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REGULAR ARTICLES

Toxoplasma gondii and *Neospora caninum* seroprevalences in domestic South American camelids of the Peruvian Andes

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Abstract The objective of this study was to investigate the presence of *Toxoplasma gondii*- and *Neospora caninum*-specific antibodies in domestic South American camelids (SAC) (llamas and alpacas) from the Peruvian Andes through a cross-sectional study. A wide panel of serum samples collected from 1,845 llamas and 2,874 alpacas from the two main SAC production areas of Peru was selected. Immunofluorescence antibody technique was employed to detect and titrate specific anti-*T. gondii* and anti-*N. caninum* immunoglobulins G in serum samples. The association between *T. gondii* and *N. caninum* seroprevalence and the geographical origin (Central and South Peruvian Andes) was evaluated. Anti-*T. gondii* antibodies were found in 460 (24.9 %) llamas and 706 (24.6 %) alpacas, whereas anti-*N. caninum* antibodies were detected in 153 (8.3 %) llamas and 425 (14.8 %) alpacas. *Toxoplasma gondii* infection was strongly associated with the South Peruvian Andes where moderate climate conditions, larger human population, compared to the Central region, and the presence of wildlife definitive hosts could favor horizontal transmission to SAC. In contrast, *N. caninum* infection was not associated with the geographical region. These results indicate that *T. gondii* and *N. caninum* infections are highly and moderately widespread, respectively, in both species of domestic SAC studied in the sampled areas and appropriate control measures should be undertaken to reduce the prevalence of both parasitic infections.

Keywords Llamas · Alpacas · Peru · *Toxoplasma* · *Neospora* · Seroprevalence

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Introduction

South American camelids (SAC) are the native ruminant species throughout the Andean mountains and play an important role in the local economy of countries in this geographical region (Quispe et al. 2009). In particular, Peru ranks first in the world in possession of alpacas and second in possession of llamas, after Bolivia, and they are the main means of subsistence of at least 1.5 million of people in the Peruvian Andean region (Fernández-Baca 2005; Quispe et al. 2009). Llamas (*Lama glama*) and alpacas (*Vicugna pacos*) are raised at altitudes between 2,000–4,000 m above sea level (m.a.s.l) and can share the environment with cattle and sheep. Two major veterinary problems affecting llamas and alpacas are high abortion and neonatal mortality rates, caused primarily by parasitic and infectious agents (Rivera et al. 1987; Fernandez-Baca 1991; Leguía 1991; Tibary et al. 2006). *Toxoplasma gondii* and *Neospora caninum* are closely related apicomplexan parasites that have worldwide distribution. Moreover, at present, *T. gondii* and *N. caninum* are two of the most threatening

Table 1 Census, sample size, and absolute error regarding geographical region of llamas and alpacas

		Central Total	South Total
Llamas	Census	241,977	537,826
	Sample size	756	1,089
	Absolute error (%)	3.57	2.97
Alpacas	Census	386,218	2,028,147
	Sample size	1,541	1,333
	Absolute error (%)	2.49	2.68

