

- (editors). Oxford: WHO/Oxford University Press, pp. 238-250.
- Cho, S. N., Kim, S. H., Cellona, R. V., Chan, G. P., Fajardo, T. T., Walsh, G. P. & Kim, J. D. (1992). Prevalence of IgM antibodies to phenolic glycolipid I among household contacts and controls in Korea and the Philippines. *Leprosy Review*, **63**, 12-20.
- De Wit, M. Y. L., Faber, W. R., Krieg, S. R., Douglas, J. T., Lucas, S. B., Montrewasuwat, N., Pattyn, S. R., Hussain, R., Ponnighaus, J. M., Hartskeerl, R. A. & Klatser, P. R. (1991). Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissues. *Journal of Clinical Microbiology*, **29**, 906-910.
- De Wit, M. Y. L., Douglas, J. T., McFadden, J. & Klatser, P. R. (1993). Polymerase chain reaction for detection of *Mycobacterium leprae* in nasal swab specimens. *Journal of Clinical Microbiology*, **31**, 502-506.
- Enarson, D. A. (1994). Why not the elimination of tuberculosis? *Mayo Clinical Proceedings*, **69**, 85-86.
- Fine, P. E. M. (1982). Leprosy: the epidemiology of a slow bacterium. *Epidemiological Reviews*, **4**, 161-188.
- Fine, P. E. M. (1992). Reflections on the elimination of leprosy. *International Journal of Leprosy*, **60**, 71-80.
- Gillis, T. P. & Williams, D. L. (1991). Polymerase chain reaction and leprosy. *International Journal of Leprosy*, **59**, 311-316.
- Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y., Sugiyama, K. & Kawatsu, K. (1990). Novel gelatin particle agglutination tests for serodiagnosis of leprosy in the field. *Journal of Clinical Microbiology*, **28**, 525-529.
- Job, C. K., Drain, V., Williams, D. L., Gillis, T. P., Truman, R. W., Deming, A. T. & Hastings, R. C. (1991). Comparison of polymerase chain reaction technique with other methods for detection of *Mycobacterium leprae* in tissues of wild nine-banded armadillos. *Leprosy Review*, **62**, 362-373.
- Klatser, P. R., Van Beers, S., Madjid, B., Day, R. & De Wit, M. Y. L. (1993). Detection of *Mycobacterium leprae* nasal carriers in populations for which leprosy is endemic. *Journal of Clinical Microbiology*, **31**, 2947-2951.
- Nordeen, S. K., Lopez Bravo, L. & Sundaresan, T. K. (1992). Estimated number of leprosy cases in the world. *Bulletin of the World Health Organization*, **143**, 381-385.
- Pattyn, S. R., Ursi, D., Leven, M., Grillone, S. & Raes, N. (1993). Detection of *Mycobacterium leprae* by the polymerase chain reaction in nasal swabs of leprosy patients and their contacts. *International Journal of Leprosy*, **61**, 389-393.
- Ridley, D. S. & Jopling, W. H. (1966). Classification of leprosy according to immunity: a five-group system. *International Journal of Leprosy*, **34**, 255-273.
- Soebono, H. & Klatser, P. R. (1991). A seroepidemiological study of leprosy in high- and low-endemic Indonesian villages. *International Journal of Leprosy*, **59**, 416-425.
- Ulrich, M., Smith, P. G., Sampson, C., Zuniga, M., Centeno, M., Garcia, V., Manrique, X., Salgado, A. & Convit, J. (1991). IgM antibodies to native glycolipid-I in contacts of leprosy patients in Venezuela: epidemiological observations and a prospective study of the risk of leprosy. *International Journal of Leprosy*, **59**, 405-415.
- WHO (1994). Progress towards eliminating leprosy as a public health problem. *Weekly Epidemiological Record*, **69**, 145-151.
- Van Beers, S. M., Izumi, S., Madjid, B., Maeda, Y., Day, R. & Klatser, P. R. (1994). An epidemiological study of leprosy infection by serology and polymerase chain reaction. *International Journal of Leprosy*, **62**, 1-9.

Received 6 October 1994; revised 18 January 1995; accepted for publication 3 February 1995

Short Report

Prevalence of leptospiral infections in humans in Cordillera Province, Bolivia

L. Ciceroni¹, A. Bartoloni², A. Pinto¹, P. Gugliemetti³, H. Gamboa Barahona⁴, M. Roselli² and F. Paradisi² ¹National Centre for Leptospirosis, Department of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, Rome, Italy; ²Clinica di Malattie Infettive, Università di Firenze, Italy; ³Istituto di Malattie Infettive, Università di Siena, Italy; ⁴Distrito de Salud de Cordillera, Unidad Sanitaria, Santa Cruz, Bolivia

Keywords: leptospirosis, prevalence, Bolivia

Leptospirosis is a world-wide zoonotic infection with high morbidity especially in developing countries. Serological studies have indicated widespread leptospiral infection in several Latin American countries but there is little information on the prevalence in Bolivia.

