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CLINICAL-EPIDEMIOLOGICAL STUDY OF LEPTOSPIROSIS IN HUMANS AND RESERVOIRS IN YUCATÁN, MÉXICO

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SUMMARY

A leptospirosis clinical-epidemiological study was made in humans and reservoirs in the state of Yucatán, México. Interviews and serological analyses were made on 400 persons from an open population, 439 probable cases of leptospirosis and 1060 animal reservoirs (cows, pigs, dogs, rats and opossums). IgM Leptospira Dipstick™ and Microscopic Agglutination Test (MAT) was used to detect human antibodies to leptospires and serovar respectively. Leptospirosis incidence in humans was 2.2/100,000 inhab. in 1998, 0.7/100,000 in 1999 and 0.9/100,000 in 2000. Overall seroprevalence was 14.2%, relatively unchanged from seroprevalences observed 20 years ago. Highest seropositivity was found in people over 56 years of age, predominating males over females. Predominant serovars in the open population were *tarassovi*, *hardjo*, *pomona* and *panama*. Leptospirosis cases were most frequent in rural areas, and the anicteric course predominated over the icteric. The *panama*, *icterohaemorrhagiae* and *pomona* serovars predominated in both anicteric and icteric courses. Dogs, pigs and rodents had the highest seropositivity among the reservoirs. Contact with rodents and natural water sources were significant factors ($p \leq 0.05$). Human cases (74%) occurred during the rainy season. It is concluded that leptospirosis is still a serious illness with important clinical and epidemiological implications in the state of Yucatán, Mexico.

KEYWORDS: Leptospirosis; Prevalence; Incidence; Cases; México.

INTRODUCTION

Leptospirosis is a common zoonosis worldwide that affects mammals, including human beings. Infection is endemic and occurs with greatest frequency in tropical and subtropical regions. Both humans and animals can be directly infected through contact with infected tissue or urine, or indirectly through contact with contaminated soil and water^{5,6}. Leptospirosis in humans occurs in two courses: anicteric or benign (between 85 and 90% of cases); and icteric or serious, also known as Weil's disease (between 10 and 15% of cases). The wide spectrum of clinical symptoms that characterize leptospirosis make its diagnosis to be easily confused with other febrile diseases¹¹.

The first leptospirosis cases in México were reported in the State of Yucatán in 1920 by NOGUCHI & KLIEGER, followed by the discovery of human cases in the State of Veracruz by BUSTAMANTE in 1937. Research continued in 1958 with the studies of VARELA *et al.*¹⁶ demonstrating leptospira antibodies in humans and animals from México City, and again in 1961 VARELA & ZAVALA¹⁸ studied 9,931 human and animal sera in Mexico. Isolated reports began to appear, mostly sero-epidemiological^{1,2,22}. As in many other countries, the study of human leptospirosis in Mexico has been hampered by a lack of laboratories with diagnostic capabilities of this disease.

After the work of NOGUCHI during the 20th no further studies were reported until 1962, when interest in the disease was renewed after VARELA & ZAVALA¹⁷ reported six positive leptospirosis cases in 56 icteric patients, mostly of the *pomona* serotype. A number of serious cases (Weil's disease) have been reported since 1977, with some of them being fatal^{21,23,25}. In 1984, it was reported that 14% of the state's population was leptospirosis seropositive²⁴. And in 1998, anicteric leptospirosis cases were found that had been erroneously diagnosed as dengue during an epidemic outbreak of this virosis²⁶. However, this isolated information is insufficient to define the epidemiological behavior of this zoonosis in Yucatán.

In response to this need, the present study is an integrated approach including analysis of seroprevalence, incidence, most common clinical course, circulating serovars and transmission factors for the 1998 to 2000 period.

MATERIALS AND METHODS

STUDY AREA: The state of Yucatán, México is located on the Yucatán Peninsula, between 19° 40' and 21° 37' North Latitude, and 87° 30' and 90° 26' West Latitude. Average daytime temperature is relatively high, generally above 30 °C, with annual rainfall of 750 mm

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and average relative humidity of 74%. Geographically, the state is within the worldwide intertropical belt, and thus experiences microclimatic and meteorological phenomena typical of this zone, such as easterly trade winds, cold north winds, hurricanes, etc. There is no surface-running water (i.e. rivers, streams) in the state, though there are a number of rainfall deposits, locally known as “aguadas” or “cenotes”. These have always been centers for human settlement. Vegetation in the area is semi-arid and fauna is typical of these zones.

