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Serologic Survey for Antibodies to *Borrelia burgdorferi* in Sheep, Goats and Dogs in Cordillera Province, Bolivia

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With 1 figure and 3 tables

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Summary

A serosurvey for antibodies to *Borrelia burgdorferi* using an enzyme-linked immunosorbent assay (ELISA) was conducted on sheep, goat and dog serum samples collected in Cordillera Province, Bolivia, in 1992. Sera from 98 sheep, 218 goats and 43 dogs were tested. The observed seroprevalence in sheep and dogs was 0.0%, whereas the seropositivity rate for goat serum samples was 5.0%. Upon analysing 10 positive sera by Western immunoblotting, five reacted against the specific protein antigens and all of them met the criteria for positivity on the basis of immunoglobulin G (IgG) bands, indicating that goats in Cordillera Province were exposed to *B. burgdorferi*. These findings, which are further proof of the existence of *B. burgdorferi* infection in Bolivia, indicate the serologic analysis of goats as a suitable tool for Lyme borreliosis surveillance.

Introduction

Lyme borreliosis, first recognized in Lyme, CT, USA in 1975, is now the most common tick-associated disease of human beings in the United States (Steere, 1989). Its aetiological agent, *Borrelia burgdorferi sensu lato*, is also a well-known pathogen of domestic animals, including dogs, horses and cows. *Borrelia burgdorferi* infection or serological evidence of infection, has been reported in these domestic species in many endemic areas in the United States and Europe (Parker and White, 1992; Kazmierczak and Sorhage, 1993). Much less is known about Lyme borreliosis in other domestic animals such as sheep and goats (Fridriksdottir et al., 1992; Angelov et al., 1993; Ciceroni et al., 1996). In a previous work the present authors reported serological data that suggested the existence of Lyme borreliosis in Bolivia (Ciceroni et al., 1994). The purpose of the present study was to ascertain the exposure of sheep, goats and dogs to *B. burgdorferi* in this country.

Materials and Methods

Sera were collected from 98 sheep and 218 goats of various herds bred in Cordillera Province at three localities, Camiri, Boyuibe and Gutierrez, where serological evidence of infection with *B. burgdorferi* in

humans had been found (CICERONI et al., 1994). Sera were also collected from 43 dogs living in the same areas in close contact with sheep and goats.

At the time of blood collection all the animals examined appeared healthy. The blood samples were taken during the summer months of 1992. The sera were stored at −20°C in Bolivia, transported to Italy in dry ice and then stored at −70°C until tested.

Sera were tested for antibodies to *B. burgdorferi* by means of ELISA using a *B. burgdorferi sensu stricto* strain (B31). The antigen for indirect ELISA was prepared following a method described by BARANTON and POSTIC (BARANTON and POSTIC, 1989) with some modifications. Briefly, the spirochetes were grown for 4–6 days at 32°C in BSK II medium and harvested by centrifugation (10 000 ***g*** for 30 min at 4°C). The pellets were washed four times in phosphate-buffered saline (PBS, pH 7.2), resuspended in PBS, and sonicated twice for 30 min (at 20% duty cycle) in an ice water bath in a high-intensity ultrasonic processor (Sonics and Materials Inc, Danbury, CT, USA) at the maximum microtip limit. The sonic extract was centrifuged (10 000 ***g*** for 30 min at 4°C) and the protein content of the supernatant was estimated. The supernatant was diluted in coating buffer (carbonate/bicarbonate, pH 9.6) as determined by chequerboard titration and divided in aliquots kept at −70°C.

The ELISA was performed as described by VOLLER (VOLLER et al., 1980). In brief, the assay was carried out with 0.1 μg of washed, sonicated B31 cell antigen per well in 96-well plates (Dynatec Laboratories, Chantilly, VA, USA). Plates were blocked with 1% bovine serum albumin in PBS (pH 7.4) for 1 h at 37°C to reduce non-specific reactivity. The serum specimen (100 μl), diluted 1:200 in PBS (pH 7.4), was added and incubated for 1 h at 37°C. The presence of anti-*B. burgdorferi* antibodies was detected with specific anti-immunoglobulin G (IgG) alkaline phosphatase conjugate (Sigma, St Louis, MO, USA), and with the substrate p-nitrophenyl phosphate (Sigma 104 phosphate substrate tablets) in diethanolamine buffer (100 μl at pH 9.8).The enzymatic reaction was stopped by adding 40 μl of 3M NaOH.

The optical density in each well was determined with a microplate photometer (BIO-RAD model 3550 Microplate Reader) at a wave length of 405 nm. Net absorbance values greater than 0.60 for goats, 0.32 for sheep and 0.10 for dogs were graded as positive. The cut-off value chosen was the mean of the samples plus three standard deviations for each animal species. All serum samples above, at or near the cut-off value in the initial ELISA were run again. Serum samples that were repeatedly reactive were considered positive.