The present study was undertaken in collaboration with the Unidad Sanitaria de Santa Cruz to determine the prevalence of anti-leptospira antibodies in human settlements in south-eastern Bolivia.

The investigation was carried out in 1989 in 3 localities in Cordillera Province in the southern part of Santa Cruz Department; the population consists mainly of *Mestizos* with some ethnically pure Guarani Indians who resort to agriculture and animal breeding for subsistence. Camiri is a city with approximately 25 000 inhabitants; some buildings in the city centre are supplied with untreated

Address for correspondence: Dr L. Ciceroni, National Centre for Leptospirosis, Department of Bacteriology and Medical Mycology, Istituto di Sanità, Viale Regina Elena 299, 00161 Roma, Italy.

water directly from the river Parapeti. The other 2 localities, Boyuibe (2 500 inhabitants, 60 km south of Camiri) and Gutierrez (850 residents, 60 km north of Camiri), are both poor communities with no infrastructure, potable water supply or sewage system. In the study area, 800-900 m above sea level, the temperature fluctuates between 17 and 26°C and the climate is sub-humid to dry (SANABRIA, 1977).

Sera were collected from 295 apparently healthy subjects (111 males and 184 females) between 1 and 70 years of age. The cohort included 122 from Camiri, 111 from Boyuibe and 62 from Gutierrez. The sample size was calculated as recommended by the World Health Organization (WHO, 1966) to obtain evidence (with 95% certainty) of the prevalence of anti-leptospira antibodies in not less than 3% of the population.

Table 1. Prevalence of anti-leptospira antibodies in human sera from Cordillera Province, Bolivia, determined by the microscopical agglutination assay

	No. of sera	
	Tested	Positive
Locality		
Camiri	122	32 (26.2%)
Boyuibe	111	31 (27.9%)
Gutierrez	62	27 (43.5%)
Sex		
Male	111	32 (28.8%)
Female	184	58 (31.5%)
Age (years)		
1-5	64	12 (18.7%)
6-10	90	23 (25.5%)
11-20	27	14 (51.8%)
21-40	94	33 (35.1%)
>40	16	6 (37.5%)
Unknown	4	2 (50.0%)
Total	295	90 (30.5%)

The sera were stored frozen (-75°C) until tested by the microscopical agglutination (MA) assay of DIKKEN & KMETY (1978) against live leptospirae in the exponential phase of growth (4–6 d old culture). A set of 20 strains kept in the National Centre for Leptospirosis in Rome (BABUDIERI, 1972) was used, representative of 19 serovars (see Table 2).

The initial dilution of serum tested (1:50) was twice that currently used for screening clinical cases of human leptospirosis. A doubling dilution series was used to titrate antibodies in positive sera. The end-point antibody titre was defined as the highest dilution that agglutinated 50% or more of the leptospirae.

Anti-leptospira antibodies were found in 90 samples of serum, with titres ranging from 1:50 to 1:800 (Table 1).

The differences in prevalence between Gutierrez and Camiri and Boyuibe were statistically significant ($P=0.02$ and $P=0.04$, respectively). Females had a slightly higher prevalence of anti-leptospira antibodies than males ($P>0.5$). Maximum prevalence occurred in the 11–20 years age group. Antibodies were directed against 14 of the 19 serovars (Table 2).

Table 2. Prevalence of agglutinins to leptospira serovars in 90 positive sera from Camiri, Boyuibe and Gutierrez in Cordillera Province, Bolivia

Serogroup	Serovar	Strain	Prevalence of agglutinins
Icterohaemorrhagiae/icterohaemorrhagiae	copenhageni	Bianchi 1	1 (1.1%)
		Wijnberg	3 (3.3%)
	canicola	Alarik	14 (15.5%)
		Mezzano	0 (-)
	pomona	Pavia 1	1 (1.1%)
		Moskva V	0 (-)
	bataviae	Ballico	0 (-)
		Riccio 37	5 (5.5%)
	grippityphosa	Riccio 2	42 (46.7%)
		lora	1 (1.1%)
australis	Zanoni	1 (1.1%)	
	Mus 24	1 (1.1%)	
Pyrogenes	Topo 1	10 (11.1%)	
	Hardjoprajitno and Farina C715	10 (11.1%)	
Sejroe	Sari	6 (6.7%)	
	Poi	4 (4.4%)	
Mini	Mitis Johnson	1 (1.1%)	
	Castellon 3	1 (1.1%)	
Javanica	Akiyami A	0 (-)	
	Hebdomadis H	0 (-)	
Tarassovi			
Ballum			
Autumnalis			
Hebdomadis			

Most positive sera (87%) had serovar-specific antibodies, but 12 had co-agglutinins to 2 or more serovars of the same (25%) or different serogroups (75%). Most positive sera (82%) had antibody titres of 1:50–1:200.