SEROPREVALENCE STUDY IN HUMANS: Seroprevalence in the state was determined using the EPI-INFO (version 6.04a) computer software program, with a total universe equal to the state’s population of approximately 1,600,000 people. Sample size was calculated using a 95% confidence level, a 5% precision level and an estimated prevalence of 50% (given that the last serological study in Yucatán was over 16 years ago). Resulting sample size was $N = 385$, which was increased to 400 for this study. The study design was submitted to and approved by the scientific and human ethics committee of the Faculty of Medicine, Universidad Autónoma de Yucatán.

To collect the necessary samples, 65 municipalities were randomly chosen from the 106 municipalities in the state. Within each municipality, people were included in the study that voluntarily participated by providing a blood sample and by being interviewed, and that met the inclusion criterion of not having had presumptive leptospirosis symptomatology at least two months before sampling. Intra-venous blood samples were obtained and coagulated by centrifuging at 1500 rpm for 15 minutes. The resulting serum was stored in Eppendorf tubes at -20°C until use.

To determine seroprevalence and serovar, the microscopic agglutination test (MAT) was used, as described by TURNER¹³ and MYERS¹⁰. Ten serovars were used as antigens based on previous studies in the region^{19,24,26}: *canicola*, *pomona*, *wolffi*, *hardjo*, *tarassovi*, *panama*, *icterohaemorrhagiae*, *grippityphosa*, *bratislava* and *pyrogenes*. The cut-off point was dilutions $\geq 1:100$ and the predominant serovar was that with the highest dilution.

For the interviews, a questionnaire that included subject name and address, usual epidemiological data such as age, sex, location, contact with potential transmission factors such as domestic animals (dogs, bovines, and pigs), peridomestic animals (rodents, opossums), and contact with natural water sources (cenotes and aguadas), and stored water was given. A statistical association analysis was made of these variables for the χ^2 test, with 0.05 significance levels (i.e. a value of $p \leq 0.05$ suggests an association between two variables).

CLINICAL CASE STUDY: Subjects were those sent to the Faculty of Medicine by various clinics and hospitals in the state due to exhibition of presumptive leptospirosis symptoms. These subjects originated from 44 different locations in Yucatán, and had clinical histories. Anti-leptospira IgM Dipsticks™ Leptospira (Integrated Diagnostics Inc. Baltimore, MD, USA) were used to detect positive cases, and MAT was used to determine serovar.

Positive cases were analyzed using epidemiological data such as age, sex, regional distribution, and serovar, as well as clinical data like signs and symptoms. Annual incidence and monthly frequency were

calculated, as was regional monthly rainfall. All data were recorded in the EPI-INFO (version 6.04a) computer software program, and descriptive statistical analyses was used to analyze the data.

Case definition: Considering the pleomorphism of the disease, confirmed cases of leptospirosis, were those in which the main symptom was fever plus three or more universally accepted clinical symptoms of leptospirosis and positive IgM dipsticks.

RESERVOIR STUDY: A study was done to identify the presence of leptospira antibodies in animal reservoirs in different farms and ranches in the state. Blood samples were taken from 375 bovines and 353 pigs through piercing of the jugular vein, from 192 dogs through piercing of the cephalic vein, and from 60 rodents (*Rattus rattus*) and 80 opossum (*Didelphis virginianus*) through piercing of the heart after anesthesia with sodium pentobarbital (Pfizer). Serum was separated by centrifugation and stored at -20°C until use. The micro agglutination test was used to detect seropositives and serovar. Antigen and cut-off value were as those used for the human seroprevalence study.

RESULTS

HUMAN SEROPREVALENCE STUDY: Of the 400 human serum samples processed for MAT, 57 (14.2%) seropositive samples were identified. Reactivity was generally high in the age groups, except in subjects younger than 15 years of age, with predominance in subjects older than 56 years of age, though without significant differences ($p \geq 0.05$) between groups. There was, however, a clear predominance of male subjects over female subjects, which was a statistically significant difference ($p \leq 0.05$) (Table 1).

Table 1
Seroreactivity frequency, by age and sex, in Yucatán, México

Sample size	400	Positives 57	% 14.2
Age groups *			
<15	25	1	4.0
15-35	161	21	13.0
36-56	160	25	15.6
>56	54	10	18.5
Sex **			
Female	266	31	11.6
Male	134	26	19.4

* $p \geq 0.05$; ** $p \leq 0.05$

In serovar frequency, the *tarassovi* was predominant, though the *hardjo*, *pomona* and *panama* serovars also had relatively high percentages (Table 2).

