



SEROPOSITIVITY TO LEPTOSPIROSIS IN DOMESTIC RESERVOIRS AND DETECTION OF *Leptospira spp.* FROM WATER SOURCES, IN FARMS OF YUCATAN, MEXICO.

[SEROPositivIDAD A LEPTOSPIROSIS EN RESERVORIOS DOMÉSTICOS Y DETECCIÓN DE *Leptospira spp.* EN DEPÓSITOS DE AGUA, DE UNIDADES PECUARIAS DE YUCATÁN, MÉXICO]

M. F. Cárdenas-Marrugo^{*a}, I. Vado-Solís^a, C. E. Pérez-Osorio^a,
J. C. Segura-Correa^b

^a Facultad de Medicina, Universidad Autónoma de Yucatán. Av. Itzaes entre 59 y 59-A AP-1225-A, Mérida, Yucatán, México. ^b Campus de Ciencias Biológicas y Agropecuarias. Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán. Km. 15.5 Carretera Mérida-Xmatkuil, AP 4-116, Mérida, Yucatán, México. Email: cmarrugo@uady.mx*, vsolis@uady.mx, cpesorio@uady.mx, scorrea@uady.mx

*Corresponding Author

SUMMARY

Leptospirosis is a zoonotic infectious disease with a worldwide distribution. WHO classifies this disease as reemergent and it represents a risk to human health with economical repercussion to animal reproduction. Leptospirosis occurs with higher frequency in countries with tropical weather. A transversal study was conducted in 35 animal production units to determine the frequency of infection of *L. interrogans* in 476 reservoir animals: 212 bovines, 203 pigs, and 61 dogs. Positivity frequency in the reservoirs was 30.5%. 31 out of 34 animal units had positive reservoirs. The most frequent serovars were Tarassovi (53.6%), and Hardjo (31.6%) in cattle; Bratislava (66%) and Icterohaemorrhagiae (18.7%) in pigs; and Canicola (79.8%) and Icterohaemorrhagiae (9.8%) in dogs. 68 pools of water samples from water tanks were analyzed by DNA amplification of a 16S rRNA fragment for *L. interrogans* detection using Lepat1-Lepat2 primers. It is recommended to use preventive measures such as vaccination to domestic animals to reduce the risk of transmission to the human population.

Key words: Leptospirosis; Prevalence; Domestic reservoirs; Detection in water.

INTRODUCTION

Leptospirosis is an infectious disease caused by *Leptospira interrogans* and it is considered a zoonosis of worldwide distribution (Vinetz, 2001; Faine *et al.*, 1999). The World Health Organization has classified it as a reemergent disease (Meslin, 1997). The disease causes a wide spectrum of clinical manifestations in humans (Plank & Dean, 2000; Zavala *et al.*, 2008),

RESUMEN

La leptospirosis es una zoonosis de amplia distribución mundial, clasificada por la OMS como re-emergente por el compromiso con la salud humana y repercusiones económicas en la reproducción animal. La leptospirosis ocurre con mayor frecuencia en los países con clima tropical, abundantes lluvias y alta temperatura. Se realizó un estudio transversal para evaluar la frecuencia de infección a *L. interrogans* en 476 animales reservorios (212 bovinos, 203 cerdos y 61 perros) en 34 unidades de producción pecuaria. La frecuencia general de reservorios positivos fue 30.5%, distribuidos en 31 de las 34 unidades pecuarias estudiadas. Los serovares más frecuentes fueron Tarassovi (53.6%) y Hardjo (31.6%) en bovinos; Bratislava (66.0%) y Icterohaemorrhagiae (18.7%) en cerdos; y Canícola (79.8%) e Icterohaemorrhagiae (9.8%) en perros. Se analizaron 68 pooles de muestras obtenidas en depósitos de agua, pero ninguno de los pooles presentó amplificación de la fracción Lepat1-Lepat2, que sugiera la presencia de *L. interrogans*. Para reducir el riesgo de contagio a la población humana se recomienda proteger a los animales domésticos mediante medidas preventivas como la vacunación.

