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Prevalence of antibodies to *Borrelia burgdorferi*, *Borrelia parkeri* and *Borrelia turicatae* in human settlements of the Cordillera Province, Bolivia

L. CICERONI[1], A. BARTOLONI[2], P. GUGLIELMETTI[3], F. PARADISI[2], H. GAMBOA BARAHONA[4], M. ROSELLI[2], S. CIARROCCHI[1] & B. CACCIAPUOTI[1]

[1]Istituto Superiore di Sanità, Laboratorio di Batteriologia e Micologia Medica, Roma, Italy
[2]Cattedra di Malattie Infettive, Università di Firenze, Italy
[3]Istituto di Malattie Infettive, Università de Siena, Italy
[4]Distrito de Salud de Cordillera, Unidad Sanitaria, Santa Cruz, Bolivia

SUMMARY

A seroepidemiological study to determine the prevalence of human Lyme borreliosis and tick-borne relapsing fever was carried out in three communities (Camiri, Boyuibe and Gutierrez) of the Cordillera Province, Santa Cruz Department, south-eastern Bolivia. Anti-*B. burgdorferi*, anti-*B. turicatae* and anti-*B. parkeri* antibodies, tested by the indirect immunofluorescent assay (IFA), were detected in 10.8, 16.1 and 8.2% of the serum samples tested, and confirmed by IFA-ABS in 1.3, 1.3 and 1.0%, respectively. This is the first report of the presence of Lyme borreliosis and tick-borne relapsing fever in Bolivia. For Lyme borreliosis these findings represent a further datum to support its existence in South America.

Keywords: Lyme borreliosis, tick-borne relapsing fever

INTRODUCTION

Autochthonous Guaranì Indians and mestizos of the Cordillera Province, in the Santa Cruz Department, south-eastern Bolivia, were previously investigated for hepatitis A virus (HAV) (Bartoloni *et al.* 1989a), human immunodeficency virus (HIV) (Bartoloni *et al.* 1989b), cytomegalovirus (CMV) (Bartoloni *et al.* 1989b), *Toxoplasma gondii* (Paradisi *et al.* 1989), and intestinal protozoa and nematodes (Cancrini *et al.* 1989) infection rates.

To further define the spectrum of microbial pathogens circulating in the Cordillera Province, population samples of Guaranì Indians and mestizos were investigated for the presence of borrelial infections. Borreliae are arthropod-borne spirochetes capable of causing different diseases in humans and animals. Relapsing fevers are human acute febrile illnesses caused by blood *Borrelia* spirochetes. They have a worldwide distribution and occur in most continents of the world including the Americas, Europe, Africa, and Asia (Butler 1991). Lyme borreliosis is a recently recognized tick-borne zoonosis caused by *Borrelia burgdorferi* (Burgdorfer *et al.* 1982). Human infections have been reported from different countries of North America, Europe, Australia, Asia and Africa, with most reported cases coming from the United States and Europe (Paleologo 1991). Lyme borreliosis has not been reported in South America. Serologic evidence of antibodies to *B. burgdorferi*, which suggests the presence of Lyme borreliosis, was recently reported from Peru (Need & Escamilla 1991), but the possibility of

Correspondence: B. CACCIAPUOTI, Istituto Superiore di Sanità, Laboratorio di Batteriologia e Micologia Medica, Roma, Italy.

cross-reactivity to other *Borrelia* species cannot be ruled out.

To our knowledge, no previous serologic studies of borrelial infections have been reported from Bolivia. The present study was undertaken to determine the seroprevalence in humans of antibodies to *Borrelia burgdorferi*, the agent of Lyme borreliosis, and to *Borrelia turicatae* and *Borrelia parkeri*, agents of tick-borne relapsing fever. An indirect immunofluorescent assay (IFA) was performed to detect anti-*Borrelia* antibodies in serum samples. Anti-*Borrelia* positive serum samples were further tested for Borrelia antibodies after absorbtion with a *Treponema phagedenis* extract.