The studied transmission factors were found to be widely distributed throughout the state. When a statistical association analysis was done, however, a significant association was only found between living with rodents ($p \leq 0.05$) and contact with natural water deposits (aguadas and cenotes) ($p \leq 0.05$) (Table 3).

Table 2
Positivity per serogroup and serovar in Yucatán, México

Serogroup	Serovar	Positives	%
<i>Tarassovi</i>	<i>tarassovi</i>	10	17.5
<i>Sejroe</i>	<i>hardjo</i>	7	12.3
<i>Pomona</i>	<i>pomona</i>	7	12.3
<i>Panama</i>	<i>panama</i>	6	10.5
<i>Icterohaemorrhagiae</i>	<i>icterohaemorrhagiae</i>	5	8.8
<i>Grippityphosa</i>	<i>grippityphosa</i>	5	8.8
<i>Pyrogenes</i>	<i>pyrogenes</i>	4	7.0
<i>Canicola</i>	<i>canicola</i>	4	7.0
<i>Sejroe</i>	<i>Wolffi</i>	2	3.5
<i>Australis</i>	<i>bratislava</i>	1	1.7
Not processed	Not processed	6	10.5
Total		57	100

Table 3
Contact frequency with transmission factors in a population from Yucatán, México

Contact with reservoirs	People surveyed	%	Negative	%	Positive	%
	400		343		57	
Rodents *	262	65.5	219	63.8	44	77.2
Dogs	286	71.5	247	72.0	39	68.4
Opossums	240	60.0	206	60.0	33	58.0
Pigs	120	30.0	102	29.7	17	29.8
Bovines	53	13.2	43	12.5	10	17.5
Inadequate water storage	267	66.7	230	67	37	65.0
Contact with natural water deposits **	93	23.2	74	21.6	19	33.3

* $p \leq 0.05$; ** $p \leq 0.05$

CASE STUDIES: 61 confirmed leptospirosis cases during the period 1998-2000 were identified in the study. A greater number of cases were present in rural areas (36 cases; 20.5%) than in urban areas (25 cases; 9.5%), which was a significant difference ($p \leq 0.05$). Women cases also predominated over those in men (39; 16.5% vs. 22; 10.8%) in both rural and urban areas, though this was not a significant difference ($p \geq 0.05$).

The most affected of the age groups was that for subjects between the ages of 15 and 35 years, though this was not statistically significant ($p \geq 0.05$).

Of the total of 61 seropositive cases, 52 (85.2%) contracted the anicteric or benign course and 9 (14.8%) contracted the icteric or serious course, this being a significant difference (Table 4). Frequency of signs and symptoms in the anicteric cases included fever (100%), cephalalgia (90.4%), and myalgia (84.6%), whereas in the icteric cases the most frequent were fever, cephalalgia, choluria and icterus (100%) (Table 5).

Serovar determination via MAT was done for only 31 of the 52 anicteric cases because serum condition did not allow for use of this technique with the remaining 21 cases. In the 31 anicteric cases subjected to MAT, the *panama* serovar was predominant, accounting for 12 cases (38.7%),

Table 4
Frequency in population by area, sex, age and clinical course for 61 leptospirosis cases from Yucatán, México (1998-2000)

Serum	Total	Positive	%
	439	61	13.9
Area *	urban	25	9.5
	rural	36	20.5
Sex **	female	39	16.5
	male	22	10.8
Age **	<15	9	8.6
	15-35	36	18
	36-55	10	10
	>56	6	17
Clinical	anicteric	52	85.2
Course *	icteric	9	14.8

* $p \leq 0.05$; ** $p \geq 0.05$

Table 5

Frequency of signs and symptoms in leptospirosis patients from Yucatán, México (1998-2000)

	# cases	%
Anicteric		
Fever	52	100
Cephalalgia	47	90.4
Myalgia	44	84.6
Arthralgia	41	78.8
Ocular syndrome	33	63.5
Nasopharyngeal symptoms	27	51.7
Gastrointestinal symptoms	16	30.7
Hepatomegaly and splenomegaly	2	3.8
Total cases	52	100
Icteric		
Fever, cephalalgia, icterus and choluria	9	100
Gastrointestinal symptoms	8	88.8
Ocular symptoms	7	77.7
Myalgia and arthralgia	6	66.6
Hepatomegaly and splenomegaly	6	66.6
Hyperazoemia	5	55.5
Total cases	9	100

followed by the *pomona*, accounting for 8 cases (25.8%). In the 9 icteric cases, the *icterohaemorrhagiae* serovar predominated with 3 cases (33.3%), followed by the *panama* with 2 cases (22.2%) (Table 6).

