The Thai Journal of Veterinary Medicine

Volume 42
Issue 1 *March, 2012*

Article 17

3-1-2012

Seroprevalence and Risk Factors associated with Leptospirosis (L. interrogans) in Bovine Cattle in Northeastern Mexico

Verónica Carvajal-de la Fuente

Cecilia Zapata-Campos

Jorge Loredo-Osti

Rigoberto López-Zavala

José Osiel Jasso-Obregón

See next page for additional authors

Seroprevalence and Risk Factors associated with Leptospirosis (L. interrogans) in Bovine Cattle in Northeastern Mexico

Authors

Verónica Carvajal-de la Fuente, Cecilia Zapata-Campos, Jorge Loredo-Osti, Rigoberto López-Zavala, José Osiel Jasso-Obregón, and Edgar Martínez-Bautista

Original Article

Seroprevalence and Risk Factors associated with Leptospirosis (*L. interrogans*) in Bovine Cattle in Northeastern Mexico

Verónica Carvajal-de la Fuente* Cecilia Zapata-Campos Jorge Loredo-Osti Rigoberto López-Zavala José Osiel Jasso-Obregón Edgar Martínez-Bautista

Abstract

The seroprevalence of *Leptospira interrogans* serovars in cattle was determined by analyzing 385 serum samples from 8 Rural Development Districts (RDD), in Tamaulipas, Mexico. A microscopic agglutination test (MAT) including 9 serovars of *L. interrogans* as antigens was used. Serum samples were considered positive when 50% or more of agglutination in a dilution of ≥ l:100 was observed. A total of 70.4% animals were reported positive for one or more serovars. The most frequent antibodies reported were those against serovars *tarassovi* (53.25%) *hardjo* (23.64%) and *canicola* (15.32%), whilst serovar *bataviae* (0.52%) was found at lower frequency. Mante district showed the highest (100%) seroprevalence followed by Laredo (87.7%) and González (76.4%), while in San Fernando district, a lower seroprevalence was noted (47.9%) with statistically significant differences between RDD´s (p<0.05). In order to identify possible risk factors related to leptospirosis, survey questionnaires were administered to herd owners. Breed and the presence of domestic and wild animals that consumed placenta and aborted fetuses were the only variables associated with the seroprevalence of leptospirosis.

Keywords: cattle, MAT, *Leptospira interrogans*, seroprevalence, serovars

Facultad de Medicina Veterinaria y Zootecnia (FMVZ) Universidad Autónoma de Tamaulipas Km 5 Carr. Cd Victoria a Mante, Cd. Victoria, Tamaulipas, México CP 87000
***Corresponding author: E-mail:** vcarvajal@uat.edu.mx*

บทคัดย่อ

ปัจจัยที่มีต่อความชุกและความเสี่ยงของโรค (*L. interrogans*) ในโคทางภาคตะวันออกเฉียงเหนือของเม็กซิโก

Verónica Carvajal-de la Fuente Cecilia Zapata-Campos Jorge Loredo-Osti*
Rigoberto López-Zavala José Osiel Jasso-Obregón Edgar Matínez-Bautista

ศึกษาความชุกของ serovars *Leptospira interrogans* ในวัว โดยวิเคราะห์ตัวอย่างจำนวน 385 ตัวอย่างจาก 8 เขตชนบท (RDD), ตาเมาลีปัส ในเม็กซิโก โดยวิธี microagglutination test (MAT) ใช้แอนติเจนของ *L. interrogans* ทั้งหมด 9 ซีโรวาร์ ตัวอย่างที่ให้ผลบวก เมื่อการตกตะกอนเกิดขึ้น 50% หรือมากกว่า เมื่อใช้ตัวอย่างเจือจางในอัตราส่วน ≥ 1:100 พบว่าสัตว์ร้อยละ70.4 ให้ผลทดสอบเป็นบวกอย่างน้อยหนึ่งซีโรวาร์ แอนติบอดีที่พบบ่อยที่สุด คือ ต่อซีโรวาร์ *tarassovi* (53.25%) *hardjo* (23.64%) และ *canicola* (15.32%) ขณะที่ซีโรวาร์ *bataviae* (0.52%) พบได้น้อย และพบว่าเขต Mante พบความชุก (100%) สูงสุดตามด้วยเขต Laredo (87.7%) และเขต González (76.4%) ในขณะที่เขต San Fernando พบน้อยกว่า (47.9%) และมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ระหว่าง RDD เพื่อระบุปัจจัยเสี่ยงที่เกี่ยวข้องกับโรค จากการสำรวจด้วยแบบสอบถามจากเจ้าของ พบว่าสายพันธุ์และการมีสัตว์เลี้ยงและสัตว์ป่าที่กินรกและตัวอ่อนที่แท้งเป็นตัวแปรที่สัมพันธ์กับความชุกของโรค