MATERIALS AND METHODS

Study area

The survey was carried out in Camiri, Boyuibe, and Gutierrez, three localities of the Cordillera Province, Santa Cruz Department, in the south-eastern part of Bolivia. The population of the province consists mainly of mestizos with some Guaranì Indians, with agricultural and animal breeding subsistence activities. Camiri is a town of about 25 000 inhabitants and is the 'petroleum capital' of Bolivia, where some employees of the Bolivian National Petroleum Agency live. It is situated in the foothills of the Andes at an altitude of about 800 m. Boyuibe has 2500 inhabitants, an altitude of 900 m, and is approximately 60 km south of Camiri. Gutierrez is a village with about 850 residents, 60 km north of Camiri. Both Boyuibe and Gutierrez are poor communities with no infrastructure, not even a potable water supply or sewage system. In the study area the temperature fluctuates between 17 and 26°C, and the climate is defined as sub-humid–dry (Sanabria 1977).

Population sample

A total of 305 subjects, 189 females and 116 males, were examined by age for prevalence of antibodies towards *B. burgdorferi*, *B. turicatae* and *B. parkeri* spp. The chi-squared test was performed to evaluate the significance of the prevalence of reactive sera in the population. The study population selectively included autochthonous Guaranì Indians and mestizos, who can be assimilated to autochthons in their very low standard of living. The study population included 132 subjects from Camiri, 111 from Boyuibe and 62 from Gutierrez. The size of the sample to be examined in order to obtain significant results was established according to the sampling methods indicated by WHO (1966) for morbidity surveys. The sample size was calculated to give significant results, with a 95% confidence limit, for prevalence of anti-*Borrelia* antibodies in not less than 3% of the population (WHO 1973).

Serological survey

A sample of 10 ml venous blood was taken from each subject. The sera were stored at −20°C, transported to Italy in dry ice, and then tested by indirect immunofluorescent assay (IFA) for anti-*Borrelia* antibodies. *B. burgdorferi* type strain B31, *B. turicatae* and *B. parkeri* strains (supplied by R.C. Johnson, University of Minnesota, Minneapolis) were used as diagnostic antigens. Sera were screened by IFA at a dilution of 1:32. A doubling dilution series, beginning at 1:64, was used to titrate anti-bodies in positive sera. FITC labelled anti-human total Ig, directed to both IgG and IgM antibodies (Welcome Diagnostics, England), was used. IFA-reactive serum samples were absorbed with a *Treponema phagedenis* extract (Bio-Merieux, France) and re-tested for anti-*Borrelia* antibodies (IFA-ABS).

RESULTS

Anti-*B. burgdorferi* antibodies were detected in 33 (10.8%) of 305 serum samples examined (Table 1). The prevalences in Camiri, Boyuibe and Gutierrez were respectively 8.3, 14.4 and 9.7%. The different prevalences observed in each locality were not statistically significant ($P>0.1$). Males had a non-significant higher prevalence of antibodies (13.8%) than had females (9.0%) ($P>0.25$). Age-specific prevalence indicated a precocious antibody response in children 1–5 years old (12.1%) with decreasing frequencies in older age groups, but with a peak (15.3%) in adults from 21 to 40 years of age.

Anti-*B. turicatae* and anti-*B. parkeri* antibodies were detected in 49 (16.1%) and 25 (8.2%) serum samples, respectively (Table 1). The different prevalences of antibodies towards these two antigens were statistically significant ($P<0.01$). Since *B. turicatae* and *B. parkeri*

Table 1. Prevalence of anti-*Borrelia* antibodies in human sera from the Cordillera Province tested by IFA

