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Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections in goats from two Argentinean provinces

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Abstract

The aim of this study was to compare the seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in goats from two Argentinean provinces raised under different management conditions. A total of 2922 serum samples from adult goats of Córdoba (n=2187) and Buenos Aires provinces (n= 735), Argentina, were assayed by indirect fluorescence antibody test (IFAT, cut-off 1:100) for antibodies to *T. gondii* and *N. caninum*. Seroprevalence was 40.8% (CI 39.0%-42.6%) and 5.5% (CI 4.7%-6.4%) for *T. gondii* and *N. caninum*, respectively. The seropositivity for both infections was higher in goats from dairy farms, resulting as follows: for *T. gondii* 32.7% (CI 30.8%-34.8%) in extensive farms and 59.3% (CI 56.1%-62.6%) in dairy farms and for *N. caninum* 4.1% (CI 3.2%-4.9%) in extensive farms and 8.8% (CI 6.9%-10.7%) in dairy farms. This is the first extensive seroepidemiology investigation for *T. gondii* and *N. caninum* in goats in Argentina.

Keywords: Goat, *Neospora caninum*, Serology, *Toxoplasma gondii*.

Introduction

The protozoans *Toxoplasma gondii* and *Neospora caninum* cause reproductive and economic losses to livestock industry and are considered an important cause of abortion in small ruminants (Dubey, 2010; van den Brom *et al.*, 2012; Dubey *et al.*, 2017). Additionally, toxoplasmosis continues to be a public health problem worldwide. Argentina has 4.7 million goats which play an important role in the family income through the provision of milk, meat, skin and manure. Most goat production in Argentina is in small herds, based on Creole breed with extensive management. In a minor proportion, the production is developed in a semi-intensive or intensive management system, where goats are mainly used for milk and cheese production (SENASA, 2016). Little is known of the importance of toxoplasmosis and neosporosis in goats in Argentina, except two old references (Venturini *et al.*, 1993; Moore *et al.*, 2007).

The aim of this study was to evaluate the seroprevalence of *T. gondii* and *N. caninum* in goats from two Argentinean provinces raised under different management conditions.

Materials and Methods

Samples

Serum samples from adult goats (n= 2922) were obtained from three districts of Córdoba (n=2187) and

Buenos Aires provinces (n= 735), Argentina. All the samples were collected as part of a national small ruminant health surveillance program, which was held in these two provinces. The total number of animals sampled for each province and per herd was estimated to detect prevalence lower than 5% with 95% of confidence.

Buenos Aires

A total of 735 samples from 17 dairy herds were collected by the *Ministerio de Asuntos Agrarios* from Buenos Aires province as part of a brucellosis surveillance program, most of them located in the northeast of the province. Most flocks were under intensive management, mainly dedicated to the production of dairy products. All herds had Saanen breed goats except one (Anglo Nubian and cross-breed).

The average herd size was 110 goats (range 12-322). Up to 50 sera per herd were randomly selected. When the number of animals per herd was less than 50 goats, serum samples from all the animals were tested (Manazza *et al.*, 2012a).

Córdoba

A total of 2187 goat serum samples were obtained from 76 herds. The sampled districts were *Cruz del Eje*, *Pocho* and *Minas*, located in the northwest of the province, which represent the 75% of goat population

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in Córdoba (Sánchez, 2013). Out of all samples, 2037 were obtained from 73 herds belonging to small goat farmers: Creole type breed, under extensive management conditions. Samples distribution: *Cruz del Eje* (n=766, 26 herds), *Pocho* (n= 675, 23 herds) and *Minas* (n= 596, 24 herds). The remaining 150 samples belong to 3 commercial dairy farms (DF) with Saanen breed goats located in *Cruz del Eje* (n=86, 1 herd) and *Pocho* (n =64, 2 herds) (Manazza et al., 2012b). Average herd size was 30 animals (range 4-86). Up to 30 sera per herd were randomly selected. When the number of animals per herd was less than 30 goats, serum samples from all the animals were tested. In DF all animals were sampled.

