

First report of seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy sheep from Humid Pampa, Argentina

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Abstract The aim of this study was to describe the occurrence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in dairy sheep from the Humid Pampa region, Argentina. Blood samples from 704 dairy sheep belonging to six flocks were collected. Using a cut off titer of 1:50, an indirect fluorescence antibody test was used. Antibodies to *T. gondii* or *N. caninum* were detected in 17.3 % ($n=122$) and 3 % ($n=21$), respectively. All the flocks had at least one seropositive animal to *T. gondii* but two of them had no seropositive sheep to *N. caninum*. Fifty-two of 122 (42.6 %) positive samples to *T. gondii* had antibody titers higher than 1:400. There was a significantly higher proportion of *T. gondii* seropositive animals in females and older sheep ($p<0.05$). Ten of 21 (52.3 %) positive samples to *N. caninum* had antibody titers higher than 1:400. This is the

first report of seroprevalence of *T. gondii* and *N. caninum* in dairy sheep from Humid Pampa, Argentina. Further research is required for a better understanding of the role of toxoplasmosis and neosporosis in dairy sheep in Argentina.

Keywords *Toxoplasma gondii* · *Neospora caninum* · Seroprevalence · Dairy sheep

Introduction

Toxoplasma gondii and *Neospora caninum* are obligate intracellular protozoan parasites that infect a large range of intermediate hosts (Dubey and Shares 2011). *T. gondii* not only causes reproductive failure in goats and sheep that are infected during pregnancy, it is also common parasitic zoonoses (Dubey 2009). Although the disease is present in Argentina, data from this country has been scarcely reported worldwide (Dubey 2009). Neosporosis has shown to be a common cause of abortion in cattle and there is evidence of *N. caninum* exposure in many animal species (Dubey and Schares 2011). Reports about *N. caninum* infections in sheep in Argentina are not available yet.

Dairy sheep flock is growing in Argentina, particularly in the Humid Pampa region, where 20 of the nation's 48 dairy sheep farms are located. Although the sheep industry has socioeconomic importance in Argentina, yet data regarding the causes of reproductive losses is limited. Considering the relevance of toxoplasmosis as a zoonoses and the lack of information concerning *N. caninum* in dairy sheep, this study aimed to determine the occurrence of antibodies for both *T. gondii* and *N. caninum* protozoa in dairy flocks located at the Humid Pampa region, Argentina.

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Table 1 Sheep flocks, animals number tested and category in each flock, and seroprevalence to *T. gondii* and *N. caninum*

Flocks	Category	Total number of animals in the flock	Number of sampled animals	Seroprevalence to <i>T. gondii</i> (CI)	Seroprevalence to <i>N. caninum</i> (CI)
1	R, E	160	58	8.6 (1.4–15.8)	8.6 (1.4–15.8)
2	R, E, Y	330	330	10.3 (7–13.6)	0.9 (0–1.9)
3	R, E, Y	300	54	51.9 (38.5–65.2)	0
4	R	375	35	8.6 (0–17.8)	0
5	R, E, Y	130	75	24 (14.3–33.7)	4 (0–8.4)
6	E	152	152	22.4 (15.7–28.9)	6.6 (2.6–10.5)

CI confidence interval, R ram, E ewe, Y yearling ewe

Materials and methods

A cross-sectional study was conducted on 6 out 20 dairy flocks located in the Humid Pampa because only the farmers that were willing to collaborate with the project were included. This study was a part of a national project by the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina, to improve the information related to the dairy sheep industry. Data about sheep sex and category (ewe: E, yearling ewe: Y, ram: R) were available. All sheep were Pampinta breed (crossbreed 3/4 Friesian and 1/4 Corriedale) bred by INTA Anguil, Argentina.

Serum samples were obtained from 704 sheep (Table 1) representing the average type of animal of the flock and were tested for *T. gondii* or *N. caninum* antibodies using indirect fluorescence antibody test (IFAT). A test was considered positive if the titer was $\geq 1:50$ (Figliuolo et al. 2004, Dubey 2009). Positive reactions that had the highest serological dilution were considered the end-point titer. A polyclonal rabbit anti-sheep IgG labelled with fluorescein isothiocyanate (SIGMA® F5137, St. Louis, USA) was used as conjugate. The associations between serological status and sex or class were evaluated by applying Chi-square test or Fisher's exact test (EpiDat v 4.0, software, Spain).

