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Risk factors and the prevalence of leptospirosis infection in a rural community of Chiapas, Mexico

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SUMMARY

A cross-sectional study was conducted in Mapastepec, a rural community of the southern state of Chiapas, Mexico. The overall prevalence of leptospirosis infection in 1169 subjects was 37·7% [95% confidence intervals (95% CI) 34·9–40·5]. The main risk factors related to leptospirosis infection were flooding, mainly if subjects had a skin cut or abrasion [odds ratio (OR) 4·2; 95% CI 3·1–5·7], having domestic animals, either dogs and/or cats (OR 1·3; 95% CI 0·96–1·8) or cattle and/or pigs (OR 1·9; 95% CI 1·3–2·7), contact with animal excreta with no protection and with a skin cut or abrasion (OR 2·3; 95% CI 1·1–4·6). Those subjects with a dengue infection in the previous year had also an excess risk (OR 1·4; 95% CI 0·9–2·0). Mapastepec is a previously unknown area with high endemicity. Specific preventive measures should be adopted to prevent any contact with infected animals, and animal immunization should also be implemented. There is need of an epidemiological surveillance system to allow proper diagnosis.

INTRODUCTION

Leptospirosis is a spirochaetal zoonosis, distributed worldwide and with a wide spectrum of clinical manifestations in humans. Fortunately, the most common syndrome is a self-limited systemic illness [1]. The true incidence of leptospirosis in Mexico is unknown, and only some series of cases have been reported [2, 3]. Since most cases of leptospirosis present with mild, 'flu-like symptoms, it is often misdiagnosed or ignored. Poor availability of diagnostic laboratory procedures in Mexican rural and urban areas contributes to misdiagnosis, although some improvement in its diagnosis has recently been achieved to differentiate febrile illness from dengue infections, mainly in the dengue epidemics that have afflicted Mexico [4].

Prevalence studies have been reported in Mexico in some selected populations. One of the few population studies that have been done in Mexico was published in the middle of last century [5]. In this study, the states with the highest reported prevalence were Tabasco (29%), Campeche (23%), Vera Cruz (18%) and Mexico City (15·3%) [5]. In those days, the prevalence in the southern state of Chiapas was 1·6%, slightly lower than in the state of Yucatán (3·4%). Some years later, a prevalence of 7% in blood donors of Mexico City [6] was reported, as well as a prevalence of 8% in an urban population and 19% in a rural population of the state of Yucatán [7].

As a zoonosis, the risk of leptospira infection in humans is closely related to contact with tissues

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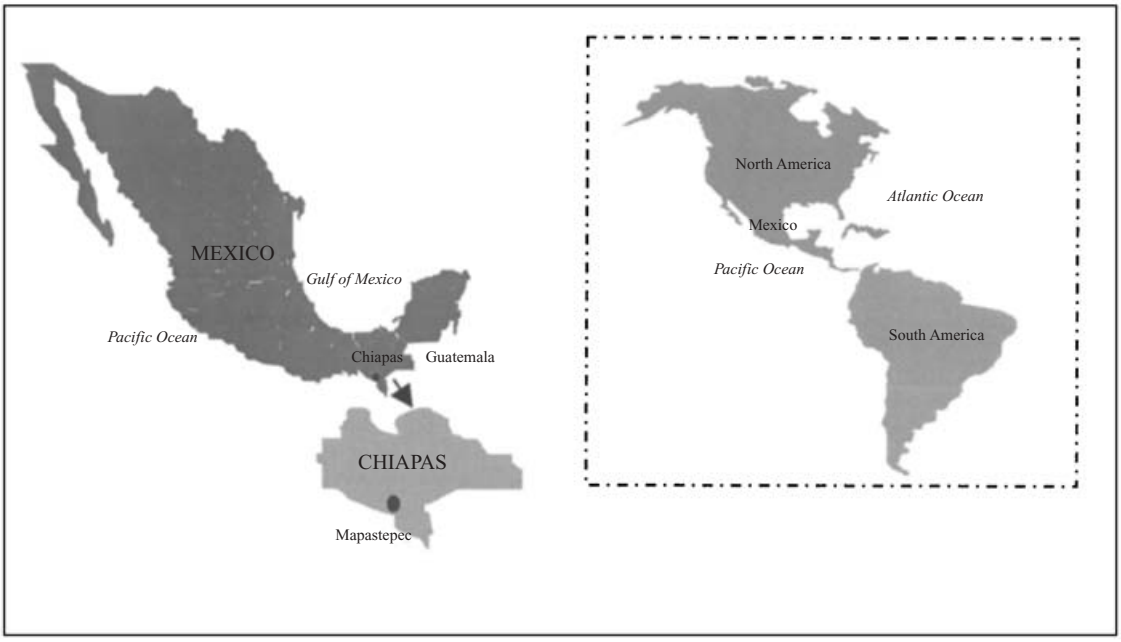