Epidemiological data	No. of sera tested	Frequency (percentage) of positive sera		
		B. burgdorferi	*B. turicatae*	*B. parkeri*
Locality				
Camiri	132	11 (8.3)	8 (6.1)	8 (6.1)
Boyuibe	111	16 (14.4)	31 (27.9)	14 (12.6)
Gutierrez	62	6 (9.7)	10 (16.1)	3 (4.8)
Sex				
Male	116	16 (13.8)	23 (19.8)	12 (10.3)
Female	189	17 (9.0)	26 (13.8)	13 (6.9)
Age (years)				
1–5	66	8 (12.1)	25 (37.9)	7 (10.6)
6–10	91	8 (8.8)	9 (9.9)	7 (7.7)
11–20	28	1 (3.6)	2 (7.1)	—
21–40	98	15 (15.3)	10 (10.2)	11 (11.2)
>40	22	1 (4.5)	3 (13.6)	—
Total	305	33 (10.8)	49 (16.1)	25 (8.2)

antibodies were almost always associated in the serum samples, they could be considered genus-specific, cross-reacting antibodies to borrelias of tick-borne relapsing fever. The higher prevalence of *B. turicatae* over *B. parkeri* antibodies suggested the presence of species-specific antibodies to *B. turicatae*. Anti-*B. turicatae* antibodies had a higher prevalence in Boyuibe (27.9%) than in Camiri (6.0%) and Gutierrez (16.1%), and the differences were statistically significant or non-significant ($P<0.001$ and $P>0.05$) respectively. The higher prevalence of anti-*B. turicatae* and anti-*B. parkeri* antibodies in females and in males respectively was statistically non-significant ($P>0.25$ for both strains). Age-specific prevalence indicated an early exposure to borrelias of tick-borne relapsing fever in children (1–5 year age group) with decreasing frequencies in older age groups.

Anti-*B. burgdorferi*, anti-*B. turicatae* and anti-*B. parkeri* cross-reacting antibodies were observed in a high prevalence of positive sera (Table 2, Figure 1). Cross-reacting sera were re-tested after absorption with a *Treponema phagedenis* extract, currently used to eliminate cross-reactivity to common antigens of *Enterobacteria, Leptospira, Treponema* and *Borrelia* spp. (Ballard *et al.* 1987) (Table 3). Anti-*Borrelia* antibodies were dramatically reduced in absorbed sera, and residual species-specific antibodies to *B. burgdorferi B. turicatae* and *B. parkeri* were present in 1.3, 1.3 and 1.0% of sera respectively.

Table 2. IFA cross-reactivity of sera to *B. burgdorferi*, *B. turicatae* and *B. parkeri*

Antigens	Frequency (percentage) of cross-reacting sera		
	B. burgdorferi	*B. turicatae*	*B. parkeri*
B. burgdorferi	33	19 (57.6)	17 (51.5)
B. turicatae	19 (38.8)	49	18 (36.7)
B. parkeri	17 (68.0)	18 (72.0)	25

DISCUSSION

Borreliae are arthropod-borne spirochetes which can be pathogenic for humans and animals. The tick-borne relapsing fever, caused by blood *Borrelia* spirochetes, has a worldwide distribution with occurrence in five main areas of the world including South America (Bell 1987; Butler 1991). As *B. venezuelensis*, the only species isolated in Central and South America (Kelly 1984), carried by the soft tick species *Ornithodoros rudis*, has not been cultivated *in vitro*, the cultivable *B. turicatae* and *B. parkeri*, agents of tick-borne relapsing fever in North America, were used as antigens in the present study. Lyme borreliosis, caused by *Borrelia burgdorferi*, has been reported in North America, Europe, Australia, Asia, and Africa (Paleologo 1991). As yet, it has not been reported in South America. A preliminary study,

Table 3. IFA and IFA-ABS reactivity of human sera to *B. burgdorferi*, *B. turicatae* and *B. parkeri* strains

Serological test	Serum titre	Frequency of positive sera		
		B. burgdorferi	*B. turicatae*	*B. parkeri*
IFA	1:32	20	35	18
	1:64	8	7	1
	1:128	3	4	4
	1:256	2	2	2
	1:512	—	1	—
	(1:32–1:512)	(33)	(49)	(25)
IFA-ABS	1:32	1	2	—
	1:64	1	3	1
	1:128	2	—	1
	1:256	—	—	1
	(1:32–1:256)	(4)	(5)	(3)

based on the serologic evidence of antibodies to *B. burgdorferi*, suggests the presence of Lyme borreliosis in Peru, even if the possibility of cross-reactivity cannot be ruled out (Need & Escamilla 1991).