abortifacient agents in small ruminants and bovines, respectively (Innes et al. 2009; Dubey and Schares 2011; Moreno et al. 2012). There is serological evidence that toxoplasmosis and neosporosis may be involved in abortion and neonatal mortality in SAC. In fact, the presence of anti-*Toxoplasma*-specific antibodies has been documented in llamas, alpacas, and vicunas (Gorman et al. 1999; Chávez-Velásquez et al. 2005; Wolf et al. 2005) and a clinical fatal case of post-natal-acquired acute toxoplasmosis has been recently described in a llama (Dubey et al. 2014). Similarly, serological evidence of infection by *N. caninum* has also been reported in adult llamas and alpacas from the Andean region (Chávez-Velásquez et al. 2004; Wolf et al. 2005). In addition, *N. caninum*-associated abortion is suspected to occur in SAC because both compatible lesions and *N. caninum* DNA have been detected in brain tissues from llama and alpaca fetuses (Serrano-Martinez et al. 2004; Serrano-Martínez et al. 2007). However, the participation of *T. gondii* in abortion in SAC is not clear yet. Cheney and Allen (1989) detected *T. gondii* antibodies in fetal fluids from two cases of abortion in llamas, whereas Serrano-Martínez et al. (2007) did not detect parasite DNA or compatible lesions in aborted alpaca and llama fetuses. In addition, the epidemiology of neosporosis and toxoplasmosis in domestic SAC (llamas and alpacas) has been poorly studied. Wolf et al. (2005) pointed out the necessity of determining the level of infection of *T. gondii* and *N. caninum* in SAC by investigating an appropriate number of animals according to the census in different areas. Thus, taking into consideration the importance of SAC in the Andean countries, the present study was conducted to determine the seroprevalence of *T. gondii* and *N. caninum* infections in a wide population of llamas and alpacas from the two main SAC-producing regions in Peru (Central and South Peruvian Andes).

Materials and methods

Target population and geographical location

In the present cross-sectional study, serum samples were collected in the Central and South Peruvian Andes, which are the

Table 2 Individual seroprevalence of *T. gondii* infection and IFAT titer distribution in SAC

	Prevalence (seropositives/analyzed)[a]	Frequency of seronegatives (negative animals/analyzed)			IFAT titer frequency (seropositives for each titer/total seropositives)					
		–	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400
Llamas	24.9 (460/1,845)	45.1 (832/1,845)	16.4 (302/1,845)	13.6 (251/1,845)	50.4 (232/460)	42.4 (195/460)	5 (23/460)	1.5 (7/460)	0	0.6 (3/460)
Alpacas	24.6 (706/2,874)	46.5 (1,337/2,874)	14.8 (428/2,874)	14 (403/2,874)	56.4 (398/706)	38.1 (269/706)	2.6 (18/706)	1.7 (12/706)	0.4 (3/706)	0.8 (6/706)
Total	24.7 (1,166/4,719)	45.9 (2,169/4,719)	15.5 (730/4,719)	13.9 (654/4,719)	54 (630/1,166)	39.8 (464/1,166)	3.5 (41/1,166)	1.6 (19/1,166)	0.3 (3/1,166)	0.8 (9/1,166)

[a] Seropositive is considered when IFAT titers are ≥1:200

most representative regions for the production of SAC in Peru. The Central Peruvian Andes has an average altitude of 3,585 m.a.s.l (INEI, http://www.inei.gob.pe/) and dry (between May and October) and rainy seasons (between November and April) with annual average temperatures ranging between 2–18ºC. This region comprises the departments of Junin, Pasco, Huanuco, and Huancavelica, with a total census of 386,218 alpacas and 241,977 llamas (Fernández-Baca 2005). The south highland region, located in the South Andes Mountains, is 3,700 m.a.s.l. It has well-defined climatic conditions, with a rainy season between December and March and a dry season between May and August. This region includes the departments of Puno and Cuzco and possesses 2,028,147 alpacas and 537,826 llamas (Fernández-Baca 2005).

In both regions, there are wild canids, such as the Andean fox (*Lycalopex culpaeus*), and wild felids, such as pumas (*Felis concolor*), pampas cats (*Oncifelis colocolo*), and Andean cats (*Oreailurus jacobita*), in addition to domestic dogs and cats associated with human settlements and villages.

SAC in both geographical regions are mainly produced by peasant communities (Fernández-Baca 2005) (representing about the 80 % of SAC production) characterized by extensive traditional management with shared pastures and mixed flocks including sheep and sometimes cattle. Males and females are not separated during the breeding, and shearing and deworming programs are applied but irregularly. The rest of SAC production (about 20 %) is managed by livestock companies which include farm cooperatives and some experimental flocks. These semi-extensive herds are characterized by a larger number of animals, sometimes with higher animal density, semi-controlled mating, and breeding with separation between males and females (Wurzinger et al. 2008) and health programs applications. There is no exchange of animals between both systems and animals remain in their original geographic region.