คำสำคัญ: โค MAT, *Leptospira interrogans*, ความชุก, serovars

Facultad de Medicina Veterinaria y Zootecnia (FMVZ). Universidad Autónoma de Tamaulipas. Km 5 Carr. Cd. Victoria a Mante, Cd. Victoria, Tamaulipas, México CP 87000
***ผู้รับผิดชอบบทความ E-mail:** vcarvajal@uat.edu.mx*

Introduction

Leptospirosis is an infectious disease caused by spirochetes of the genus *Leptospira* that are capable of infecting a large variety of domestic and wild mammals (Evangelista and Coburn, 2010). Initially, two species were recognized, *L. interrogans* (pathogenic) and *L. biflexa* (saprophytic) (World Health Organization, 2003). Recent DNA studies prove that at least 12 pathogenic and 4 saprophyte species exist. These species are divided in more than 250 serovars distributed in 24 serogroups (Alder and de la Peña, 2010). However, for diagnostic and epidemiologic purposes, the antigenic classification is still used (Palaniappan et al., 2007). Leptospirosis is considered one of the major zoonosis world-wide, widespread mainly in countries with subtropical or tropical climates since *Leptospira* grows best in warm and humid conditions (Vijayachari et al., 2008). This disease is responsible for significant economic losses to the livestock production, largely due to negative impacts on reproductive functions (abortion, embryonic death, stillbirths and infertility), decreased milk production and growth rates, as well as indirect costs associated with treatments (Ellis, 1994). However, it is complicated to estimate the real economic impact due to infected animals that often have no clinical signs of the diseases. In Mexico, leptospirosis has been an important issue of study in diverse regions, especially from an epidemiological point of view. In this regard, it has been reported that cattle herds from at least 17 Mexican states, have been exposed to this bacterium, revealing high seroprevalence rates (up to 49.7%) (Luna et al., 2005). Particularly, in Tamaulipas, information about accurate prevalence and epidemiological distribution of this disease is unknown. The present study was therefore carried out to establish the seroprevalence and risk factors associated with leptospirosis in cattle from Tamaulipas, in order to know which the most affected districts are, and the most frequent serovars involved.

Materials and Methods

Area of study: The state of Tamaulipas lies in the northeast corner of Mexico between 22º 13´ and 27º 40´ N and 97º 09´ and 99º 58´ W. Climate can vary considerably from humid to semi-dry depending on the region. According to SAGARPA's information (Mexican Agricultural Department), Tamaulipas State is divided into 9 Rural Development Districts (RDD) (Figure 1); however, only 8 RDD were included in this study due to external circumstances.

Figure 1 Map of Tamaulipas, México showing the 8 Rural Development Districts from which cattle serum samples were obtained.

Sample collection: To estimate seroprevalence, the total cattle population of Tamaulipas was taken into account (985, 896 heads) (INEGI, 2007). A sample size for proportions was used (Daniel, 1995) at confidence intervals of 95% with maximum margin of error 5% and hypothetical prevalence expected of 50%. From this calculation, the sample size was 385; they were distributed to obtain representative samples, in proportion to the cattle-head number in the different districts. Cattle were selected by systematic random sampling. Blood samples were collected from cattle of reproductive age (both sexes). All included animals were not previously vaccinated against *Leptospira*.

