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Seroprevalence of Leptospirosis in Beef Cattle of Nuevo Leon, Mexico

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Abstract: A serological study for several serovars of *Leptospira* sp. was undertaken on a randomly selected population of beef cattle in 22 municipalities of Nuevo Leon, Mexico. Blood samples were collected from 1400 animals and sera were tested for antibodies against 12 serovars of *Leptospira* sp. (*bratislava*, *canicola*, *gryppotyphosa*, *hardjo*, *hebdomadis*, *icterohaemohrragiae*, *panama*, *pomona*, *pyrogenes*, *shermani*, *tarassovi*, *wolffi*) using the microscopic agglutination test. Antibodies against one or more serovars were detected in 646 sera (46%) of the 1383 samples tested. The most prevalent serovars detected were *hardjo* (19.8%), *wolffi* (18.6%) and tarassovi (6.5%).

Key words: Beef cattle, leptospirosis, mexico, serovars

INTRODUCTION

Leptospirosis is an important economic disease in many countries around the world and constitutes a public health risk (Espi *et al*., 2000). It is a zoonotic disease caused by the spirochete, *Leptospira interrogans*, occurring in humans and in a wide variety of wild and domestic animals (Alonso-Andicoberry *et al*., 2001; Hamir *et al*., 2001; Adler *et al*., 2002). According to the traditional classification system, strains of *Leptospira interrogans* are divided into serogroups and these in serovars; at least 212 serovars belonging to 23 serogroups are recognized (Kmety and Dikken, 1988; Guitian *et al*., 2001). The epidemiology of the disease for a specific region and domestic species could be summarized as a high frequency infection by the adapted serovar and a low frequency of infection by serovars that are adapted to other species, the so-called accidental infections (Guitian *et al*., 2001). Leptospirosis is primarily a disease of wild and domestic animals and humans are infected through contact with infected urine (Faine, 1995; Thornley, 2002). Transmission of leptospirosis can be direct or indirect. Direct mechanism is essential for adapted serovars while indirect mechanisms are crucial for accidental infections caused by non-adapted serovars (Gitton *et al*., 1994; Martinez *et al*., 1999; Alonso-Andicoberry *et al*., 2001; Guitian *et al*., 2001). Small animals are like mice are the main sources of leptospirosis (Adler *et al*., 2002). *Leptospira* sp. can enter the body skin through abraded skin or mucous membranes (Adler *et al*., 2002). The contamination occurs after contact with water and soil containing urine of infected rats and other animals (Bovet *et al*., 1999). Occupational exposure workers like those in the sewer and abattoir services, veterinarians and farmers are at high risk of leptospirosis and this disease has also been reported to be associated with recreational activities (Teichmann *et al*., 2001; Adler *et al*., 2002). *Leptospira interrogans* serovar *hardjo* is one of the causal agents of disease and abortion in humans (Godinez *et al*., 1999).

Although many serovars of this bacterium have been described, the infection on the animals is usually produced by endemic serovars closely linked to ecological and environmental factors (Alonso-Andicoberry *et al*., 2001). The infection in cattle has been classified into two etiological groups: one due to strains adapted to and maintained by cattle (*Leptospira interrogans* serovar *hardjo*) and a second group of incidental infections caused by strains maintained by other domestic and wild animals (Ellis, 1994; Alonso-Andicoberry *et al*., 2001). In tropical countries the second group appears to cause more frequent problems, due to environmental and farming conditions (Lilienbaum and Santos, 1996).

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Leptospira hardjo is considered to be the serovar maintained by cattle, but infections by other serovars like *L. pomona* and *L. grippotyphosa* have also been associated with losses on beef and dairy cattle (Guitian *et al*., 2001). The animals affected with leptospirosis may present a variety of signs and symptoms that causes important losses because of the effects on production performance (Alonso-Andicoberry *et al*., 2001). *Leptospira interrogans* serovar *hardjo* infection in cattle has been associated with various clinical manifestations including abortion, stillbirth, infertility and agalactia, birth of weak calves (Guitian *et al*., 1999; Guitian *et al*., 2001). The animals infected with leptospirosis may continue to shed the organism for its entire life (Colagross-Schouten *et al*., 2002). It may serve as a source of infection to other member of the same species, creating more maintenance hosts (Colagross-Schouten *et al*., 2002).

In Mexico few studies exist concerning seroprevalence of leptospirosis disease in cattle. Luna-Alvarez *et al*. (2005) reported prevalences from 22-84.6% in 16 states of Mexico. In Europe, it has been observed that prevalences of leptospirosis in cattle ranges from 2.8% in France to 34% in Great Britain (Alonso-Andicoberry *et al*., 2001).

The present study was carried out in order to evaluate the frequency of 12 serovars of *Leptospira* in beef cattle from 22 municipalities of Nuevo Leon, Mexico.

MATERIALS AND METHODS

Location and climate: The state of Nuevo Leon is located in the northern of Mexico, between 27° 49' - 23° 11' N and 98° 26' and 101° 14' W. The climate is dry with monthly average temperature of 26°; relative humidity of 70% and annual rainfall from 200-800 mm.

A seroprevalence study was carried out in different farms from 22 municipalities of Nuevo Leon, México, from April 2000-April 2001. The municipalities selected were considered to be representative of beef cattle production in the region. Cattle were of several beef-breeds and its crosses and were managed mainly as pasture-based herds (extensive system). Ten percent of the animals older than 1 year of each selected ranch were considered for this study.

