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Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in goats from north-western Spain

Pablo Díaz[1], Eva Cabanelas[1], José Manuel Díaz[1], Miguel Viña[1], Juan Pablo Béjar[1], Ana Pérez-Creo[1], Alberto Prieto[1], Ceferino Manuel López[1], Rosario Panadero[1], Gonzalo Fernández[1], Pablo Díez-Baños[1], Patrocinio Morrondo[1]

[1] *Animal Pathology Department (INVESAGA Group), Faculty of Veterinary Medicine. Universidade de Santiago de Compostela. Campus Universitario s/n, Lugo, Spain*

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Abstract

Introduction and objective. *Toxoplasma gondii* and *Neospora caninum* are protozoans involved in reproductive failure especially in ruminant livestock. The objective was to estimate the seroprevalence of both parasites in goats from north-western Spain and to study the influence of some factors on seropositivity.

Materials and method. Blood samples from 638 goats were collected in 50 farms. Presence of *T. gondii* and *N. caninum* antibodies were detected by direct agglutination and competitiveELISA techniques, respectively. The risk factor analysis was performed using a mixed-effects logistic regression.

Results. Individual (48%) and herd-level (74%) *T. gondii* seroprevalence values were high; the within-herd prevalence was 53%. In contrast, 6% of animals tested positive to *N. caninum* and 38% of the herds had at least one positive animal, with a true within-herd prevalence of 10%. Mixed infections were limited; 91% of *N. caninum* seropositive goats were also positive to *T. gondii*. The risk factor analysis showed that *T. gondii* seroprevalence is influenced by the presence of sheep in the farm (OR=4.9) and the seropositivity to *N. caninum* (OR=16.5); goats from the Central-coastal area, more humid and warm, had a 15.7-fold probability of being seropositive to *T. gondii* than those from the Mountainous area. Cross-breed goats (OR=4.5) and the seropositivity to *T. gondii* (OR= 9.5) were factors associated with *N. caninum* seropositivity.

Conclusions. The high *T. gondii* seroprevalence in goats constitute a noticeable zoonotic risk. The consideration of the risk factors identified in designing *T. gondii* and *N. caninum* control programs in goat herds should allow the implementation of more efficient measures, avoiding the appearance of outbreaks of reproductive disorders by both protozoans in goats.

Key words

Toxoplasma gondii, *Neospora caninum*, goat, Spain, risk factors

INTRODUCTION

Toxoplasma gondii and *Neospora caninum* are worldwide-distributed apicomplexan parasites strongly associated with reproductive problems in ruminants, such as foetal reabsorption, abortions, foetal mummification, still birth and neonatal losses, leading to substantial economic losses in livestock production [1, 2].

Toxoplasmosis is considered one of the major causes of infectious reproductive failure in ovine and caprine livestock [3]. The zoonotic role of *T. gondii* has been demonstrated since the consumption of infected raw/undercooked meat or milk causes human infection [4]. *N. caninum* is responsible for reproductive failure in several animal species; studies on *N. caninum* infections in caprine livestock are limited when compared to other ruminant species, especially cattle [3, 5]. Although several investigations have isolated *N. caninum* and described *Neospora*-associated lesions from aborted goat foetuses [6, 7, 8], the real epidemiological importance of neosporosis in this small ruminant have not been completely elucidated.

Address for correspondence: Pablo Díaz, Facultad de Veterinaria. Pabellón I, Planta Baja, Campus Universitario s/n, 27002, Lugo, Spain
E-mail: pablo.diaz@usc.es

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Studies carried out in north-western Spain have revealed noteworthy seroprevalence rates of *T. gondii* and *N. caninum* in sheep (38–58%; 6–10%) and cattle (7%; 16%-24%) [9, 10, 11, 12], but no data are available on goats. This epidemiological information could be useful for studying outbreaks of reproductive disorders on goat farms, since animals could be exposed to *T. gondii* or *N. caninum* without apparent reproductive problems; consequently, performing serological studies after abortion outbreaks could lead to erroneous conclusions if the analysis does not consider the population seroprevalence [9].