The sera classed positive by ELISA were tested by Western immunoblotting for antibodies to *B. burgdorferi* to further characterize the antibody response. The procedure for the Western immunoblotting has been described elsewhere (CACCIAPUOTI et al., 1995). *Borrelia burgdorferi* B31 strain was used as antigen. Proteins were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a 12% polyacrylamide gel and elecrophoretically transferred to nitrocellulose sheets. The nitrocellulose sheets were cut in strips about 10 mm wide, that were probed with sera diluted 1:100 in PBS with 0.15% Tween for 1 h at room temperature. Reactive antigen bands were detected using the specific anti-IgG (whole molecule) alkaline phosphatase conjugate (Sigma) and the Fast Red/naphthol phosphate colour development solution (BIO-RAD Laboratories, Richmond, CA, USA). Non-specific binding was blocked with 1% gelatine in PBS.

All sera classed positive by ELISA were also tested (at a dilution of 1:64) for antibodies to *Borrelia turicatae* (a relapsing fever borrelia) by using the immunofluorescence assay (IFA) performed according to the standard method.

Furthermore, all ELISA-positive sera were tested for antibodies to *Leptospira interrogans sensu lato* by the microscopic agglutination (MA) test (DIKKEN and KMETY, 1978). The MA test was performed (at dilutions of 1:100 and 1:200) using the reference strains of all *L. interrogans* serogroups (KMETY and DIKKEN, 1988) except Manhao, and *hardjo* Hardjoprajitno, *hardjo* Hardiobovis and *poi* Poi.

Results

A total of 359 animal serum samples were collected from the three localities under examination and analysed by ELISA. Of the 98 sheep that were ELISA tested, none was seropositive to *B. burgdorferi* (Table 1). No seropositive dogs were found either. Eleven goats (5.0%) had antibodies to *B. burgdorferi*. The highest percentage of positive goats (6.2%) was recorded in Camiri, but no significant differences were found between the prevalence of anti-borrelia antibodies detected in Camiri and those of Boyuibe (1.9%) and Gutierrez (5.6%) (P-values higher than 0.05).

All sera that were positive by ELISA, when the amount was significant, were examined by Western immunoblotting. Of the 10 caprine sera examined five did not exhibit any reaction, and five (50.0%) reacted against the specific protein antigens of *B. burgdorferi* (Table 2). The

Table 1. ELISA result for sheep, goat and dog serum samples collected in 1992 from different areas in the Cordillera Province

Animal species	Geographic location	Serum sample	
		Tested (n)	Positive (%)
Sheep	Camiri	28	— (0.0%)
	Boyuibe	38	— (0.0%)
	Gutierrez	32	— (0.0%)
	Total	98	— (0.0%)
Goats	Camiri	113	7 (6.2%)
	Boyuibe	52	1 (1.9%)
	Gutierrez	53	3 (5.6%)
	Total	218	11 (5.0%)
Dogs	Camiri	19	— (0.0%)
	Boyuibe	7	— (0.0%)
	Gutierrez	17	— (0.0%)
	Total	43	— (0.0%)

apparent molecular mass of the antigens, and the frequency of reactivity of the antigens on Western immunoblotting, are given in Table 3. Representative IgG immunoblots are shown in Figure 1. Interestingly, no response to the major outer surface proteins OspA was seen, while all specimens that were reactive by Western immunoblotting had antibodies to five or more of the following 10 significant bands: 18, 21, 28, 30, 39, 41, 45, 58, 66 and 93 KDa, and were interpreted as positive (CDC/ASTPHLD, 1994). However, all Western immunoblotting-positive sera reacted with the major outer surface protein OspB.

To assess the specificity of ELISA, all specimens with antibodies to *B. burgdorferi* in this assay were also screened against the relapsing fever borrelia, *B. turicatae*, and 25 strains of *L. interrogans sensu lato*. None of the sera which were positive in the *B. burgdorferi* ELISA had IFA-antibodies to *B. turicatae*. Only three were positive (at dilution 1:100) in the MA test for antibodies to *Leptospira* serovars, but none of them reacted with whole cell lysates of *B. burgdorferi* in the Western immunoblotting.

Discussion

In a previous study conducted in human settlements in the south-eastern part of Bolivia the present authors found serological evidence of Lyme borreliosis infection among the population (Cicheroni et al., 1994). In the present study, carried out in the same areas on sheep, goats and dogs, it was found found that 5.0% of the caprine sera had antibodies to *B. burgdorferi* in ELISA.