In order to identify possible risk factors related to leptospirosis, survey questionnaires were administered to herd owners. These documents collected information on the farmer's management and health practices, socioeconomic factors, feeding, water supply, contact with dogs, rodents and other wild animals, as well as individual animal data (breed, sex and age).

Blood samples were collected from the coccygeal vein with Vacutainer® red-stopper tubes. The samples were identified and transported on ice (less than four hours) to the Diagnostic Laboratory (Facultad de Medicina Veterinaria de la Universidad Autónoma de Tamaulipas). The blood samples were centrifuged at 2,500 rpm for 10 min and the serum was transferred into disposable microcentrifuge tubes (Eppendorf®) and stored at –20^{0}C until analysis was performed.

Microscopic agglutination test: Sera samples were processed using a microscopic agglutination test (MAT) which has a sensitivity of 98.2% and a specificity of 96.4% (Bajani et al., 2003). A panel of 9 serovars (*L. bataviae, L. canícola, L. hardjo, L. grippothyphosa, L. icterohaemorrhagiae, L. pyrogenes, L. pomona, L. tarassovi, L. wolffi*), representing the most frequent serogroups of Leptospira in Mexico, was used (Luna et al., 2005). Sera were considered positive when 50% or more *Leptospira* were agglutinated with one or more serovars in a ≥ 1:100 serum dilution as observed by dark-field microscopy (OIE, 2008).

Data analysis: Data and all variables (including variables form the questionnaires) of tested animals were analyzed through descriptive statistics. Because some variables evaluated were qualitative, proportions and rates were obtained, and thus, the frequency of animals according to sex, age, and breed was established. Additionally, chi-square and odds ratio tests were used to establish the association between each of the variables studied and the presence of antibodies against *Leptospira*. A p value < 0.05 was considered statistically significant. The data were analyzed using the statistical program EPI INFO (Ver. 3.5.3).

Table 1 Frequency distribution of Leptospira seroprevalence according to Rural Development Districts (RDD), age and breed.

		Positive		Negative		Total	
		n	%	n	%	n	%
RDD	Laredo	48	85.7	8	14.3	56	100
	Matamoros	5	50.0	5	50.0	10	100
	San Fernando	23	47.9	25	52.1	48	100
	Abasolo	46	63.9	26	36.1	72	100
	Victoria	35	62.5	21	37.5	56	100
	Jaumave	7	63.6	4	36.4	11	100
	Mante	26	100.0	0	0.0	26	100
	Gonzalez	81	76.4	25	23.6	106	100
Age groups (Months)	0-20	54	68.4	25	31.6	79	100
	21-40	39	66.1	20	33.9	59	100
	41-60	92	69.7	40	30.3	132	100
	61-80	39	79.6	10	20.4	49	100
	81-100	29	82.9	6	17.1	35	100
	101-120	8	72.7	3	27.3	11	100
	More than 120	10	50.0	10	50.0	20	100
Breed	Beef Master	23	48.9	24	51.1	47	100
	Brangus	23	71.9	9	28.1	32	100
	Cross breed	148	74.7	50	25.3	198	100
	Charolais	18	60.0	12	40	30	100
	Holstein	7	70.0	3	30.0	10	100
	Simbrah	25	75.8	8	24.2	33	100
	Swiss	19	95.0	1	5.0	20	100
	Others	8	53.3	7	46.7	15	100
	Total	**271**	**70.4**	**114**	**29.6**	**385**	**100**

Results

Out of 385 serum samples tested, 271 were found positive to at least one serovar of *Leptospira* with an overall seroprevalence rate of 70.4% (Confidence Interval 65.6-75.8%).

Mante was the RDD with the highest seroprevalence (100%), followed by Laredo, González, Abasolo, Jaumave and Victoria with more than 60% of positive cases, while the RDD of Matamoros and San Fernando showed the least seroprevalence (50.0 and 47.9%, respectively). The difference in RDD seroprevalence was statistically significant (p<0.05; Table 1).