The aim of this study was to estimate the seroprevalence and risk factors for *T. gondii* and *N. caninum* in goats from north-western Spain. This information would be of great interest for implementing new preventive strategies against these protozoans in goats.

MATERIALS AND METHOD

Study area and goat population. Galicia (north-western Spain) is a major Spanish livestock production area where the goat sector is poorly developed and professionalized and represents a small percentage of the Galician livestock sector; in 2012, the goat population was only 44,624 animals [13].

Goats are mainly managed under a traditional husbandry system and commonly reared mixed with sheep. The highest goat census and largest herds are located in mountainous areas, owing to goat rusticity and adaptability to unfavourable environments where pastures are scarce and other species may not be economically productive. The autochthonous 'Cabra Galega' breed is especially adapted to such zones and is present throughout the entire study area.

In north-western Spain, goats are mainly reared using two different management systems, they generally graze near the farm during the day and are brought indoors at night in the semi-extensive system, and during mild seasons go to large grazing areas, passing long periods outdoors in the extensive season.

According to geographic and climatic conditions, two different areas were previously considered in the study area [14]: a central-coastal area with moderate rainfall (<1,500 mm/year)and warm temperature ($\bar{x}$=12.3 °C) and a mountainous area with lower temperatures ($\bar{x}$=10.1 °C) and higher precipitation (>1,500 mm/year).

Serological study. Sample size was calculated for a confidence interval of 99% and 95% precision, establishing an estimated seroprevalence of 58% according to previous reports in sheep from the same study area [9, 12]. Thus, 638 goat blood samples were randomly taken from 50 farms between 2010–2013. Samples were taken by jugular puncture and sera stored at -20 °C until analysed.

Toxoplasma-specific IgG antibodies were detected by a direct agglutination commercial kit (Toxo-Screen DA, BioMérieux, Lyon, France) using the manufacturer's instructions. All samples were also analysed for the presence of *N. caninum* antibodies using a commercial competitive ELISA (cELISA-VMRD, VMRD, Pullman, USA). According to the product leaflet, serum samples were considered positive when the inhibition percentage was greater than 30%. Sensitivity and specificity values provided by the manufacturer were used to calculate the true seroprevalence [15]. Farms were considered positive when their true seroprevalence was >0.

Risk factors considered and statistical analysis. In order to investigate the possible influence of some factors on *T. gondii* and *N. caninum* seroprevalence values, farmers were asked to complete a herd management questionnaire on the day of sampling. Gender, breed and date of birth of animals were obtained from official individual registers. Variables and their categorization are summarized in Table 1.

Herd size categories were chosen in order to obtain similar number of cases in each herd; the number included goats and sheep present on the farm. The influence of *N. caninum* seropositivity on the presence of *T. gondii* antibodies and *vice versa* was also studied. Although it has been demonstrated that the presence of cats and dogs in the farm represent a risk factor to acquire *T. gondii* and *N. caninum* infections, respectively, these variables were not considered in the present study as both species of domestic animals were present on all studied farms.

Seropositivity was analyzed with a Mixed-effects Logistic Regression algorithm. The dependent variable was the seropositivity to *T. gondii* or *N. caninum* at the individual level. Herd was introduced as a random factor to control its effect over factors. The variables defined previously were introduced in a backward conditional method and removed from the model one by one on the basis of the AIC value until the best model was built. Next, all pairwise interactions that were biologically plausible were evaluated. These statistical analyses were performed with glmer() function from lme4 package in the R statistical programme (R v.3.1.1; R Development Core Team, 2014).

Table 1. *T. gondii* and *N. caninum* seropositivity values in goats from north-western Spain considering the different variables included in risk factor analysis