Serum samples: Blood samples were collected from the coccigeal vein of each animal using Vacutainer® tubes containing separator gel. Samples were centrifuged, aliquoted and stored at -20°C until the serological test for antibodies against *Leptospira* sp. was performed (Meri *et al*., 1995; Sunyballi *et al*., 1997). Sera were inactivated at 60°C for 30 min. Seventeen samples were hemolized and were not tested. Animals in the herds were not vaccinated with any commercial vaccine containing Leptospira serovars.

All samples used in this study were referred to the Regional Central Laboratory (Laboratorio Central Regional) of Monterrey, belonging to the Comite para el Fomento y Proteccion Pecuaria del Estado de Nuevo Leon, A.C., for leptospiral serology. Serological test was carried out using the Microscopic Agglutination Test (MAT) in microtitre plates according to standard methodology (Meri *et al*., 1995; Sunyballi *et al*., 1997; Winslow *et al*., 1997). Live antigens of 12 pathogenic serovars of *Leptospira* sp. (*bratislava, canicola, gryppotyphosa, hardjo, hebdomadis, icterohaemo-hrragiae, panama, pomona, pyrogenes, shermani, tarassovi, wolffi*) were used. Sera were initially tested at 1:25, 1:50 and 1:100 dilutions against each of the 12 serovars. Dilutions of the sera were made in 0.01M PBS. MAT was performing by incubating the sera at 30°C for 60 min with suspensions of live organisms of each *Leptospira* strain (Segura-Correa *et al*., 2003).

All sera for which MAT titer were = 100 against one or more of the 12 *Leptospira interrogans* serovars were considered to have positive results (Meri *et al*., 1995). The MAT titer was the reciprocal of the highest dilution of the serum in which = 50% of the antigen was agglutinated. In all cases positive and negative control sera were used.

Statistical analysis: The overall serological frequency was calculated as the number of animals with positive reaction between the animals tested. For each serovar, the serological frequency was calculated as the percentage of animals with a positive reaction against any specific serovar. The municipalities were clustered into three regions (northern, central and southern region) for comparison of presence of antibodies against *Leptospira* sp.

RESULTS AND DISCUSSION

Antibodies against one or more serovars of *Leptospira* sp. were detected in 646 (46%) samples of the 1383 sera tested. Table 1 shows the number of municipalities with at least one seropositive animal and the relative frequencies for each serovar. The most frequent serovars detected in the animals were *hardjo* (19.8%), *wolffi* (18.6%) and *tarassovi* (6.5%). All animals were negative to Leptospira serovars *gryppotyphosa*, *panama*, *pomona*, *pyrogenes* and *shermani*.

The results of this study demonstrated high leptospiral reactor rates among cattle in Nuevo Leon, Mexico. This serological response probably reflects

Table 1: Municipality and individual seroprevalences for different serovars of *Leptospira* sp. in beef cattle in the state of Nuevo Leon, Mexico

	Seroprevalence			
	Municipalities (n = 22)		Animals (n=1383)	
Serovar	Positives	(%)	Positives	(%)
Bratislava	2	9.1	2	0.14
Canicola	5	22.7	6	0.43
Gryppotyphosa	0	0.0	0	0.0
Hardjo	14	63.6	274	19.80
Hebdomadis	1	4.5	2	0.14
Icterohaemohrragiae	7	31.8	15	1.08
Panama	0	0.0	0	0.0
Pomona	0	0.0	0	0.0
Pyrogenes	0	0.0	0	0.0
Shermani	0	0.0	0	0.0
Tarassovi	12	54.5	90	6.51
Wolffi	13	59.1	257	18.58

natural exposure because vaccination of cattle against leptospiral serovars was not practiced in Nuevo Leon. As in the present study, antibodies against multiple leptospiral serovars have frequently been identified in individual animals. The leptospirosis seroprevalence found in this study (46%) is higher than that reported in an earlier study in the region (37.8%) (Luna-Alvárez *et al*., 2005). However, is lower than the seroprevalence estimated in beef cattle in Yucatan, Mexico (Segura-Correa *et al*., 2003), in which the percentage of positive animals was 62.8%. Seroprevalences reported in other regions of Mexico varies from 39.4-63.8% (Luna-Alvárez *et al*., 2005). On the other hand, our results show a higher serological frequency than that informed in other countries, in which the seroprevalences varied from 7-18.3% (Alonso-Andicoberry *et al*., 2001; Guitian *et al*., 2001; Aslantas and Ozdemir, 2005). Leptospirosis transmission is influenced by climatic factors like temperature and humidity, which allow the bacteria to survive out of the host, favoring in this way the indirect transmission. This may explain the differences in seroprevalences among municipalities and regions of Nuevo Leon.

A literature review in Mexico (Luna-Alvárez *et al*., 2005) stated that the most common serovars are *hardjo*, *wolffi and tarassovi* which agree with the results of the present study. Similarly, Ellis (1994) reported that the most frequent serovars were *hardjo*, *wolffi*, *bratislava* and *pomona*. *Hardjo* is usually the most-prevalent serovar in cattle, since it is adapted to this specie (Lilienbaum and Santos, 1996; Alonso-Andicoberry *et al*., 2001; Guitian *et al*., 2001). However, in some regions of Spain, the more prevalent serovars seems to be the serovars *pomona* and *grippotyphosa* (Espi *et al*., 2000). A study in humans in Mexico showed a higher seroprevalence for the serovars *shermani* (53%), *canicola* (33%), *pyrogens* (20%), pomona (13%) which indicates that Leptospira serovars infecting human and cattle are different. The identification of the most frequent serovars can be used to made vaccines appropriated for each region or country.

CONCLUSION

The results of this study indicate that Leptospirosis is widespread in Nuevo Leon, Mexico and that the most common serovars are L. *hardjo* and *wolffi*.

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