to keep in growth cultures, since it needs special media (Barbour-Stoenner-Kelly) and long incubation periods of up to 3 weeks (Mouristen *et al*., 1996). For this reason attention has been placed in using serological tests such as ELISA (Enzyme-Linked Immunoabsorbent Assay), indirect immunofluoresence (IFI) and Western Blot as confirmatory tests for its diagnosis. Currently, polymerase chain reaction is considered a very useful and specific tool for the detection of *B. burgdorferi* in blood, urine, synovial fluid and cephaloraquideum fluid and biopsies of affected organs (Bauerfeind *et al*., 1998; Malloy *et al*., 1990; Salinas *et al*., 1999; Salinas *et al*., 2001; Sheets *et al*., 2000). The most common tests used for diagnosis are serological. In Mexico, there are reports about the disease existence in both domestic and wild animals as well as in humans at several states, some related with prevalence studies and others only with the disease presence. Even at the state of Nuevo Leon, the presence of the disease has been reported however, no prevalence studies exists. Therefore, the objective of this study was to estimate the prevalence of Lyme disease in dogs from Monterrey by determining antibodies against *Borrelia burgdorferi* based in a quadrant sampling.

MATERIALS AND METHODS

Animal population: Blood samples were obtained from 391 dogs of various breeds at different areas of the city of Monterrey, using as inclusion factor only animals with fixed address, age >6 months known owner and in apparently healthy state. As exclusion factors were considered an age <6 months; furthermore, it was decided to sample only one animal per house in case of having >1 dog. The examination of the dogs started with physical evaluation followed by blood sampling. All dogs showed no symptoms of any disease.

Localization and climate: This study was carried out at the city of Monterrey, Nuevo Leon located in the Northeast of Mexico with a territorial extension of 451.30 km^2. Location coordinates are 25°40'17" N, 100° 18'31"W. Altitude is 530 m above sea level.

The climate of the region has an average of 21°C but because of annual thermal oscillation of 18°C with important contrast among seasons. In summer time, temperatures >30°C are common with an average in July and August of 34°C. In Winter, cold air arrive constantly to the region often accompanied of humidity from the coast, making the temperature descend drastically and every year at least 2-3 days are recorded with 0°C or less. The average annual precipitation is of 600 mL spread mainly in summer with September as the rainiest month.

Study design and sample taking: The city was divided in quadrants in accordance with its cartographic plan. From this map, the 15 most urbanized quadrants were chosen, since the others belonged to non well developed neighborhoods and few human population. Sampling was performed according the dog population density and owner cooperation, sampling only one animal per city block and only one animal per house.

Sample size: To determine the sample size, calculations were made in basis of the population's representative sample (infinite) with precision level of 5%, confidence level of 95% and test strength of 80% in order to ensure reliability of the results and that they could be translated to the population under study using a 16% prevalence, according to previous studies in the country. Sample size was determined using Epidat 3.1. Blood was extracted from the jugular vein with Vacutainer vacuum tubes and sterile needles, drawing close to 5 mL from each animal. No anesthetic or tranquilizer was used. The samples were carried in a container with refrigerant material to the laboratory and kept al 4°C until they were centrifugated at 3000 rpm for 5 min to separate the serum, after which were processed to determine the presence of antibodies against Lyme disease.

Diagnosis procedure: For the *in vitro* diagnosis for detection of antibodies against *Borrelia burgdorferi* in the samples a commercial kit canine SNAP*4D×(IDEXX labs, Inc. USA) was used.

Before starting the procedure, samples must be at room temperature. The sera, either fresh or refrigerated was utilized after no more than week from the sampling. Sensibility and specificity of the kit for the disease are reported with a minimum of 98.8 and 100%, respectively.

RESULTS AND DISCUSSION

Results obtained in the present study show that only four animals had a positive result (Table 1). Two of these animals were located in the South area of the city. Regarding the breed of the positive dogs, two belongs to a mix-breed, one was English Bulldog and other was Boxer. In this sense, due to the low level of prevalence observed (1%), it was not possible to make a relationship

Table 1: Prevalence of antibodies against *Borrelia burgdorferi* in dogs from Monterrey, Nuevo Leon, Mexico

Sex	No. of animals sampled	Positives	(%)
Females	218	2	0.9
Males	173	2	1.1
Total	391	4	1.0

with a risk factor. Several studies exist aimed to proving the presence of *B. burgdorferi* in canines from the metropolitan area of Monterrey, Mexico, using PCR test and IFI (Indirect Immunofluorescence). In one of this studies performed in 1995 by PCR, primer pairs designed for the detection of a specific portion a 224 base pairs DNA coding the 16S rRNA gen from the variable V4 region of *B. burgdorferi*, a sample of synovial fluid of an arthritic dog was found to be positive (Salinas *et al*., 1995).

In a second research samples from 850 dogs located in the metropolitan area of Monterrey were analized serologically (IFI) for the detection of antibodies against *B. burgdorferi* and 16% (Salinas *et al*., 1999). Other studies performed at the same area but in different animal species (horses and deer) showed also the presence of the disease (Martinez *et al*., 1999; Salinas *et al*., 2001). These results have shown that exposition to the microorganism is common and its prevalence is minimal at the region.

Prevalence observed in this study is <1 found in the year 1991 (16%) in dogs by immunofluoresence and is also lower than the prevalence informed by recent studies at other parts of the country such as Mexicali, Baja California were a 12% prevalence was reported using the ELISA technique (Salinas *et al*., 1999; Barreras *et al*., 2009).

The low prevalence observed could be due to the fact that Lyme disease has lately taken importance among the people and veterinarians have taken conscience about the importance of the disease that affects both humans and animals which has leaded to preventive sanitary actions for the animals such as the more often planning of anti-tick baths and fumigation of areas used for pets, aimed to the interruption of the tick's biological cycle main transmitter of the disease.

CONCLUSION

The present research confirms that presence of the aetealogical agent in dogs is still present despite what was studied above. Therefore, researchers estimate that it will be convenient the realization of this kind of studies in order to determine future changes that could mean a danger of both human and animal health.

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