Palabras clave: Leptospirosis; Prevalencia; Reservorios domesticos; Detección en agua.

while in domestic animals is cause of economical losses due to the animal reproduction impact (Vinetz, 2001) such as abortous, infertility and death (Thierman, 1995). Leptospirosis occurs more frequently in countries with tropical weather, high rainy seasons, and high temperature (Vinetz, 2001). The leptospires colonize the kidney tubules in several domestic animals such as dogs, cows, pigs, and horses; and wild animal reservoirs as rodents, possums, and

raccoons. They are excreted through the urine and transmitted to humans by direct contact or through contaminated soil, water and feedstuff (Vinetz, 2001, Faine, *et al.*, 1999). However, transmission occurs mainly by contaminated water with animal excreta where leptospires survive for long periods of time (Western, 1982). Leptospirosis was demonstrated in the State of Yucatan, Mexico in 1920 (Noguchi & Klieger, 1920). Since then, many different studies involving human beings and reservoir animals have confirmed its endemicity (Zavala *et al.*, 1984; Vado *et al.*, 2002a; Vado *et al.*, 2002b). The objective of this study was to estimate the seroprevalence of leptospirosis in animal reservoirs and to detect the presence of *Leptospira interrogans* in water deposits of animal production units in the State of Yucatan, Mexico.

MATERIAL AND METHODS

The present study was carried out from august 2004 to december 2005. Samples from cattle, pigs and dogs were collected taking into account the economical activity of the rural communities of Yucatan that is based on cattle and pork raise, considering as well the importance of dogs as domestic reservoirs which are commonly found in the farms and stables as company and surveillance. A sample size of 492 animals was calculated using an expected prevalence of 20%, a confidence level of 95%, and arbitrary design effect of 2. Thirty five animal production units from 35 counties were selected randomly, and each animal production unit was visited once; however, one of the animal production units was missing. The average population size on each unit was 200 animals, of which 14 were sampled (Segura & Honhold, 2000).

Five ml of blood was obtained from each animal as follow: for pigs, blood was collected from the jugular vein; for cattle, the caudal vein was used to collect the blood; and dogs were bled from the cubital vein. Blood samples were kept in refrigeration until they were centrifuged and the serum was separated. Serum was maintained frozen until tested. The Microagglutination Test (MAT) was used for serology and for serovar detection accordingly to WHO and considered as the reference test for the diagnosis of leptospirosis, using as live antigen leptospires of the serovars: Pomona, Canicola, Hardjo, Tarassovi, Panama, Icterohaemorrhagiae, Gryppotyphosa, Pyrogenes, and Bratislava. A title of 1:100 was considered as the cut-off to determine the positivity (Mayers, 1985). When antibodies to two or more serovars were detected, the serovar with the highest titer was considered as the infective serovar (Mayers, 1985).

Fourteen water samples from natural reservoirs – cenotes, dwells, and lagoons, and water deposits from

each animal production units were collected. Sampling was performed collecting 50 ml of superficial waters at a 15 cm depth in sterile containers. The pH was measured ($\text{pH } 7.43 \pm 0.252$) and the samples were kept refrigerated for transportation to the laboratory. At arrival to the laboratory, the samples were filtered in sterile gauze to eliminate coarse residues. Then, 2 pools per animal production unit were formed by mixing 7 samples at random and a total of 68 water samples were obtained. The water samples were analyzed by PCR using a protocol described by Murguía *et al.* (1997). To standardize the test, DNA extractions from pure cultures of several serovars of *L. interrogans* were used as positive controls. The efficiency of the protocol to detect leptospires in water samples was evaluated by contaminating sterile water with pure cultures of *L. interrogans* at different logarithmic concentrations. For the DNA extraction from water samples, they were pretreated by an initial centrifugation of samples with 1ml of 7% LiCl in a Beckman Coulter mod. Avanti J2 at 25,000 rpm for 60 min and at 18°C. The pellet was resuspended in 0.5 ml. DNA was extracted from this suspension using the DNeasy®Tissue kit (QIAGEN, California, E.U.A.) following the instructions from the manufacturer. 5 µl of DNA extraction was mixed with 46 µl of PCRSupermix (Invitrogen, E.U.A.) containing 0.2 µM of each primer (Lepat 1 and Lepat2) as described by Murguía *et al.* (1997). Amplicons were detected in 1.5% agarose gels stained with ethidium bromide and the results were registered in a gel documentation system.