Fig. 1. Geographical location of Mapastepec, Chiapas in Mexico.

or urine, from infected animals. Some occupational activities related to handling animal tissues thus have an increased risk of infection [8]. Other risk factors include contact with water or mud during work or recreational activities, mainly if the water is contaminated with animal excreta, including rodent urine. The risk of infection increases in the presence of skin cuts or abrasions [9] since leptospires rapidly invade the bloodstream after penetrating skin or mucous membranes [1]. Natural disasters associated with flooding, have been related to an increase in the occurrence of leptospirosis in humans [10, 11].

A high prevalence of leptospirosis infection has been reported in Mexican animals. In central Mexico, the prevalence of *Leptospira* serovars in commercial pig herds ranged from 28 to 34% [12] and it was over 35% in Holstein race milk cows in the state of Mexico [13].

The purpose of this study was to determine the prevalence of leptospirosis infection for specific antibody detection in the bloodstream of humans in a rural area of the southern state of Chiapas, Mexico. A further objective was to identify potential risk factors related to the occurrence of leptospirosis infection.

METHODS

A cross-sectional serological survey of humans was conducted in Mapastepec (Fig. 1), a rural community of the southern state of Chiapas, from 1 March to 31 July 2000. Mapastepec is a 1085 km² municipality, with a central urban area, where 37% of the population reside, there are 314 settlements throughout the municipality that host the remainder of the population. The total population of Mapastepec was 44 000 inhabitants in 2000 (16 280 living in the urban area and 27 720 in the rural areas). Nearly two-thirds of the population work in agriculture, growing maize, beans, sesame, mangos, watermelons and avocados. There are also inhabitants who own or work on ranches that produce beef, milk and poultry.

Study population

There is a rural 40 bed-hospital in Mapastepec, governed by the 'IMSS–Solidaridad' programme, which provides medical care to all inhabitants in the city districts (*barrios*) of Mapastepec (urban areas), as well as to other communities called 'intensive action areas' belonging to the municipality of Mapastepec (rural areas). The hospital maintains an annual census of all households in its catchment area. A total of 1170 householders out of 2878 living in the area, were randomly selected and included in this study. If the householder was not at home, his or her wife/husband was selected, failing that any other family member older than 14 years. If no one was available or there was nobody at home, the householder was given an

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Table 1. *Prevalence of* Leptospira *antibodies in Mapastepec, Chiapas*

	Subjects with antibodies	Total (*n*)	Prevalence (%)	95% CI
		Mapastepec *barrios* (urban communities)		
Embarcadero	15	76	19·7	11·5–30·5
Santa Cruz	61	162	37·3	30·2–45·1
Santa Cecilia	70	164	42·7	35·1–50·3
Olímpico	44	129	34·1	25·9–42·9
Generación 2000	5	13	38·5	13·9–68·4
Benito Juárez	9	25	36·0	18·0–57·5
Juan Becerra	7	14	50·0	23·0–77·0
Llanito	12	22	54·5	32·2–75·6
Unidad Mexicana	26	72	36·1	25·1–48·3
15 de Enero	16	78	20·5	12·2–31·2
San Miguel	6	16	37·5	15·2–64·6
San Agustín	7	14	50·0	23·0–77·0
Total	278	785	35·4	32·1–38·8
		Intensive action areas (rural communities)		
Gpe. Victoria	44	96	45·8	35·6–56·3
Limones	29	67	43·3	31·2–56·0
Costa Rica	3	8	33·3	7·4–70·1
Mariitas	5	34	14·7	4·9–31·1
Huamuche	27	37	73·0	55·9–86·2
Barrancón	26	40	65·0	48·3–79·4
Nuevo Milenio	29	102	28·4	19·7–37·2
Total	163	384	42·4	37·5–47·4
Total	441	1169	37·7	34·9–40·5

appointment at the hospital for interview. No one living in the area for less than 6 months was included in the study.

The study was approved by the Local Research Committee of the Coordination of Community Health at the Mexican Institute of Social Security.