Of the 385 animals screened, 347 were females and 38 were males. A prevalence of 71.8% in females and 57.9% in males was recorded. There was no association between *Leptospira* infection and sex (p>0.05). Although no significant differences in antibody seroprevalence were observed among age groups (p>0.05), there was a tendency in the group of 81-100 month old to be more seropositive than those over 120 months of age (Table 1).

Results on breed showed that Swiss cattle had the highest seroprevalence (95%) followed by Simbrah, Brangus, Holstein and Cross breeds (range; 70-75%). On the other hand, Beef Master was the breed that recorded the lowest seropositivity with less than 50%. An association between *Leptospira* infection and breed was found (p<0.05; Table 1). A statistically significant difference was also found for the presence of domestic and wild animals that consumed placenta and aborted fetuses (OR = 5.76, 95% CI = 3.54 to 9.36, p<0.05).

Of all positive animals, the highest frequency was for the serovar t*arassovi* (205/385, 53.2%), followed by *hardjo* (91/385, 23.6%), *canicola* (59/385, 15.3%) and *pomona* (53/385, 13.7%). On the other hand, only 2 samples (2/385, 0.5%) were found positive for serovar *bataviae*. Positive titers against more than one serovar were detected in 134 sera samples (134/385, 34.8%). Therefore, there were 525 positive reactions against different serovars of *L. interrogans*. The most common serovar pair found in the same animal was for *hardjo* and *tarassovi*, whilst *hardjo*, *tarassovi* and *canicola* were the most common three serovars found in the same animal (Table 2).

Of the 271 positive samples it was found that 16 (5.9%) showed antibody titers equal to or greater than 1:1600, where *tarassovi* registered 7 positive samples with this dilution, followed by *wolfi* (4 samples), *Icterohaemorragiae* (2 samples), *hardjo*, *pomona* and *grippothyphosa* (1 sample).

Table 2 Frequency and seroprevalence for different serovars of *Leptospira* sp.

Serovar	Frequency	Seroprevalence
bataviae	2	0,52
canicola	59	15,32
grippothyphosa	13	3,38
hardjo	91	23,64
icterohaemorragiae	31	8,05
pomona	53	13,77
pyrogenes	25	6,49
tarassovi	205	53,25
wolffi	46	11,95
hardjo + tarassovi	60	15,58
canicola + tarassovi	51	13,25
pomona + tarassovi	38	9,87
wolffi + tarassovi	35	9,09
hardjo+ canicola	27	7,01
hardjo+ pomona	23	5,97
icterohaemorragiae + tarassovi	22	5,71
pyrogenes +tarassovi	19	4,94
tarassovi + hardjo + canicola	27	7,01
tarassovi + canicola + pomona	20	5,19
tarassovi + hardjo + wolffi	19	4,94

Discussion

The results of this investigation demonstrated that cattle farms in the State of Tamaulipas have been exposed to the Leptospirosis with a high seroprevalence rate (70.4%). This percentage was slightly lower than those found in previous studies that have been carried out in Tamaulipas, where a 77.9% was reported (Moles et al., 1997). On the other hand, lower seroprevalence rates (31.2%) were detected by Cantú and Banda in 1995. However, it is important to mention that the latter study was undertaken only in the southern region of Tamaulipas, which does not necessarily reflect the problem in the entire state. In another study, conducted by Salinas et al. (2007) in another state in northeastern Mexico (Nuevo León), they recorded a lower leptospirosis seroprevalence rate (46%). The high seroprevalence rate found in the present study, might partly be due to lack of vaccination programs (vaccination was not practiced in evaluated herds), inappropriate hygiene and sanitation practices for the disposal of aborted fetuses, placenta and vaginal discharges which contain *Leptospira*, thus contaminating other domestic and wild animals which serve as reservoirs of infection to cattle (Millán et al., 2009; Evangelista and Coburn, 2010).