RESULTS

Thirty one of the 34 farms and an equal number of counties had at least one animal positive to *L. interrogans* antibodies (Table 1). A prevalence of 30.5% was observed in the animal reservoirs and positivity per specie is shown in Table 2. As shown, cattle had the highest frequency of antibodies to *L. interrogans* followed by dogs (45.8 and 36% respectively). The serovars Tarassovi (53.6%) and Hardjo (31.6%) were the most frequent in cattle and Canicola and Icterohaemorrhagiae (79.8 and 9.8% respectively) were found more frequently in dogs (Table 3). Pigs had the lowest prevalence (13%) of *L. interrogans* and Bratislava and Icterohaemorrhagiae were the most common serovars (66 and 18.7% respectively). Antibody titers varied among the reservoir species ranging from 1:100 to 1:3200 in cattle, 1:100 to 1600 in dogs, and from 1:100 to 1:400 in pigs.

Table 1. Seroprevalence of *Leptospira spp.* in animals by region, from 34 animal units in the State of Yucatan, Mexico.

Region	Number of Municipalities	Positive Municipalities	Total animals	Seroprevalence in animals
Central	22	19	264	21.21
Eastern	4	4	56	71.42
South	8	8	112	41.07
Total	34	31	476	30.46

The result of the standardization of the PCR was that (with the exception of *Gryppotyphosa* and Panama) all serovars produced an amplicon with the expected size of ~310 bp with the primers Lepat1 and Lepat2. All but the 10⁻⁴ dilution (the lowest dilution) had a positive result from the intentionally contaminated water samples. However, none of the 68 pools obtained from the water samples collected in the field had a positive amplicon of the expected size.

Table 2. Frequency of seropositive animals to *Leptospira interrogans* from animal units of Yucatan, Mexico by species.

Animal species	Number of Animals	Positives	%
Bovine	212	97	45.8
Canine	61	22	36.0
Porcine	203	26	13.0
Total	478	145	30.5

Table 3. Frequency of serovares of *Leptospira interrogans* by animal species*.

Serovar	Bovines	Canines	Porcines
	(%)	(%)	(%)
Tarassovi	53.6	0.0	4.5
Hardjo	31.6	0.0	0.0
Wolffi	9.4	0.0	0.0
Bratislava	3.8	0.0	66.0
Gryppotyphosa	1.6	3.6	6.9
Canicola	0.0	79.8	0.0
Icterohaemorrhagiae	0.0	9.8	18.7
Panama	0.0	4.0	0.0
Pyrogenes	0.0	2.8	3.9
Pomona	0.0	0.0	0.0

* The total number of samples was 478.

DISCUSSION

Thirty one of the 34 animal production units (counties) had at least one positive animal to *L. interrogans*, which indicates that the bacterium is widely distributed in our region and we confirm the endemicity of infection (Zavala *et al.*, 1984; Vado *et*

al., 2002a; Vado *et al.*, 2002b). The seropositivity frequency in bovines (45.8%) is higher than other parts of the world, and therefore bovine leptospirosis might represent an economical risk to the cattle industry of Yucatan. In this animal reservoir, Hardjo was the most frequent serovar and it is associated with reduced fertility, increase of abortions, weak off-springs, retention of placenta, and dramatic drop in milk production (Ellis *et al.*, 1985). This high frequency is consistent with other findings worldwide, where it is considered that bovines are the main reservoir of Hardjo (Ellis *et al.*, 1985; Ellis, 1984). Animals infected with *Leptospira spp.* are the main source of persistent infection by transmission to other domestic and wildlife reservoirs, as well as to human beings, through the intermittent urine excretion of the microorganism which contaminate water, soil and pastures (Ellis, 1984).

In Mexico, Hardjo, Wolffi and Tarassovi are still the most important serovars identified, although some difference in prevalence have been found among different ecological regions (Luna *et al.*, 2005). Hardjo and Tarassovi are predominant in cattle populations in Yucatan (Segura, Solís, & Segura, 2003) and from the public health point of view, Tarassovi has been documented in human populations, which might indicate that this serovar is playing an important role in a zoonotic transmission from bovines to humans (Vado *et al.*, 2002b).

In many tropical countries, dogs are considered a significant reservoir for the transmission of infection to human beings and they may be the most important source of epidemic outbreaks (Levett, 2001); not only because the dogs' prolonged leptospiuria, but also because their close relationship and contact with man. Dogs are recognized as the host of Canicola and Icterohaemorrhagiae (Levett, 2001), which is consistent with the results obtained herein. In this study, a 79.8% and a 9.8% frequency was obtained for serovars Canicola and Icterohaemorrhagiae, respectively. In a previous study, 35% (140/400) antibody prevalence was found in feral dogs of Merida, Yucatan. Canicola was the most frequent serovar followed by Icterohaemorrhagiae (Jimenez *et al.*, 2008). The data from the previous report in addition to the 36% of

seropositivity obtained in this study, indicate that dogs may be responsible of the dissemination of *Leptospira interrogans* in urban and rural (in animal production units) areas in the State of Yucatan.