Samples and data

The sampling units were llamas or alpacas over 2 years old from the Central and South Peruvian Andes. A total of 1,845 llamas and 2,874 alpacas from peasant communities or livestock companies were sampled.

Convenience sampling was undertaken due to difficulties in accessing some areas. However, based on the census, the number of animals sampled was large enough in both geographical regions to give an absolute error less than 4 %, (with a CI of 95 % and an expected prevalence of 50 %) (Table 1). Ninety-five percent of samples corresponded to females and 5 % to males. Reproductive data were not available.

Blood samples were collected from the jugular vein using vacuum collecting tubes (10-ml BD Vacutainer®, Plymouth, UK). The serum was separated, aliquoted, and stored at −20ºC until analysis.

Immunofluorescence antibody test (IFAT)

In this study, the protocol for immunofluorescence antibody test (IFAT) described by Chavez-Velasquez et al. (2004) was used to detect the existence of specific anti-*T. gondii* and anti-*N. caninum* IgGs in serum samples from domestic SAC. Tachyzoites of *T. gondii* ME 49 strain and *N. caninum* Nc-1 isolate were employed. A cut-off of 1:200 was considered for *Toxoplasma* infection in domestic SAC (alpacas and llamas) (Chávez-Velásquez et al. 2005), whereas for *N. caninum* infection, a cut-off of 1:100 was considered (Chávez-Velásquez et al. 2004) based on previous western blot—IFAT comparisons.

Samples were titrated by means of double serial dilutions, and a goat anti-llama IgG conjugated with fluorescein isothiocyanate (Lab. VMRD Inc., Pullman, WA, USA) was used. A pool of positive sera from *Toxoplasma*- and *Neospora*-infected camelids tested previously by western blot (Chávez-Velásquez et al. 2004, 2005) was employed as a positive control. A pool of negative sera from camelids tested previously by WB was also employed as a negative control.

Data analysis

Absolute errors (%) of sample sizes were calculated using basic epidemiologic software (Win Episcope V. 2.0.). A 95 % confidence interval (CI) was used. Because no previous

Table 3 Individual seroprevalence of *T. gondii* infection in SAC according to the geographical region

	Geographical region	Seroprevalence (seropositives/analyzed)	Chi-squared test	OR (CI 95 %)
LLAMAS (*Lama glama*)	Central	17.7 (134/756)	χ^2=34.9; p<0.05	1.98 (1.58–2.49)
	South	30.0 (326/1,089)		
ALPACAS (*Vicugna pacos*)	Central	20.2 (311/1,541)	χ^2=33.9; p<0.05	1.67 (1.40–1.98)
	South	29.6 (395/1,333)		

OR odds ratio; *CI* confidence interval

data about expected prevalence were available, a 50 % expected prevalence was employed.

The differences in prevalence rates of *T. gondii* and *N. caninum* infections observed according to the geographical region (Central and South Peruvian Andes) were analyzed by the chi-squared test and the odds ratio (OR) using a 95 % CI.

For statistical analysis, two programs were used (StatCalc program of the EPI INFO V.6 and STATGRAPHICS Plus v.5.1, StatPoint, Inc., Herndon, VA, USA) (Thrusfield et al. 2001).

Results

Toxoplasma gondii seroprevalence and influence of geographical region

The individual prevalence rates of anti-*T. gondii*-specific antibodies were similarly high in both species of SAC (24.9 and 24.6 % for llamas and alpacas, respectively) (Table 2). According to IFAT titers, most seropositive animals showed titers close to the cut-off, and only 6.2 % (72 out of 1,166) showed titers equal to or higher than 1:800.