The 72% of the cases occurred during the rainy season in the region (June to October), along all three years of the study.

Recorded incidence varied from year to year, being 2.2/100,000 inhab. in 1998, 0.7/100,000 inhab. in 1999, and 0.9/100,000 inhab. in 2000. None of the studied cases throughout the three-year period resulted in mortality.

Table 6

Seropositivity and microagglutination by serogroup and serovar in diseased patients from Yucatán, México (1998-2000)

Serogroup	Serovar	Positive	
		Anicteric	Icteric
<i>Panama</i>	<i>panama</i>	12 (38.7%)	2 (22.2%)
<i>Pomona</i>	<i>Pomona</i>	8 (25.8%)	1 (11.1%)
<i>Australis</i>	<i>bratislava</i>	3 (9.7%)	0
<i>Icterohaemorrhagiae</i>	<i>icterohaemorrhagiae</i>	2 (6.5%)	3 (33.3%)
<i>Grippotyphosa</i>	<i>grippotyphosa</i>	2 (6.5%)	1 (11.1%)
<i>Sejroe</i>	<i>Wolffi</i>	2 (6.5%)	0
<i>Sejroe</i>	<i>Hardjo</i>	1 (3.2%)	1 (11.1%)
<i>Tarassovi</i>	<i>tarassovi</i>	1 (3.2%)	0
<i>Canicola</i>	<i>canicola</i>	0	1 (11.1%)
<i>Pyrogenes</i>	<i>pyrogenes</i>	0	0
Total		31 (100%)	9 (100%)

21 Serum samples not processed.

RESERVOIR STUDIES: The three species found to have the highest seropositivity for leptospira antibodies were pigs 88 (25%), dogs 36 (19%) and rodents 9 (15%), followed by bovines 21 (5.6%), and opossums 4 (5%). The predominant serotypes in pigs were the *bratislava*, *icterohaemorrhagiae* and *panama*; in bovines they were *hardjo* and *tarassovi*; in dogs they were *grippotyphosa* and *pomona*; in rodents they were *icterohaemorrhagiae*, *wolffi* and *bratislava*; and in opossums they were *pomona* and *wolffi* (Table 7).

Table 7

Seropositivity and serovar reservoirs from Yucatán, México

Reservoirs	# of animals	# positive	%		
Pigs	353	88	25.0		
Dogs	192	36	19.0		
Rodents	60	9	15.0		
Bovines	375	21	5.6		
Opossums	80	4	5.0		
Serovar	Bovines %	Pigs %	Dogs %	Rodents %	Opossums %
<i>Pomona</i>	4.7	3	22	0	75
<i>Canicola</i>	0	0	14	0	0
<i>Hardjo</i>	43.0	4	0	0	0
<i>Panama</i>	0	10	3	0	0
<i>grippotyphosa</i>	9.5	6	55.5	0	0
<i>Wolffi</i>	9.5	8	0	14.2	25
<i>icterohaemorrhagiae</i>	0	12	0	71.4	0
<i>Bratislava</i>	4.7	51	0	14.2	0
<i>Pyrogenes</i>	0	1	5.5	0	0
<i>Tarassovi</i>	28.6	5	0	0	0

DISCUSSION

When comparing the last reported seroprevalence for the human population in Yucatán in 1984 (14.1%)²⁴, with that of the present study (14.2%), it is clear that this has changed little over the past 16 years. *Leptospira* continues to circulate at the same level, which is greater than in other states in México².

In this region, infection occurs at early ages, which is facilitated by patterns of work and recreation in the young and adult population¹⁰. The relatively high seropositive frequency in the elderly population can be partially explained by the long-term persistence of IgG antibodies¹⁴ though it may also be due to recent infections. The predominance of infection in men coincides with results reported for other regions of México and in other countries^{2,9}.