The leptospiral infection in pigs can occur in the subclinical form, but febrile reaction may be observed in some animals for a short period. Other subpopulations can produce miscarriage, and in some occasions meningitis and nervous system symptoms (Cisneros *et al.*, 2002). The lowest frequency of positivity was found in this animal species. This can be attributed to the origin of the samples since all the pig population was from the feeding cycle with a short life cycle, that is, of 3 to 4 months. This fact reduces the probability of coinfection. In addition, the sanitary management is stricter in pig production systems. However, 13% of the positivity in pigs indicates that the bacterium is circulating within the farms. The serovar Bratislava was detected with the highest frequency followed by icterohaemorrhagiae. Bratislava has been associated with reproductive failure in pigs form several countries around the world, including Mexico (Levett, 2001; Cisneros *et al.*, 2002).

Transmission of *Leptospira spp.* from wild and domestic animal reservoirs to human beings has already been documented, therefore serological surveys on leptospirosis to detect prevalence of serovars in animal hosts are necessary to understand the epidemiology of the disease on the region. Because the animals tested were not vaccinated against *Leptospira spp.*, the results in this paper show that the serovars detected in the Yucatan are species specific. Reports indicate that prevalence variation among the maintenance host and the common serovar produce a trend change in the epidemiology of the disease (Levett, 2001), and this is probably due to vaccine immunity.

In human beings, leptospiral infection is produced accidentally. The disease is occupational and is more common among rice rural workers, veterinarians, soldiers, and persons in close contact with contaminated urine or direct contact with fetal-maternal fluids of infected animals, or the indirect contact with contaminated water, soil and pastures (Plank & Dean, 2000). The potential risk of infection by swimming in ponds, lagoons or rivers where cattle and wild animals feed has been demonstrated (Vinetz, 2001, Faine *et al.*, 1999; Plank & Dean, 2000). However, from the 68 pools of water samples and tested by PCR for the detection of *L. interrogans* in this study, none had a positive result. Several factors could adversely affect the assay, including enzymatic inhibitors from the environment, damaged or degraded DNA from the sample (Miller *et al.*, 1999), or the season in which the samples were obtained. Although in tropical regions, as in Yucatan, transmission of

leptospiras may occur at any season, it is recognized that a variation in the distribution of rainfall and environmental temperature may affect the survival of *Leptospira interrogans*. As a consequence, it may be observed a reduction in the number of bacterial cells present in the superficial waters.

CONCLUSIONS

The bacteria causing leptospirosis are present in cattle, pigs and dogs from farms of the State of Yucatan. These animal species are in close contact with humans and consequently they are of high risk for the zoonotical transmission. In order to reduce the infection to the human population, it is recommended to protect the domestic animal through routine vaccination using prevalent serovars in the region. Also, adequate health promotion among the population about proper hygiene practices and appropriate animal health management should be implemented. Through a rodent population control program, it will be reduced retransmission to domestic animals. It should also be considered the introduction of serological negative animals to reduce reinfection to local herds. An important aspect of hygienic practices is to properly dispose all farm wastes and biohazards such as death animals, placenta and fetuses. These preventive measures will control leptospirosis in farm animals and reduce the risk of transmission to the human population.

ACKNOWLEDGEMENT

This study was supported by the grant YUC-2002-CO1-8707 from "Fondo Mixto de Fomento a la Investigación Científica y Tecnológica CONACYT – Gobierno del Estado de Yucatán.

REFERENCES

- Cisneros Puebla M.A., Moles Cervantes L.P., Gavaldon-Rosas D., Rojas Serrania N., Torres Barranca J.I. 2002. Serología diagnóstica de leptospirosis porcina en México 1995-2000. Revista Cubana de Medicina Tropical. 54: 28-31.
- Ellis W.A., O'Brien J.J., Bryson D.G., Mackie D.P. 1985. Bovine leptospirosis: Some clinical feature of serovar *hardjo* infection. Veterinary Record. 117: 101-104.
- Ellis, W.A. 1984. Bovine leptospirosis in the tropics: prevalence, patogénesis and control. Preventive Veterinary Medicine. 2: 411-421.