The association between the presence of *T. gondii*-specific antibodies and the geographical region in both SAC species are summarized in Table 3. A significant higher seroprevalence was observed in the South Peruvian Andes for both llamas and alpacas (χ^2=34.9 and χ^2=33.9, respectively, $p<0.05$) (Table 3). According to OR values, there is also a strong association between *T. gondii* seroprevalence and the South Peruvian Andes in llamas (OR=1.98) and alpacas (OR=1.67) (Table 3).

Neospora caninum seroprevalence and influence of geographical region

N. caninum-specific antibodies were found in 8.3 % of llamas and 14.8 % of alpacas. Individual prevalence data and IFAT titers are summarized in Table 4. Most predominant IFAT titers were close to the cut-off, and only 12.9 % (75 out of 578) showed titers equal to or higher than 1:800. Only 1.03 % (19/1845) of llamas and 1.29 % (37/2874) of alpacas exhibited specific antibodies against both *T. gondii* and *N. caninum*.

Contrary to the observations made in *T. gondii* infection, similar seroprevalence rates in both species of SAC were detected in Central and South Peruvian Andes (χ^2=1.7 and χ^2=0.9; $p>0.05$) (Table 5). OR values also showed no association between seroprevalence and geographical region for both species (OR=1.26 in llamas; OR=1.10 in alpacas).

Table 4 Individual seroprevalence of *N. caninum* infection and IFAT titer distribution in SAC

	Prevalence (seropositives/analyzed)[a]	Frequency of seronegatives (negative animals/analyzed)	IFAT titer frequency (seropositives for each titer/total seropositives)						
		–	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:6,400
Llamas	8.3 (153/1,845)	85.1 (1,571/1,845)	6.6 (121/1,845)	66.7 (102/153)	13.7 (21/153)	9.2 (14/153)	10.5 (16/153)	0	0	0
Alpacas	14.8 (425/2,874)	75.9 (2,182/2874)	9.3 (267/2,874)	54.4 (231/425)	30.1 (128/425)	1.6 (7/425)	12.2 (52/425)	1.6 (7/425)	0	0
Total	12.2 (578/4,719)	79.5 (3,753/4,719)	8.2 (388/4,719)	57.6 (333/578)	25.8 (149/578)	3.6 (21/578)	11.8 (68/578)	1.2 (7/578)	0	0

[a] Seropositive is considered when IFAT titers are ≥1:100

Table 5 Individual seroprevalence of *N. caninum* infection in SAC according to the geographical region

	Geographical region	Seroprevalence (seropositives/analyzed)	Chi-squared test	OR (CI 95 %)
LLAMAS (*Lama glama*)	Central South	7.2 (55/756) 9.0 (98/1,089)	χ^2=1.7; p>0.05	1.26 (0.89–1.77)
ALPACAS (*Vicugna pacos*)	Central South	20.2 (311/1,541) 29.6 (395/1,333)	χ^2=0.9; p>0.05	1.10 (0.89–1.35)

OR odds ratio; *CI* confidence interval

Discussion

At first, large-scale serosurvey was conducted to determine the prevalence of *T. gondii* and *N. caninum* infection in llamas and alpacas from the two main SAC-producing regions in Peru, allowing a representative overview of the importance of both infections in Peruvian domestic SAC. The presence of *T. gondii* and *N. caninum* infections in adult domestic SAC from Peru has been already reported (Chávez-Velásquez et al. 2004, 2005; Wolf et al. 2005). In addition, *N. caninum* seems to be implicated in abortions in these species (Serrano-Martinez et al. 2004; 2007). Although there are no data on sensitivity and specificity values of *T. gondii* and *N. caninum* IFAT on SAC, cut-offs were selected based on IFAT and western blot comparisons showing non-specific binding by western blot in negative sera and sera with IFAT titers equal to 1:50 (*N. caninum*) or equal to 1:100 (*T. gondii*) (Chávez-Velásquez et al. 2004, 2005).