In the present study, carried out in a rural area of the Cordillera Province in south-eastern Bolivia, anti-*B. burgdorferi*, anti-*B. turicatae*, and anti-*B. parkeri* antibodies, tested by the indirect immunofluorescent assay (IFA), were detected in 10.8, 16.1 and 8.2% of the serum samples tested. The prevalence of anti-*Borrelia* antibodies decreased dramatically in absorbed sera: anti-*B. burgdorferi*, anti-*B. turicatae*, and anti-*B. parkeri* were confirmed by IFA-ABS in 1.3, 1.3 and 1.0% of the sera tested, respectively.

This is a first report of the presence of Lyme borreliosis and tick-borne relapsing fever in Bolivia. For Lyme borreliosis these findings represent a further datum to support its existence in South America. The reactivity of sera of Bolivians to *B. parkeri* and *B. turicatae*, carried by the North American soft tick species *O. parkeri* and *O. turicatae* and not carried by *O. rudis*, is not very surprising. The tick-spirochete specificity theory, which states that borreliae carried by a given species of tick constitute individual species, is flawed by the fact that some borreliae isolated from one species of tick are capable of infecting other species of tick (Kelly 1984). Moreover, DNA–DNA reassociation studies, to assess the species-specificity of North and South American *Borrelia* isolates, have not been performed. In fact, North and South American *Borrelia* isolates cross-react with reciprocal immune sera.

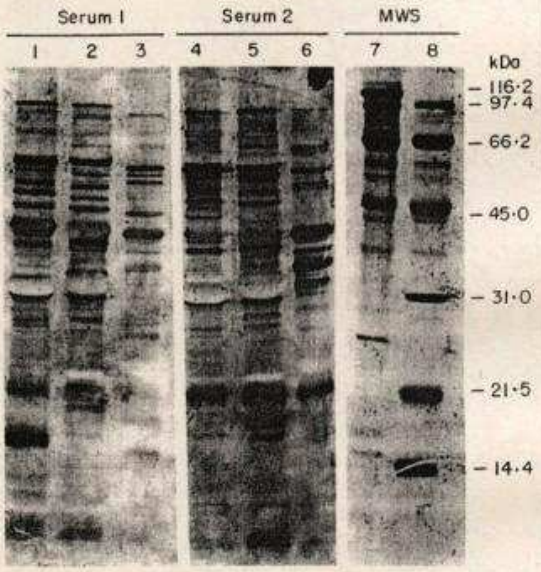

Figure 1. Western blot assay (WBA) of whole-cell lysates of *Borrelia burgdorferi* B31 (lanes 1, 4) N34 (lanes 2, 5) and *Borrelia turicatae* (lanes 3, 6) run on a 10.0% SDS polyacrylamide gel and transferred to nitrocellulose for WBAs. The blots were reacted with two sera (lanes 1–3, serum 1; lanes 4–6, serum 2) IFA-ABS positive to *B. burgdorferi* B31. High and low molecular weight standards (MWS) were beta-galactosidase (116.2 kDa), phosphorylase (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa) and hen egg lysozyme (14.4 kDa).

Our serological data showed a high frequency of cross-reacting sera with *B. burgdorferi*, *B. parkeri* and *B. turicatae* strains. The presence of cross-reacting antibodies and their disappearance after absorption with *T. phagedenis* indicated that common bacterial antigens, responsible for false-reactivities of sera to *Borrelia* antigens, were widely circulating in the sample population of the Cordillera Province. The high prevalence of oro-faecal infections previously noticed in the Cordillera Province (Bartoloni *et al.* 1989a; Paradisi *et al.* 1989c; Cancrini *et al.* 1989) suggested that common antigens could be mainly carried in the population sample by strains of the Enterobacteriaceae family (Ballard *et al.* 1987).

In conclusion, the serological data indicated that Lyme borreliosis and tick-borne relapsing fever circulate with a relatively low prevalence in the population of the Cordillera Province.

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