Frequency and distribution of different serovars in cattle depend on several factors such as ecological regions, an increased migration of wildlife, and forms of bovine production (reproduction practices, hygienic measures, introduction of new animals, etc.). In Mexico, for example, a frequency of 37.8% has been reported for arid and semi-arid regions; 45.9% for the dry tropical region; and 63.8% for the humid tropical region. Regarding the predominating serovars recorded in those regions, it was found that strain H-89 (*hardjo* genotype *hardjoprajitno*), *hardjo, wolffi* and *tarassovi* were the most commonly reported but with different frequencies (Cantú et al., 1997; Luna et al., 2005). In the present research, *tarassovi* was the serovar most frequently reported with a seroprevalence of 53.2%. Nevertheless, in a national study, *tarassovi* only showed a seoprevalence of 9.7% and was considered to be the fourth most significant serovar affecting cattle in Mexico (Moles et al., 2002).

Although MAT cannot be utilized to determinate the infecting serovar in a single infection or outbreak, it has been reported that, in an endemic areas a single titer of ≥ 1:1600 or a four-fold rise in

titers between the acute and convalescent samples can be compatible with a positive diagnosis. In a non endemic area an inferior titer is considerable (Bajani et al., 2003; OIE, 2008). In this paper 5.9% of positive sera recorded titers of ≥ 1:1600 suggesting the occurrence of the disease; nevertheless this needs further confirmation.

The fact that a high number of animals had a seropositivity reaction for one or more serovars in this study might be due to animals carrying mixed serovar infection or a serological cross-reactivity between *Leptospira* serovars and serogroups. These reactions are well documented, especially when an early immune response exists, since it is possible to have antibodies against the infecting serovar that cross-react with other serovars, although at a lower level (Levette, 2003; Scanziani et al., 2002). As an alternative, culture isolation can be used in order to establish specific serovars; however, this assay has its own limitations given that this test is laborious and time-consuming (it has to be examined weekly by dark-field microscopy for up to 13 weeks), technically demanding, and in particular, requires laboratories with special safety regulations (Ellis, 1990). Consequently, MAT technique is still considered worldwide as the gold standard test to diagnose leptospirosis (Palaniappan et al., 2007; OIE, 2008).

The results obtained in this research provide important information since current vaccinations rely on knowing which serovars are the most frequently involved in a region. Different serological studies on cattle involve *L. pomona* and *L. hardjo* as the prevalent serovars responsible for bovine leptospirosis in the United States (Ellis, 1986). Following this premise, existing commercial vaccines which protect against these serovars would not be successful in providing immunity against leptospirosis caused by *L. tarassovi* and other serovars reported in the present study, since current vaccines provide protection only against closely related serovars (Deveson et al., 2011). Therefore, using specific vaccines will allow establishing an adequate vaccination programs to control and reduce reproductive problems associated to bovine leptospirosis. In conclusion, cattle from rural districts of Tamaulipas, México, have been exposed to leptospirosis, where *tarassovi, hardjo, canicola* and *pomona* are the most frequently involved serovars. However, specific antigen detection tests need to be conducted to investigate if these serovars may have a direct impact on reproductive disorders in this area. Mante district showed the highest (100%) seroprevalence. The presence of domestic and wild animals that consumed placenta and aborted fetuses has been shown to be a risk factor for leptospirosis.

Acknowledgements

The authors would like to acknowledge the FOMIX-Tamaulipas/CONACYT for financial support of this project (TAMPS-2008-C17-107297)

References

Adler, B. and de la Peña, M.A. 2010. Leptospira and leptospirosis. Vet Microbiol. 140: 287-296.

Bajani, M.D., Ashford, D.A., Bragg, S.L., Woods, C.W., Aye, T., Spiegel, R.A., Plikaytis, B.D., Perkins, B.A., Phelan, M., Levett, P.N. and Weyant, R.S. 2003. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol. 41: 803-809.

Cantú, C.A. and Banda- Ruiz, V.M. 1995. Seroprevalence of bovine leptospirosis in three southern cities of Tamaulipas. (Technical note). Tecnica Pecuaria en Mexico. 33: 121-124.