Our results provide evidence that *T. gondii* infection is widely present in both SAC species in the sampled area, which could be explained by breeding close to human shelters where cats kept by the breeders can act as the definitive host. The lower individual seroprevalence of *N. caninum* infection could be explained by a reduced presence of cattle livestock in this region of the Peruvian Andes, thus reducing the main source of infection for dogs, the definitive host of *N. caninum* (Moré et al. 2008). In Argentina, a similar high individual seroprevalence of *T. gondii* infection and a lower prevalence of *N. caninum* infection were also reported in llamas (Moré et al. 2008). Moreover, most IFAT titers were close to the cut-off in both infections, in agreement with previous works in cattle indicating a possible predominance of subclinical chronic infections since acute infections are normally associated to high IFAT titers (Quintanilla-Gozalo et al. 2000).

Our results showed a significant influence of the geographical region in *T. gondii* infection. Thus, a significantly higher prevalence rate in both alpacas and llamas was obtained in the South Peruvian Andes. In this region a larger human population is found (2,600,000 in the South Peruvian Andes versus 1,700,000 in the Central Peruvian Andes) (INEI, http://www.inei.gob.pe/), and, therefore, frequent contact with domestic cats occur. In addition, in this region, milder climatic conditions could favor the survival of oocysts, in contrast to the dry climate of the central highland region that may adversely influence the oocysts' viability. Similar differences attributed to climate conditions have been found by Panadero et al. (2010) in sheep from coast or center regions of Galicia, Spain. Although there is only one study reporting the detection of oocysts in pampas cats (Elmore et al. 2010), it may be indicative of the presence of sylvatic cycle of *T. gondii* in the Andean region, and it is expected that other wild felid species may be involved as in other parts of the world. Indeed, the south highland region has a higher density of wild felids (E. Serrano-Martínez, personal communication, 2012) that could also act as definitive hosts and contribute to higher prevalence rates of *T. gondii* infection in intermediate hosts.

As for *T. gondii*, both llamas and alpacas are susceptible to *N. caninum* infection but the seroprevalence is substantially lower than for *T. gondii*. In addition, no significant association between the prevalence of *N. caninum* infection and geographical origin was found. It is difficult to assess the predominant transmission route of *N. caninum* in these populations and studies including SAC age or dog prevalence as risk factors must be undertaken. However, in contrast to *T. gondii* infection, our results provide evidence that climate or human factors associated with the geographical region does not influence *N. caninum* infection, which could suggest that infection spread is mainly governed by vertical transmission. The predominant vertical transmission of *N. caninum* has been widely described in cattle where a 1.4 % horizontal transmission incidence was reported by Bartels et al. (2007).

Our study is pioneering in determining the seroprevalence of *T. gondii* and *N. caninum* in domestic SAC from the two main SAC-producing areas of Peru. The results show that both parasitic infections are spread in llamas and alpacas from Peruvian Andes. In *T. gondii* infection, a strong effect of the environment suggests the dependence of definitive hosts (domestic or wildlife) in the epidemiology of this infection in SAC, whereas *N. caninum* infection could be maintained by mainly vertical transmission in these populations. However, the common presence of sylvatic and domestic cycles cannot be discarded in both llamas and alpacas. This interaction has been previously evidenced in *T. gondii* and *N. caninum* infections by Panadero et al. (2010), as specific antibodies

were found in different ruminant species that shared pastures. Further work should be done involving the presence of sheep and cattle, SAC age, SAC gender, and prevalence in felids and canids as potential risk factors in these areas to extend the knowledge on the epidemiology of both parasitic infections in SAC. Appropriate control measures to diminish the dissemination of these agents in the domestic SAC-rearing areas could be the same as those recommended for sheep and cattle (Conraths 2007; Buxton 2007).

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Conflict of interest All authors have given informed consent prior to their inclusion in the study and certify that there is no conflict of interest with any financial organization.

Ethical statement All the animal studies were carried out according to the international standards for animal experimentation and accomplish with Spanish and EU legislation about the use of animals for Science purposes (Spanish law 6/2013 and Council Directive 2010/63/UE).

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