Table 2. Western immunoblot analysis of goat serum samples positive in ELISA collected in 1992 from different areas in the Cordillera Province

Geographic location	Serum samples	
	Tested (n)	Positive (%)
Camiri	6	1 (16.7%)
Boyuibe	1	1 (100.0%)
Gutierrez	3	3 (100.0%)
Total	10	5 (50.0%)

Table 3. Reactive bands on Western immunoblots probed with goat sera positive in ELISA

Number of samples tested	Samples reactive to antigens with a molecular mass (KDa) of: 93	66	58	45	41	39	30	28	21	18
5	—	5	5	1	5	5	4	2	4	—

Half of these samples had IgG bands when analysed by Western immunoblotting and met the criteria for positivity on the basis of IgG bands, indicating antigenic exposure of goats to *B. burgdorferi* in Cordillera Province. Lyme borreliosis in goats has never been described and nothing is known about the role of goats in the life cycle of *B. burgdorferi*. The only previous studies involving goats were from Bulgaria and Italy, where in areas endemic for Lyme borreliosis 48.0% and 36.8% of animals, respectively, were found to be positive (ANGELOV et al., 1993; CICERONI et al., 1996). Despite the fact that the goat seropositivity rate in ELISA was 5.0% none of the sheep or the dogs examined had antibodies to *B. burgdorferi*. One possible explanation for this could be that sheep and dogs in the study areas have a tick distribution pattern different from that of goats. An alternative possible explanation might be that the samples of sheep and goats did not adequately represent the regional population of these two species.

Five out of the 10 goat samples tested by IgG Western immunoblotting were reactive but all reacted with the major outer surface protein OspB. Interestingly, no response to the other major surface protein OspA was seen. This is not uncommon since either very low or no

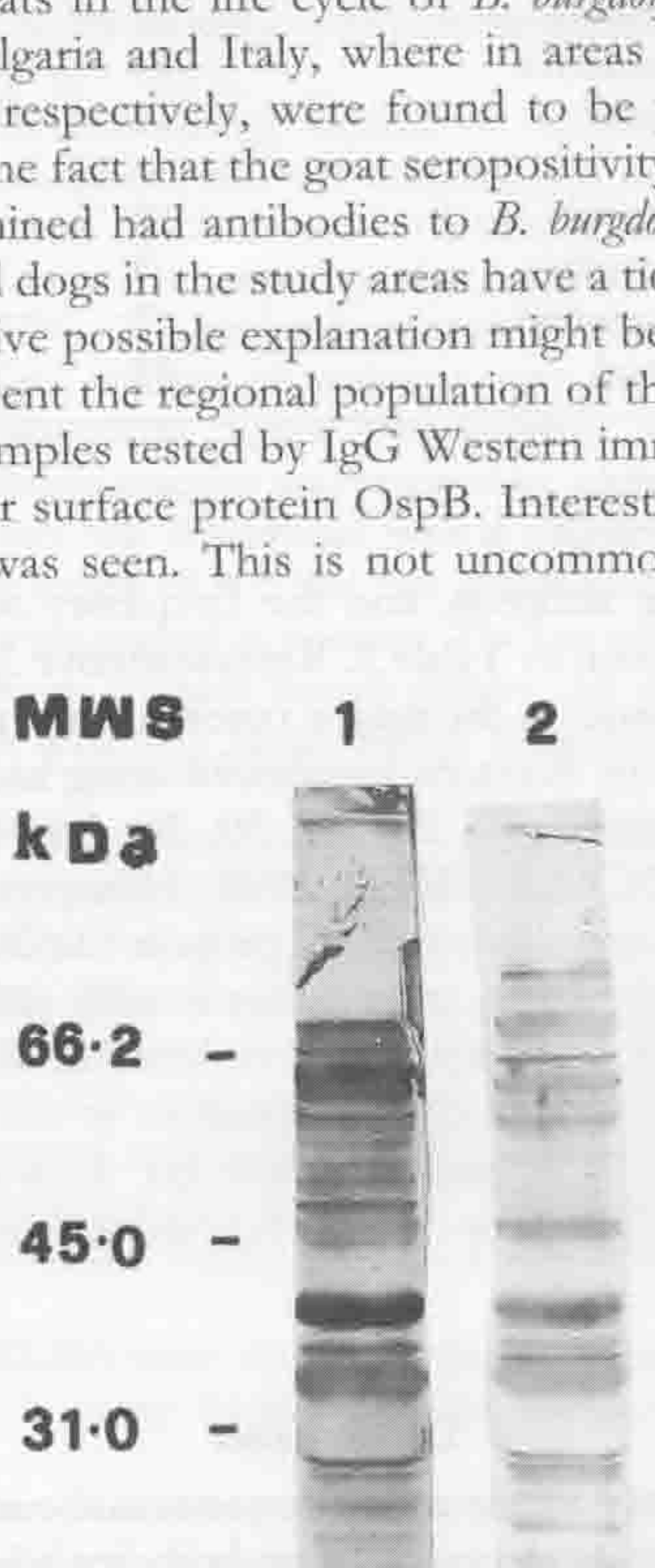

Fig. 1. Western immunoblots of whole-cell lygates of *B. burgdorferi* B31 probed with two different goat sera. Lanes: 1, goat sample from Camiri that was positive by ELISA; 2, goat sample from Gutierrez that was positive by ELISA. MWS, molecular weight standards

response to OspA has been reported for both animals and humans (Craft et al., 1986; Greene et al., 1988; Grodzicki and Steere, 1988; Gill et al., 1993).

