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Veterinary Parasitology 130 (2005) 93–97
veterinary parasitology
www.elsevier.com/locate/vetpar

Toxoplasma gondii infection in adult llamas (*Lama glama*) and vicunas (*Vicugna vicugna*) in the Peruvian Andean region

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Received 2 December 2004; received in revised form 28 February 2005; accepted 21 March 2005

Abstract

The present study was designed to investigate *Toxoplasma gondii* infection in adult llamas (*Lama glama*) and vicunas (*Vicugna vicugna*) in the Peruvian Andean region, for which to date no information has been available. Serum samples from 43 llamas (*L. glama*) and 200 vicunas were tested by IFAT detecting titres of 1:50 or higher in 55.8% (33.9–70.9%) and 5.5% (2.8–9.6%), respectively. IFAT titres ranged from 1:50 to 1:6400. In order to avoid cross reactions with closely related coccidian parasites and to confirm the existence of *T. gondii* specific antibodies, IFAT positive sera from both ruminant species were also analysed by western blot. *T. gondii* specific antigens were recognised by IFAT positive sera, although different IFAT cut-off points could be selected for llamas (1:200) and vicunas (1:50) meaning seroprevalence of 44.2% (29.1–60.1%) and 5.5% (2.8–9.6%), respectively. Based on the frequency and intensity of tachyzoite antigen recognition, at least three immunodominant antigens with apparent molecular weights of 22–24, 30, and 38–40 kDa were detected, together with other minor protein fractions located in the 18–73 kDa range. This study documents for the first time the presence of *T. gondii* infection and reports the target *T. gondii* antigens in adult llamas and vicunas in Peru.

Keywords: *Toxoplasma gondii*; Llama; Vicuna; Peru; Seroprevalence; Antigens recognition

1. Introduction

Toxoplasma gondii is a widespread, cyst-forming coccidian parasite that can cause severe disease in humans and domestic animals. Whilst domestic and wild cats are the only definitive host, a wide range of intermediate hosts, including South American camelids have been described (Fernández-Baca, 1975; Leguía, 1991). Preliminary data have been published on serology of alpacas (*Vicugna pacos*) and llamas (*Lama glama*) assessed by DAT methodology (Dubey et al., 1992; Gorman et al., 1999). Local reports provided preliminary data on the seroprevalence of toxoplasmosis based on agglutination assays in

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doi:10.1016/j.vetpar.2005.03.023

alpacas, llamas and vicunas (*Vicugna vicugna*) in Peru (Leguía and Casas, 1999). These animals play an important economic role in the Andean region. However, no attempts have been made to evaluate the utility for the diagnosis in South American camelids of the serological methods currently in use for toxoplasmosis in humans or domestic ruminants.

The objective was contribute to knowledge about the serological diagnosis of *T. gondii* infection in South American camelids in Peru. IFAT and western blot were used to evaluate IgG responses and to characterise the target antigens for *T. gondii*-specific IgG immune responses in domesticated llama and wild vicuna, two species representing different families of camelids. Moreover, the present work reports data on the seroprevalence of *T. gondii* infection in adult llama and vicuna in Peru.

2. Materials and methods

The adult llamas (1–3 years old) and vicunas (2–4 years old) included in the present study belonged to flocks reared under an extensive husbandry system in the main productive areas of South American camelids in Peru, Central and South Sierra. Serum samples were collected from December 2002 to July 2003 and kept in a serum bank at the School of Veterinary Medicine in Lima. Llama sera ($n = 43$) and vicuna sera ($n = 200$) were screened for *T. gondii* antibodies on ME49 *T. gondii* tachyzoite-coated IFAT slides at a unique dilution of 1:50. Positive sera were titrated by IFAT in two-fold serial dilutions from 1:50 to 1:6400. To ensure that antibodies detected by IFAT were specific against *T. gondii*, western blot was carried out on all positive and eight negative serum samples from both llama and vicuna species. Sera from *Neospora* infected llamas with IFAT titres of 1:50 ($n = 2$), 1:100 ($n = 2$) and 1:800 ($n = 2$) were also tested by western blot in order to detect possible cross reacting antigens. Since *Neospora* infection has not been detected in vicunas (data not shown), sera from this species were not included in this part of the study. In both cases, a goat antibody anti-llama IgG, FITC-conjugated (VMRD, Pullman, Washington) (1:1 dilution) was employed. Confidence intervals for the prevalence estimates were calculated using a 95% confidence level.

Soluble protein for electrophoresis was prepared as follows: isolated tachyzoites of *T. gondii* (ME49 strain) were disrupted at a concentration of 10^8 tachyzoites per milliliter in sample buffer by heating at 100 °C for 5 min in a water bath. Thereafter, insoluble material was removed by centrifugation at 12,000 × *g* for 30 min, and the supernatant containing aqueous buffer soluble proteins was collected and heated at 100 °C for 5 min under reducing conditions with 5% β-mercaptoethanol (v/v). Electrophoresis in 12.5% SDS-PAGE, electrotransference to nitrocellulose membranes, and western blots were performed as previously described (Álvarez-García et al., 2002a; Chávez-Velásquez et al., 2004). Following incubation with sera (1:25 dilution), the membranes were exposed to goat antibody anti-llama IgG (1:7 dilution) and then incubated again with anti-goat IgG conjugated with peroxidase (Sigma, St. Louis, MO) at 1:1000 dilution. Western blot results were based on the reactivities of the sera with major proteins located in the 18–40 kDa range, according to a previous work conducted with small ruminants (Conde et al., 2001).

The relationship between IFAT titres and *Toxoplasma* antigen recognition in the western blot was also studied in llamas and vicunas, and in both cases IFAT titres were compared with the total number of antigens recognised by serum samples.

3. Results

The IFAT results showed that 24 llamas (55.8%; 33.9–70.9%) and 11 vicunas (5.5%; 2.8–9.6%) had detectable *T. gondii* antibody titres. As shown in Table 1, IFAT titres ranged from 1:50 to 1:6400, with a predominance of titre equal to or higher than 1:200 in both species. All positive IFAT sera from vicunas

Table 1
Frequency distribution of IFAT titres to *T. gondii* found in 43 llamas and 200 vicunas

	Number of animals							
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400
Llama (19)[a]	3	2	3	1	7	5	0	3
Vicuna (189)[a]	1	1	0	0	4	1	2	2

[a] Animals negative by IFAT.