Subjects numbering 1171 between the ages of 15 and 86 years participated in this study. One blood sample was inadequate for antibody measurement, so the final sample was 1169 subjects, including 223 men (19%) and 946 women (81%). Table 1 shows the distribution per community. There were 785 inhabitants from the *barrios* of Mapastepec, and 1169 subjects from other communities served by the rural hospital (intensive action areas).

Questionnaires

A structured questionnaire was applied to all participants. Information from these questionnaires included demographics, education, habitual occupation, occupational handling or contact with animal tissues or excreta, domestic animal ownership, contact with puddles, owning silos or other places to keep cereal grains, the presence of rodents at home or in the silo. Information was also collected regarding antecedents of flooding in the community or contact with flood water. Subjects were also questioned in relation to the presence of skin cuts or abrasions or the use of any preventive measure (such as wearing special footwear or using disinfectants) while in contact with puddles or animal excreta. Since there are freshwater pools in the area, subjects were asked if they were used for swimming and if cattle drank there. The presence in the previous year of any symptom related to leptospirosis (fever, jaundice, acholia or choluria) or dengue fever, was also registered.

Determination of serological markers

Venous blood samples were taken from the antecubital vein, and serum was separated and stored at 2–8 °C before being transported to the laboratories of the National Institute of Diagnostic and Epidemiologic Reference of the Ministry of Health, in Mexico City, where all analyses were carried out.

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The microscopic agglutination test (MAT) was used to detect antibody titres to pathogenic *Leptospira* species, using live leptospires as antigen: hardjo, bratislava, autumnalis, ballum, australis, pomona, cannicola, pyrogenes, wolffi, tarassovi, cynopteri, grippothyposa, batavie, icterohaemorrhagiae, shermani, javanica and borincana. Serum was inactivated at 56 °C, for 30 min, and then double dilutions were performed from 1:40 to 1:2560, and tested against each one of 17 different serovars at a concentration of 1:1. The leptospiral antibody titres were defined as the highest dilution of serum at which 50% or more of leptospires were agglutinated. A titre of 1:80 or more was considered positive. To identify acute infection a PCR analysis was performed in those serums with antibody titres >1:600.

Statistical analysis

Global, as well as age-specific, and gender-specific antibody prevalence, with 95% confidence intervals (CI) were calculated. The χ^2 test was used to compare categorical data and to evaluate association with risk factors. The strength of the association with the studied risk factors was measured by prevalence odds ratios (OR), with 95% CIs. To control for potential confounders and to assess the independent effect of each risk factor, an explicative model was constructed with a non-conditional multivariate logistic regression analysis, and the corresponding ORs with 95% CIs were also estimated. Variables that were related to leptospira infection in the bivariate analysis were included in the multivariate analysis by a forward procedure.

RESULTS

Crude prevalence of leptospira infection was 37·7% (95% CI 34·9–40·5). Prevalence was slightly higher in rural communities, the so-called intensive action areas (42·4%; 95% CI 37·5–47·4), than in urban communities (35·4%, 95% CI 32·1–38·8), as shown in Table 1. Rural communities had lower socio-economic levels than urban ones. The proportion of illiterates in rural areas was higher (35·9 *vs.* 25·1%), whereas the proportion of households with sewerage was lower (21·1 *vs.* 75·7%). Only 41·4% of rural households had potable tubed water, compared to 74·1% in urban areas.

Seventeen different serovars were identified in the population studied (Table 2). Some patients had antibodies to more than one different serovar. There were 24 subjects with antibody titres >1:600, but only 7 of them had shown any symptom related to leptospirosis within the previous year. Only one serum was positive to PCR. She was a 28-year-old female, who had reported choluria 2 months prior

Table 2. *Distribution of* Leptospira interrogans *serovars in Mapastepec, Chiapas*

Serovar	Total (*n*)	Percent
hardjo	254	29·7
bratislava	172	20·2
autumnalis	99	11·5
ballum	82	9·6
australis	56	6·6
pomona	55	6·4
cannicola	45	5·3
pyrogenes	41	4·8
wolffi	12	1·4
tarassovi	10	1·4
cynopteri	7	0·8
grippothyposa	7	0·8
batavie	5	0·6
icterohaemorrhagiae	4	0·5
shermani	2	0·2
javanica	2	0·2
borincana	1	0·1
Total	854	100

Table 3. *Specific prevalence of antibodies against* Leptospira *sp. by age and sex*