In conclusion, these results demonstrate that Lyme borreliosis is present in Bolivia and that goats can be used as a suitable sentinel for the serological surveillance of *B. burgdorferi*. However, it would be useful to extend the study to tick populations to which goats are exposed to assess the role this animal species may have as a maintenance host of *B. burgdorferi*, and to support serological evidence of *B. burgdorferi* infection by isolation and characterization of spirochetes in study areas.

References

Angelov, L., D. Arnaudov, T. Rakadzhieva, E. Kostova, and E. Lipkev, 1993: Study of the epizootiology of Lyme borreliosis in Bulgaria. Infectivology **30,** 12–14.

Baranton, G., and D. Postic, 1989: Methodes de laboratoire leptospirose-borreliose de Lyme. Commission des Laboratoires de Reference et d'Expertise de l'Institut Pasteur, Paris, 1–107.

Cacciapuoti, B., L. Ciceroni, S. Ciarrocchi, C. Khoury, and J. Simeoni, 1995: Genetic and phenotypic characterization of *Borrelia burgdorferi* strains isolated from *Ixodes ricinus* ticks in the province of Bolzano, Italy. Microbiologica **18,** 169–181.

CDC/ASTPHLD, 1994: Lyme Disease Workgroup Recommendations. Deaborn, Michigan.

Ciceroni, L., A. Bartoloni, P. Guglielmetti, F. Paradisi, H. Gamboa barahona, M. Roselli, S. Ciarrocchi, and B. Cacciapuoti, 1994: Prevalence of antibodies to *Borrelia burgdorferi, Borrelia parkeri* and *Borrelia turicatae* in human settlements of the Cordillera Province, Bolivia. J. Trop. Med. Hyg. **97,** 13–17.

Ciceroni, L., J. Simeoni, A. I. Pacetti, S. Ciarrocchi, and B. Cacciapuoti, 1996: Antibodies to *Borrelia burgdorferi* in sheep and goats. Microbiologica **19,** 171–174.

Craft, J. E., D. K. Fischer, G. T. Shinamoto, and A. C. Steere, 1986: Antigens of *Borrelia burgdorferi* recognized during Lyme disease. J. Clin. Invest. **78,** 934–939.

Dikken, H., and E. Kmety, 1978: Serological typing methods of leptospiras. In: Bergan, T., and J. R. Norris (eds), Methods in Microbiology. Academic Press, London. **11,** pp. 259–308.

Fridriksdottir, V., L. L. Nesse, and R. Gudding, 1992: Seroepidemiological studies of *Borrelia burgdorferi* infection in sheep in Norway. J. Clin. Microbiol. **30,** 1271–1277.

Gill, J. S., R. G. McLean, D. F. Neitzel, and R. C. Johnson, 1993: Serologic analysis of white-tailed deer sera for antibodies to *Borrelia burgdorferi* by enzyme-linked immunosorbent assay and Western immunoblotting. J. Clin. Microbiol. **31,** 318–322.

Greene, R. T., R. L. Walker, W. L. Nicholson, H. W. Heidner, J. F. Levine, E. C. Burgess, M. Wyand, E. B. Breitshwerdt, and H. A. Berkhoff, 1988: Immunoblot analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. J. Clin. Microbiol. **26,** 648–653.

Grodzicki, R. L., and A. C. Steere, 1988: Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigens preparations for diagnosing early Lyme disease. J. Infect. Dis. **157,** 790–797.

Kazmierczak, J. J., and F. E. Sorhage, 1993: Current understanding of *Borrelia burgdorferi* infection, with emphasis on its prevention in dogs. J. Am. Vet. Med. Assoc. **203,** 1524–1528.

Kmety, E., and H. Dikken, 1988: Revised list of *Leptospira* serovars (accepted by the Subcommittee on the Taxonomy of *Leptospira* at the Manchester meeting, 5–6 September, 1986). University Press, Groningen.

Parker, J. L., and K. K. White, 1992: Lyme borreliosis in cattle and horses: a review of the literature. Cornell Vet. **82,** 253–274.

Steer, A. C., 1989: Lyme disease. New England J. Med. **321,** 586–594.

Voller, A., D. Bidwell, and A. Bartlett, 1980: Enzyme-linked immunosorbent assay. In: Manual of Clinical Immunology, 2nd ed. American Society for Microbiology, Washington DC. pp. 359–371.