Serology

Serum samples were stored at -20°C until serological studies were performed. Presence of antibodies to *T. gondii* and *N. caninum* from serum samples was determined by indirect fluorescent antibody test (IFAT) proceeding as described previously (Unzaga et al., 2014).

T. gondii (RH strain) and *N. caninum* (NC1 strain) tachyzoites were used as antigen. Sera were tested at 1:100 PBS dilution using rabbit anti-goat Ig G-FITC conjugate (Sigma-Aldrich, St Louis, USA). Positive and negative goat sera for each protozoan infection were used as controls. Complete peripheral fluorescence of tachyzoites was considered as a positive reaction (Paré et al., 1995).

Data analysis

Seroprevalence and Confidence Interval (CI) at 95% for antibodies to *T. gondii* and *N. caninum* were calculated for all samples from both provinces. Differences among serologic prevalences from the different Córdoba province districts, as well between management conditions were analyzed by Chi-square test (χ^2) (<http://www.winepi.net/sp/index.htm>).

Results and Discussion

Overall seroprevalence was 40.8% (1192/2922) (CI 39.0%-42.6%) and 5.5% (162/2922) (CI 4.7%-6.4%) for *T. gondii* and *N. caninum*, respectively.

In Buenos Aires province, seroprevalence for *T. gondii* was 63% (463/735) (CI 59.5%-66.5%) and all herds were seropositive, with a range of intra-herd seroprevalence from 19.2 to 100%. The seroprevalence for *N. caninum* was 9.7% (71/735) (CI 7.5%-11.8%) and 14 herds (82.4%) were seropositive, with a range from 3.2 to 22.2% of intra-herd seroprevalence.

In Córdoba province, antibodies to *T. gondii* were found in 33% (CI 31.4%-35.3%) of 2187 goats and 94.7% of the herds were seropositive, with an intra-herd seroprevalence range of 3.4 to 100% (Table 1). Seroprevalence of district *Pocho* was significantly lower ($p \leq 0.05$). In districts *Cruz del Eje* and *Minas* 3 herds had 100% of seropositive animals and 3 with 0% of seropositive animals to *T. gondii*. Seroprevalence for

N. caninum was 4.2% (91/2187) (CI 3.32%-5.0%). Positive animals to *N. caninum* were found in 20 of 73 small farms: *Cruz del Eje* (15; with a range of intra-herd seroprevalence from 7.1 to 41.9%), *Minas* (4; with a range from 1.8 to 3.7%) and *Pocho* (1; 25%). Seroprevalence in *Pocho* and *Minas* was significantly lower than in *Cruz del Eje* and DF ($p \leq 0.05$). All commercial dairy farms were seropositive to *T. gondii* and *N. caninum* (Table 1).

The seroprevalences for *N. caninum* and *T. gondii* in goats from Buenos Aires province were significantly higher than in goats from Córdoba province ($p \leq 0.001$). The seroprevalence for *T. gondii* was 32.7% (667/2037) (CI 30.8%-34.8%) in extensive farms and 59.3% (525/885) (CI 56.1%-62.6%) in dairy farms; whereas the seroprevalence for *N. caninum* was 4.1% (83/2037) (CI 3.2%-4.9%) in extensive farms and 8.8% (78/885) (CI 6.9%-10.7%) in dairy farms. The seropositivity for both infections was higher in goats from dairy farms ($p \leq 0.001$).

The present work reports higher seroprevalence for *T. gondii* in goats (40.8%) compared to previous studies conducted in Argentina (Venturini et al., 1993) and many other countries (Dubey, 2010). The overall seroprevalence for *N. caninum* detected (5.5%) in the present study was similar to the 6.6% reported from La Rioja province in Argentina (Moore et al., 2007).