- Faine S., Adler B., Bolin C., Perolat P. 1999. *Leptospira* and leptospirosis, 2nd Ed. MediSci. Melbourne, Australia.
- Levett PN. 2001. Leptospirosis. *Clinical Microbiology Reviews* 14: 296-326.
- Jimenez-Coello M., Vado-Solis I., Cárdenas-Marrufo M.F., Rodríguez-Buenfil J., Ortega-Pacheco A. 2008. Serological survey of canine leptospirosis in the tropics of Yucatan Mexico using two different tests. *Acta Tropica*. 106: 22-26.
- Luna-Álvarez M.A., Moles-Cervantes L.P., Gavaldon-Rosas D., Nava-Vázquez C., Salazar-García F. 2005. Estudio retrospectivo de seroprevalencia de leptospirosis bovina en México considerando las regiones ecológicas. *Revista Cubana de Medicina Tropical*. 57: 28-31.
- Mayers D.M. 1985. Manual de métodos para el diagnóstico de laboratorio de la leptospirosis. Nota técnica 30. CEPANZO OPS. Buenos Aires, Argentina, p.7-8.
- Meslin F.X. 1997. Global aspects of emerging and potencial zoonoses: WHO perspective. *Emerging Infectious Disease*. 3: 223-228.
- Miller D.N., Bryant J.E., Madsen E.L., and Ghiorse W.C. 1999. Evaluation and Optimization of DNA extraction and purification procedures for soil and sediment samples. *Applied Environmental Microbiology*. 65: 4715-4724.
- Murguia R., Riquelme N., Baranton G., Cinco M. 1997. Oligonucleotides specific for pathogenic and saprofitic leptospira occurring in water. *FEMS Microbiology Letters*. 148: 27-34.
- Noguchi H., Klieger J. 1920. Immunological studies with a strain of leptospira isolated from a case of yellow fever in Mérida, Yucatan. *Journal of Experimental Medicine*. 32: 627-637.
- Plank, R. & Dean, D. 2000. Overview of the epidemiology, microbiology and pathogenesis of *Leptospira spp.* in humans. *Microbes and Infection*. 2: 1265-1276.
- Segura Correa J., Honhold N. 2000. Métodos de Muestreo para la producción y la salud animal. Ediciones de la Universidad Autónoma de Yucatán. Mexico, p. 75.
- Segura Correa V.M, Solís Calderon J.J, and Segura Correa J.C. 2003. Seroprevalence of and risk factor for leptospiral antibodies among cattle in the State of Yucatán, Mexico. *Tropical animal health and Production*. 35: 293-299.
- Thierman A.B. 1995. Leptospirosis. *Clinical Infectious Disease*. 21: 1-6.
- Vado Solís I., Cárdenas Marrufo M.F., Jiménez Delgadillo B., Alzina López A., Laviada Molina H., Suárez Solís V., Zavala Velázquez J. 2002a. Clinical epidemiological study of Leptospirosis in human and reservoirs in Yucatán, México. *Revista del Instituto de Medicina Tropical, Sao Paulo*. 44: 335-340.
- Vado Solís I., Cárdenas Marrufo M.F., Laviada Molina H., Vargas Puerto F., Jiménez Delgadillo B., Zavala Velázquez J. 2002b. Estudio de casos clínicos e incidencia de Leptospirosis humana en el estado de Yucatán, México durante el periodo 1998 a 2000. *Revista Biomédica*. 13: 157-164.
- Vinetz J.M. 2001. Leptospirosis. *Current Opinion in Infectious Disease*. 14: 527- 538.
- Western, K. 1982. Vigilancia epidemiológica con posterioridad a los desastres naturales. Organización Panamericana de la Salud/Organización Mundial de la Salud. Publicación Científica No. 420. Washington, D.C., U.S.
- Zavala Velázquez J., Pinzón Cantarell J., Flores Castillo M., Damián Centeno A.G. 1984. La Leptospirosis en Yucatán. Estudio serológico en humanos y animales. *Salud Pública de México*. 26: 254-59.
- Zavala-Velazquez J., Cárdenas-Marrufo M., Vado-Solis I., Cetina-Cámara M., Cano-Tur J. and Laviada-Molina H. 2008. Hemorrhagic pulmonary leptospirosis: Three cases from the Yucatan Peninsula, Mexico. *Revista de la Sociedad Brasileña de Medicina Tropical*. 41: 404-408.

Submitted April 22, 2010 – Accepted May 25, 2010
Revised received June 17, 2010