Age groups (years)	Subjects with antibodies	Total (*n*)	Prevalence (%)	95% CI
Men				
15–24	9	31	29·0	13·1–45·0
25–34	21	50	42·0	28·2–56·8
35–44	19	38	50·0	34·4–66·6
45–54	17	36	47·2	30·4–64·5
55–64	13	28	46·4	27·5–66·1
⩾65	21	40	52·5	36·1–68·5
Total	100	223	44·8	38·3–51·4
Women				
15–24	58	182	31·9	25·1–38·6
25–34	90	221	40·7	34·2–47·2
35–44	75	202	37·1	30·5–43·8
45–54	60	156	38·5	30·8–46·1
55–64	38	118	32·2	23·3–40·6
⩾65	20	67	29·9	19·3–42·3
Total	341	946	36·0	33·0–39·1
Total	441	1169	37·7	34·9–40·5

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Table 4. *Association between risk factors and* Leptospira *infection according to the bivariate analysis* (*prevalence odds ratios*)

Variable	OR	95% CI
Gender: Male	1·44	1·07–1·93
Native from Mapastepec	1·21	0·95–1·52
Having lived lifelong in the community	1·30	1·03–1·67
Water in puddles nearby home	1·37	1·06–1·77
Occupational contact with water in puddles	1·78	1·28–2·47
Walking barefoot	1·63	1·23–2·17
Previous flooding in the community		
No previous flooding	1·00	
Previous flooding once	1·49	1·13–1·96
Previous flooding twice	2·40	1·43–4·02
Previous flooding more than twice	2·33	1·21–4·52
Skin cuts or abrasion during flooding	4·76	3·59–6·29
Swimming in freshwater pools		
No swimming	1·00	
Swimming but cattle do not drink there	0·96	0·64–1·43
Swimming and cattle do drink there	1·80	1·14–2·84
Domestic animals		
None	1·00	
Dog and/or cat	1·50	1·12–2·01
Pig and/or cattle	2·49	1·80–3·46
Handling animal tissues		
No handling	1·00	
Handling chicken tissues	1·18	0·85–1·66
Handling cattle tissues	1·83	1·15–2·90
Handling pig tissues	1·98	1·12–3·51
Handling cattle and pig tissues	3·09	1·45–6·64
Contact with animal excreta		
No contact	1·00	
Contact with any protection and no skin cuts or abrasion	0·69	0·45–1·06
Contact with protection but with skin cuts or abrasion	1·77	0·91–3·44
Contact with no protection and no skin cuts or abrasion	2·39	1·59–3·62
Contact with no protection and with skin cuts or abrasion	4·97	2·74–9·09

to the interview. She was asymptomatic by the time of the study.

There was no significant difference among different age groups on the prevalence of antibodies against leptospira (Table 3). Prevalence was higher among men.

Table 4 shows the results of the analysis to identify risk factors for infection in this population. Contact with water in puddles or from flooding was a risk factor related to leptospira infection. The risk was higher if the contact was barefooted or the subject had any skin cut or abrasion. Swimming in freshwater pools was only a risk factor if cattle drank there. Owning animals was also a risk factor for leptospira infection, mainly if the animals were pigs or cattle. Other important identified risk factors were occupational handling of animal tissues, mainly if they were cattle or pigs. One of the risk factors with the highest strength of association was occupational contact with animal excreta, and the risk was higher if subjects had no protection against this contact, and also if they had a skin cut or abrasion in any extremity.

In the multivariate analysis (Table 5), while controlling for age, gender and socioeconomic level, the strongest association was observed with occupational exposure to animal excreta as well as contact with water in puddles. In this model, the detected presence of rodents in the home, the presence of choluria and/or an illness resembling dengue virus infection in the previous year, were strong predictors to explain leptospira infection.

DISCUSSION

The estimated prevalence of leptospira infection in Mapastepec, Chiapas is high, mainly when compared to previous reports from Mexico [5–7] and other highly endemic areas outside Mexico [8, 14, 15]. The observed value is in fact considerably higher than the only previous report in Chiapas (1·6% in 1985) [5] and it is even more important since there have been no acute cases reported in the region in recent years. It could be considered a high rate of underreporting of leptospira infection, since it has been considered that 5–10% of cases are severe and potentially fatal. It should be expected, therefore, that among 400 infected subjects at least 20–40 were undiagnosed severe cases of acute infection.