The seroprevalence for *T. gondii* and *N. caninum* in Argentinean goats were significantly higher in dairy farms with intensive management conditions than in farms with extensive management conditions. In Buenos Aires province, *T. gondii* seropositive animals were found in all herds and most herds (82.4%) showed at least one positive animal for *N. caninum*. Additionally, the seroprevalence of both protozoans were higher than in Córdoba suggesting a wide distribution of both infections in the region. These differences could be attributed to the production system of the analyzed herds, because in Buenos Aires all of them are mainly dedicated to the production of dairy products. It is known that intensification of management practices as well as the greater contact with cats and dogs, which are maintained as pets and for the prevention of rodents, are suggested as potential risk factors which could justify such differences (Uzêda et al., 2007; Topazio et al., 2014). It is also important to point out the environmental conditions of Buenos Aires province where the humidity is higher than in the northwest of Córdoba; humidity favors the longer survival of the oocysts in the soil, which often can remain viable and infective for more than 18 months (Dubey, 2010; Dubey et al., 2017).

In Córdoba province, although most of the goat breeding is carried out under extensive management conditions, *T. gondii* seropositive goats were detected in most of the extensive farms analyzed (94.5%).

Table 1. Distribution of seropositive goats and herds for toxoplasmosis and neosporosis in Córdoba province.

District	Herds	No. of goats	Toxoplasmosis					Neosporosis				
			Pos.	%	±	H+	%	Pos.	%	±	H+	%
<i>Cruz del Eje</i>	26	766	296	38.6 ^a	3.4	24	92.3	73	9.5 ^a	2.1	15	57.7
<i>Pocho</i>	23	675	151	22.4 ^b	3.1	21	91.3	6	0.9 ^b	0.7	1	4.3
<i>Minas</i>	24	596	220	36.9 ^a	3.9	23	95.8	4	0.8 ^b	0.7	4	16.7
DF	3	150	62	41.3 ^a	7.9	3	100	7	4.7 ^a	3.3	3	100
Total	76	2187	729	33.3	2.0	71	93.4	91	4.2	0.8	23	30.3

(±): CI 95%; (H+): positive herds; (DF): Dairy farms; different letters indicate significant differences.

Despite the fact that in the Northwest of Córdoba the weather is dry and arid, animals are confined in pens and therefore the concentration of urine and faeces could change the environment conditions and create a propitious situation for oocysts survival. During the day, goats are grazing freely in distant areas where wild cats, such as puma (*Felis concolor*) and gato montés sudamericano (*Leopardus geoffroyi*) are frequently observed. The role of wild felines in toxoplasmosis has not yet been studied for this region. Noteworthy differences were observed in *T. gondii* seroprevalence among districts, and intra-herds within the same district, because some herds had all seropositive or all seronegative animals. These differences may be influenced by climate and altitude since an important mountainous area is located in the region (Sánchez, 2013). Studies on georeferentiation should be conducted to properly assessed the concentration and distribution of positive animals.

Although in Córdoba the prevalence of neosporosis is low, there are some individual herds with high prevalence; this may be due to a higher contact with dogs since in some farms dogs are used in daily work activities along with farmers, and also stay together with goats at pens and at grazing time.

Through the present study we identified the status of infection of *T. gondii* and *N. caninum* in herds from an important area of goat production in Argentina. We conclude that is necessary to create the conditions for small holder family farmers to improve training and have access to a better knowledge about the prevention and control of toxoplasmosis and neosporosis as well as to alert them to improve the management conditions. Since toxoplasmosis is a zoonotic disease, it is highly important to properly educate farmers about the potential risk factors and how to reduce them, such us: correct cooking and conservation of meat and milk derivates, the implication of domestic and wild felids in the area and the potential risk of oocysts contamination of soil and water sources. Finally, hygienic measures to reduce these diseases will improve their subsistence production and increase the opportunity of obtaining safety products.

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Conflict of interest

The authors declare that there is no conflict of interest.

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