Results and discussion

The seroprevalence for *T. gondii* was 17.3 % (IC_{95%}, 14.5–20.1 %). Our results are similar to those obtained by studies in Brazil and Sweden (Lundén et al. 1992; Gondim et al. 1999). All flocks had at least one seropositive animal and the prevalence ranged from 5.5 to 61.8 %. From the 122 positive samples to *T. gondii*, 52 (42.6 %) had titers higher than 1:400 (Table 2). These high titers indicate currently active infection (Dubey 2009).

According to class, the seroprevalence of *T. gondii* was significantly higher in adults (≥ 1 year) ($p < 0.05$) in conjunction with previous studies (Figliuolo et al. 2004; Rossi et al. 2011) indicating that horizontal transmission of sporulated oocysts by ingestion seems to be a main route of infection. In addition, the prevalence in females (20 %) were

significantly higher than in males (6 %) ($p < 0.05$) with seroprevalence rate of 3.37 (IC_{95%}, 1.75 to 6.48). These results are in contrast to other studies (Soares et al. 2009; Ueno et al. 2009) but in agreement with those reported by Van Der Puije et al. (2000).

The seroprevalence to *N. caninum* was 3 % (IC_{95%}, 1.7–4.2 %) which is in agreement with previous studies (Špilovská et al. 2009; Martins de Brito Moraes et al. 2011). Higher frequencies (ranged from 9.2 % of 597 to 63 % of 339) were found in Brazil and Jordan (Figliuolo et al. 2004; Abo-Shehada and Abu-Halaweh 2010). In contrast, other authors reported lower prevalence in northeastern of Brazil (Soares et al. 2009). Four out of six (66.6 %) flocks had at least one seropositive animal. The flock prevalence ranged from 3 to 10 % with 21 positive sheep. Of these sheep, 11 (52.3 %) had titers higher than 1:400 (Table 2). No significant association was observed when variables were examined via Chi-square or Fisher's exact test due to limited positive samples preempting *N. caninum* analysis. In this study, 52.3 % animals expressed end titers of *N. caninum* that were higher than 1:400. Unlike *T. gondii*, where positive serology is indicative

Table 2 Distribution of IgG antibody titers or levels anti-*T. gondii* and anti-*N. caninum* determined by IFAT in 704 sheep from flocks located in the Humid Pampas, Argentina

Titer	<i>T. gondii</i> positive, n (%)	<i>N. caninum</i> positive, n (%)
50	2 (1.6)	6 (28.6)
100	53 (43.4)	0 (0)
200	15 (12.3)	4 (19.0)
400	10 (8.2)	0 (0)
800	12 (9.8)	5 (23.8)
1,600	11 (9.0)	2 (9.5)
3,200	15 (12.3)	4 (19.0)
6,400	4 (3.3)	0 (0)
Total positive	122 (17.3)	21 (3.0)
Total number	704 (100)	704 (100)

IFAT titers considered positive for *T. gondii* and *N. caninum* were IFAT ≥ 50

of active infection, positive serology to *N. caninum* only provides evidence of exposure (Figliuolo et al. 2004).

Positive samples to both *N. caninum* and *T. gondii* in three sheep were observed: one in flock 5 and two from flock 6. The antibodies titers were lower than 1:200 for both parasites. IFAT is considered as a reference test for *N. caninum* in several animal species and has no cross-reactivity with related protozoan parasites (Dubey and Schares 2011).

This is the first report of seroprevalence of *T. gondii* and *N. caninum* in dairy sheep from Humid Pampa, Argentina. Our data displayed a higher prevalence of infection by *T. gondii* than *N. caninum* in Humid Pampa dairy flocks. The low prevalence of *N. caninum* suggests that there is low reproductive impact from this parasite in sheep farms within the Humid Pampa region. To better understand infection by these agents in this region, further investigations are required that sample larger sheep populations, and provide detailed information on reproductive loss and etiological characterization.

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