Of the various serovars detected in the present, the *hardjo*, *panama* and *wolffi* serovars were not identified in the last leptospirosis study in Yucatán²⁴. This introduction of new serovars may be due to the introduction of animals from other regions of México and other countries where these serovars persist. It may also result from natural disasters (e.g. hurricanes, forest fires and floods) which cause the migration of wild animals into new areas, thus creating a new epizootiological environment for transmission of new *Leptospira* serovars. Worth noting

is that even though the *tarassovi* and *hardjo* serovars are the most common in bovine leptospirosis, they were the two least serologically identified serovars in the human cases.

Of the studied transmission factors, the seropositive subjects interviewed for this study most commonly reported having rodents in their house or in their surroundings, and being exposed to natural water deposits (aguadas and cenotes). This suggests that the two most common ways of transmission in Yucatán are natural waters, that have been contaminated by wild and/or domestic animal urine, and contact with rodents.

On this matter clinical cases, it has been shown that IgM antibodies appear first in the early stages of infection and/or illness. However, high IgM antibody titers do not persist as long as IgG antibody titers. It is for this reason that the presence of IgM antibodies is considered as a marker of illness when it is associated to clinical signs and symptoms presented by the patient.

It is quite possible that the 61 recorded cases over the three years of this study do not represent all leptospirosis cases in the population during this time period. This is probable because clinically diagnosing leptospirosis can be quite difficult. In Yucatán, as in other tropical regions, its symptoms are easily confused with those of other endemic febrile etiologies from the region^{8,11,20}, in particular with those of classic dengue in Yucatán²⁶.

Though leptospirosis occurs in all people of all ages, it was found to be more frequent among youths and young adults. As has been reported for other regions, this is likely related to professional and recreational activities^{7,12}. No significant differences were found between men and women, with both sexes being equally likely to contract this disease¹⁵. The largest number of cases in this study were identified from rural areas. Here, the predominant cultural characteristics of the region, such as the close coexistence of domestic animals and people, and domestic hygiene conditions that attract peridomestic species (e.g. rodents and opossums), play an important role in creating a propitious environment for transmission of leptospirosis.

The results reported in the present study confirm the predominance of the *panama* serovar in confirmed anicteric and icteric cases of leptospirosis, as reported for other regions^{3,4,15}.

Comparable to other tropical regions, the occurrence of leptospirosis demonstrates a marked seasonal variance, with the greatest number of cases recorded during the season of highest rainfall and temperature, that is, during optimum survival conditions for leptospires^{8,9,11}.

In comparing serovar frequency between humans and reservoirs, probable transmission routes can be drawn. Given the results, the *panama* serovar transmits to humans most frequently via pigs and dogs; the *pomona* via opossums and dogs; the *icterohaemorrhagiae* via rodents and pigs; the *grippityphosa* via dogs and cows and the *tarassovi* and *hardjo* via cows.

As demonstrated by these results, leptospirosis is still an important pathological entity, both clinically and epidemiologically, in the State of Yucatán, México.

RESUMEN

Estudio clínico-epidemiológico de leptospirosis en humanos y reservorios en Yucatán, México

Un estudio clínico-epidemiológico de leptospirosis fue realizado en humanos y reservorios en el estado de Yucatán, México. Se entrevistaron y analizaron mediante serología 400 personas en población abierta, 439 pacientes sospechosos y 1060 reservorios (bovinos, cerdos, perros, roedores y zarigüeyas). En humanos para la detección de anticuerpos se utilizó IgM Dipstick Leptospira™ y la prueba de aglutinación microscópica (MAT) para detectar serovar. La incidencia en humanos fue de 2.2/100,000, 0.7/100,000 y 0.9/100,000 habitantes para los años 1998, 1999 y 2000 respectivamente y la seroprevalencia de 14.2%. La frecuencia más alta de seropositivos se encontró en mayores de 56 años, predominando el sexo masculino sobre el femenino. Predominó en población abierta el serovar *tarassovi* siguiendo en importancia *hardjo*, *pomona* y *panamá*. Los casos fueron más frecuentes en el área rural y el curso anictérico predominó sobre el icterico. *Panamá*, *icterohaemorrhagiae* y *pomona* predominaron en ambos cursos de la enfermedad. Los perros cerdos y roedores tuvieron mayor positividad. El contacto con roedores y depósitos naturales de agua fue significativo ($p \leq 0.05$). El 74.3% de los casos se presentaron en época de lluvia.

En conclusión la leptospirosis en Yucatán, México, sigue siendo una entidad patológica de importancia tanto clínica como epidemiológica.

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