To our knowledge there has been no epidemiological study of human leptospirosis in Bolivia for the past 20 years. However, an epidemiological survey carried out from October 1977 to April 1980 on dairy cattle in Santa Cruz and San Javier provinces indicated that leptospirosis was endemic, with antibodies in 67% of animals mainly to *hardjo*, *sejroe* and *tarassovi* serovars (NICHOLLS, 1980).

Since the presence of anti-leptospira antibodies in sera can be ascribed only to *Leptospira* infection, our data suggest that infection is widespread in the population examined. Similarly high prevalences of leptospira antibodies in humans have also been reported from Somalia (CACCIAPUOTI *et al.*, 1982) and some Italian regions (CACCIAPUOTI *et al.*, 1994).

The titres we found indicate that, for the most part, infections were not recent. It was not possible to establish what percentage of seropositive subjects had experienced clinical leptospirosis.

Traditionally, leptospirosis is considered to be an infection of adulthood. Our results demonstrated that leptospirosis was common in infants and schoolchildren in Cordillera Province. The unusually high prevalence among children under 10 years old can be ascribed to early contact with polluted soil and water. Similarly, RATNAM *et al.* (1983) found 26 (47%) of 55 children aged 4–11 years to be positive in a village near Madras, India, and EVERARD *et al.* (1989) found serological evidence of *Leptospira* infection in 12.5% of Barbadian schoolchildren and 9.5% of Trinidadian schoolchildren aged 7–14 years.

The percentage of positive subjects in Gutierrez was twice that found the other localities, but we could find no satisfactory explanation for this.

The prevailing infecting serovars were *bratislava*, *canicola*, *hardjo* and *sejroe*. The prevalence of antibodies to *bratislava* may be peculiar to Cordillera Province, as serological evidence of exposure to this serovar has not been found in previous studies on leptospirosis in Bolivia (LIMPIAS & MARCUS, 1973; NICHOLLS, 1980). Antibodies to *icterohaemorrhagiae* serovar, commonly responsible for human leptospirosis in other countries, seem to be rare in this area.

Leptospirosis is widespread among the population of Cordillera Province and, in contrast with what is normally found in developed countries, young children presented a remarkably high prevalence of anti-leptospira antibodies.

References

- Babudieri, B. (1972). List of *Leptospira* strains kept in the WHO/FAO *Leptospira* Reference Laboratory in Rome. *Annali dell'Istituto Superiore di Sanità*, 8, 159–196.
- Cacciapuoti, B., Nuti, M., Pinto, A. & Sabrie, A. M. (1982). Human leptospirosis in Somalia: a serological survey. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 76, 178–182.
- Cacciapuoti, B., Ciceroni, L., Pinto, A., Apollini, M., Rondinella, V., Bonomi, U., Benedetti, E., Cinco, M., Dessi, S., Dettori, G., Grillo, R., Falomo, R., Mansueto, S., Miceli, D., Marcuccio, L., Marcuccio, C., Pizzocaro, P., Schivo, M. L., Varaldo, E., Lupidi, R., Ioli, A., Marzolini, A. & Rosmini, F. (1994). Survey on the prevalence of leptospira infections in the Italian population. *European Journal of Epidemiology*, 10, 173–180.
- Dikken, H. & Kmetz, E. (1978). Serological typing methods of leptospirae. In: *Methods in Microbiology*, Bergan, T. & Norris, J. R. (editors). London: Academic Press, vol. 11, pp. 259–308.
- Everard, C. O. R., Hayes, R. J. & Edwards, C. N. (1989). Leptospirosis infection in schoolchildren from Trinidad and Barbados. *Epidemiology and Infection*, 103, 143–156.
- Limpas, E. V. & Marcus, S. J. (1973). Encuesta serologica de la leptospirosis en Santa Cruz, Bolivia. *Boletín de la Oficina Sanitaria Panamericana*, 75, 139–145.
- Nicholls, M. J. (1980). Health of cattle in dairy herds of Santa Cruz and San Javier provinces of Bolivia. *Mision Britanica en Agricultura Tropical*, 359, 1–67.
- Ratnam, S., Sundararaj, T. & Subramanian, S. (1983). Serological evidence of leptospirosis in a human population following an outbreak of the disease in cattle. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 77, 94–98.
- Sanabria, H. (1977). Monografía del Departamento de Santa Cruz. *Boletín de la Sociedad de Estudios Geográficos e Históricos*, 39, 60–77.
- WHO (1966). *Sampling Methods in Morbidity Surveys and Public Health Investigations*. 10th Report of the Expert Committee on Health Statistics. Geneva: World Health Organization, Technical Report Series, no. 336.

Received 9 February 1995; revised 28 March 1995; accepted for publication 28 March 1995