Leptospirosis is frequently misdiagnosed as dengue virus infection [10] and in places where leptospirosis is highly endemic, dengue virus infections are misdiagnosed as leptospirosis [16]. Recent dengue infection epidemics in Mexico, have triggered an intensive search for leptospira infection. The similar clinical patterns between leptospira and dengue virus infection, underlies the need of differential diagnosis

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Table 5. *Relative risk* (*odds ratios*) *of* Leptospira *infection, derived from an explicative multivariate non-conditional logistic regression analysis**

	OR	95% CI
Domestic animals		
None	1·00	
Dog and/or cat	1·31	0·96–1·80
Pig and/or cattle	1·87	1·29–2·72
Contact with animal excreta		
None	1·00	
Contact with any protection and no skin cuts or abrasion	0·53	0·32–0·89
Contact with protection but with skin cuts or abrasion	0·87	0·41–1·84
Contact with no protection and no skin cuts or abrasion	1·50	0·88–2·54
Contact with no protection and with skin cuts or abrasion	2·25	1·10–4·56
Occupation		
Non-risk occupation	1·00	
Farmer	1·95	1·03–3·71
Cattle breeder, cheese-maker or butcher	1·04	0·55–1·98
Pig farmers	1·41	0·85–2·33
Handling animal tissues		
No handling	1·00	
Handling chicken tissues	1·14	0·78–1·65
Handing cattle or pig tissues	1·52	0·86–2·70
Foot skin cuts or abrasion during flooding	4·23	3·12–5·75
Type of footwear		
Closed footwear	1·00	
Open footwear	1·80	1·32–2·45
Rodents in home	1·30	0·98–1·73
Choluria in the previous year	2·19	1·23–3·90
Dengue infection in the previous year	1·37	0·95–1·97

* All variables in the table were included in the model, as well as gender, sex and socioeconomic level.
Maximum likelihood estimator (MLE) = 308·6, $P < 0·001$.

through a reference diagnostic laboratory, especially when treatment for both diseases is quite different.

Prevalence of specific antibodies was slightly higher in the rural areas of Mapastepec municipality. Even though leptospirosis outbreaks have been reported in urban areas [17], leptospirosis infection is more common in rural areas, where risk factors are more prevalent.

As in previous seroprevalence studies [8, 15, 17–19], a relatively low cut-off value (1:80) was used to identify infected subjects. Previous studies in Mexico have reported antibody titres between 1:100 and 1:190 in acute cases [2]. Nevertheless, we restricted PCR analysis to those with titres >1:600. The only PCR-positive female was asymptomatic, but she may well have been a convalescent infected patient, since she had reported choluria 2 months prior to the study, and her antibody titres were >1:1280.

Since no age-effect was observed, it should be considered that the studied region is endemic for leptospira infection and exposure to related risk factors begins early in life. A higher prevalence in men must be related to occupational exposure to risk factors, especially handling animal tissues and excreta, and this activity is more common in men in this region. Leptospira infection is frequent in cattle and pigs in some regions of Mexico [12, 13] and although there are no available studies from Chiapas, it may be that the occurrence is similar to other places in the country. The increased risk observed when handling animal tissues and excreta has been previously reported as being mainly related to cattle and pigs [18–20]. Exposure at home to cats and dogs considerably increases the risk of infection which could be due to lack of vaccination in domestic animals, in whom the prevalence of infection may be high [21]. Vaccination against leptospirosis could be considered in high occupational risk subjects [22] but there is no efficient vaccine available that protects against different serovars and that does not require frequent administration. Nevertheless some specific protective measures, such as wearing special footwear (e.g. boots) or using disinfectants while in contact with animal excreta, should be recommended, since they significantly reduce the risk of infection. These easily adopted measures should be promoted among high occupational risk subjects.

Recent natural phenomena, characterized by flooding of extensive inhabited areas, increases the risk of exposure to leptospira from animal excreta [10, 23] especially from rodents [24] as was evident in the increased risk in subjects that reported having been exposed to flooding of their homes or the surrounding area. This risk is even higher if subjects are barefooted [24].

Domestic animals at home in Mexican rural areas, are a risk factor for leptospira infection [24]. A public health policy could be the extension of animal vaccination against leptospira, both in domestic animals (cats and dogs), as well as in cattle and pigs. Rodents close to the home are a risk factor that has been identified [24] and any measure taken to eradicate

this pest will contribute to reducing exposure to leptospira infection.

There was an increased risk of infection in subjects that reported an antecedent of choluria or dengue virus infection, this emphasizes the importance of clinical identification of probable infection and also stresses the need of diagnostic support in an epidemiological reference diagnostic laboratory. Leptospirosis may be an endemic disease in Mexico, rarely diagnosed, and its true incidence must be identified.

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