Daniel, W.W. 1995. Biostatistics: A Foundation for Analysis in the Health Sciences. 6th ed. John Wiley & Sons Inc. 205-207.

Deveson, Lucas, D.S., Cullen, P.A., Lo M, Srikram, A., Sermswan, R.W. and Adler, B. 2011. Recombinant LipL32 and LigA from Leptospira are unable to stimulate protective immunity against leptospirosis in the hamster model. Vaccine. 29: 3413-3418.

Ellis, W.A., O'Brien, J.J., Bryson, D.G. and Mackie, D.P. 1985. Bovine leptospirosis: Some clinical feature of serovar *hardjo* infection. Vet Rec. 117: 101-104.

Ellis, W.A. and Thiermann, A.B. 1986. Isolation of leptospiras from the genital tracts of Iowa cows. Am J Vet Res. 47: 1694-1696.

Ellis, W.A. 1990. Leptospirosis. In: OIE Manual of Recommended Diagnostic Techniques and Requirements for Biological Products for List A and B Diseases. Vol 2. Paris, France: Office International des Epizooties. 1-11.

Ellis, W.A. 1994. Leptospirosis as a cause of reproductive failure. Vet Clin North Am. 10: 463-478.

Evangelista, K.V. and Coburn, J. 2010. Leptospira as an emerging pathogen: A review of its biology, pathogenesis and host immune responses. Future Microbiol. 9: 1413-1425.

INEGI. 2007. Statistical Yearbook of the United Mexican States.

Levett, P. 2003. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. Clin Infect Dis. 36: 447-452.

Levett, P. 2006. Leptospirosis. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 6th ed. Philadelphia, PA: Churchill Livingstone. 2495-2500.

Luna, M., Moles, L., Gavaldón, D., Nava, C. and Salazar, F. 2005. Estudio retrospectivo de seroprevalencia de leptospirosis bovina en México considerando las regiones ecológicas. Rev Cub Med Trop. 57: 28-31.

Millán, J., Candela, M.G., López-Bao, J.V., Pereira, M., Jiménez, M.A. and León-Viscaíno, L. 2009. Leptospirosis in wild and domestic carnivores in natural areas in Andalusia, Spain. Vector Borne Zoonotic Dis. 9: 549-54.

Moles, C.L.P., Aguirre, E.J., Gavaldón, R.D. and Torres, B.J. 1997. Analysis of bovine leptospirosis results in five states of central and

northern zones of Mexico. XXI National Congress of Buiatrics of the Mexican Association of Specialist Veterinarians in Bovine. Colima, México.

Moles, C.L.P., Cisneros, M.A., Gavaldon, R.D., Rojas, R.N.S. and Torres, B.J. 2002. Estudio Serológico de Leptospirosis Bovina en México. Revista Cubana Med Trop. 54: 24-27.

Palaniappan, R.U., Ramanujam, S. and Chang, Y.F. 2007. Leptospirosis: Pathogenesis, immunity, and diagnosis. Curr Opin Infect Dis. 20: 284-292.

Salinas, M.J.A., Narvaez, A.C., Riojas, V.V., Cantú, C.A., Avalos, R.R. and Segura, C.J.C. 2007. Seroprevalence of leptospirosis in beef cattle of Nuevo León, México. J Anim Vet Adv. 6: 1265-1268.

Scanziani, E., Origgi, F., Giusti A., Iacchia G., Vasino A., Pirovano, G., Scarpa, P. and Tagliabue, S. 2002. Serological survey of leptospiral infection in kennelled dogs in Italy. J Small Anim Pract. 43: 154-157.

Vijayachari, P., Sugunan, A.P. and Shriram, A.N. 2008. Leptospirosis: An emerging global, public Health problem. J Biosci. 33: 557-569

World Health Organization. 2003. Human Leptospirosis: Guidance for diagnosis, surveillance and control. Geneva: International Leptospirosis Society, 2003 (ISBN 92 4 154589 5; http://whqlibdoc.who.int/hq/2003/WHO_CDS_CSR_EPH_2002.23.pdf).