Variables		***T. gondii*** positive/total (%)	***N. caninum*** positive/total (%)
Age	≤12 months	31/64 (48%)	2/64 (3%)
	>12 months	268/574 (47%)	43/574 (8%)
Sex	Female	280/599 (47%)	43/599 (7%)
	Male	19/39 (49%)	2/39 (5%)
Climatic area	Central-coastal	279/492 (57%)	38/492 (8%)
	Mountain	20/146 (14%)	7/146 (5%)
Breed	Cross-breed	256/460 (56%)	42/460 (9%)
	"Cabra Galega" Pure breed	43/178 (24%)	3/178 (2%)
Husbandry system	Semi-extensive	285/574 (50%)	42/574 (7%)
	Extensive	14/64 (22%)	3/64 (5%)
Sheep presence	At least one sheep	241/455 (53%)	40/455 (9%)
	Purely goat flock	58/183 (32%)	5/183 (3%)
Flock size	< 96 animals	72/208 (35%)	9/208 (4%)
	96-194 animals	110/169 (65%)	22/169 (13%)
	> 194 animals	117/261 (45%)	14/261 (5%)
Seropositivity	to *N. caninum*	41/299 (14%)	-
	to *T. gondii*	-	41/45 (91%)
TOTAL		299/638 (48%)	45/638 (6%)

RESULTS

Individual and flock seroprevalence of both parasites. The individual true seroprevalence to *T. gondii* was high (48%), as well as the herd-level (37/50; 74%) and the true within-herd (53%) prevalence values. In contrast, a low percentage of animals tested positive to *N. caninum* (6%) and 19/50 of the herds (38%) had at least one positive animal, with a true within-herd prevalence of 10%. At the individual-level, mixed infections were not common, since only 41/638 of the examined goats (6%) were seropositive to both *T. gondii* and *N. caninum*. Interestingly, all *N. caninum* positive herds were also positive to *T. gondii*.

Risk factors. Seroprevalence for each factor considered in the study are summarized in Table 1. Cross-breed goats and those from coastal-central areas and medium-size herds presented the highest seroprevalence values for both protozoans. It was also observed that *N. caninum* and *T. gondii* seroprevalence was higher in animals reared together with sheep, and in a semi-extensive management system than the other; on the contrary, similar seropositivity percentages were observed in relation to gender. In contrast to that observed for *T. gondii*, an age-related increase in the *N. caninum* seroprevalence was recorded.

Table 2. Results of Mixed-effects Logistic Regression (backward conditional method) of risk factors associated with individual *T. gondii* and *N. caninum* seropositivity in goats from north-western Spain

	Estimate	Std. Error	P
***T. gondii* (step 6)**			
(Intercept)	0.626	0.385	0.010
Seropositivity to *Neospora*	2.802	0.683	< 0.001
Zone	-2.752	0.639	< 0.001
Sheep presence	-1.604	0.596	< 0.001
***N. caninum* (step 7)**			
(Intercept)	-4.080	0.583	< 0.001
Seropositivity to *Toxoplasma*	2.249	0.610	< 0.001
Breed	-1.504	0.666	0.024

Mixed-effects logistic regression results (Tab. 2) indicated that *T. gondii* seroprevalence in goats is mainly influenced by the climatic area, the presence of sheep on the farm and seropositivity to *N. caninum*. Thus, goats from the Central-coastal area had a 15.7-fold probability (95% CI 4.5–54.9) of being seropositive than those from the mountainous area. In addition, the risk of being seropositive was 4.9 times higher (95% CI 1.5–16.0) in those goat herds including sheep and 16.5 times higher (95% CI 4.3–62.8) in those animals tested positive for *N. caninum*.

For *N. caninum*, the multivariate analysis results indicated that the breed and the seropositivity to *T. gondii* were factors associated with goat seropositivity. In this case, the probability of being seropositive was 4.5-fold higher (95% CI 1.2–16.6) in cross-breed goats and 9.5-fold higher (95% CI 2.9–31.3) in those positive to *T. gondii*.

DISCUSSION

The presented study is the first population study on the seroprevalence of *T. gondii* and *N. caninum* in caprine livestock from northern Spain. The results obtained reveal a broad dissemination of both protozoans amongst goat farms and coincide with previous reports performed in sheep and cattle from the same area [9, 10, 11, 12]. This suggests that climatic and management factors facilitate the survival of oocysts in the environment and the contact between definitive and intermediate hosts. The high seroprevalence values of *T. gondii* found in this present study also implies a real risk for public health [4].The higher individual prevalence observed for *T. gondii* than for *N. caninum* in Galician caprine livestock is in agreement with several European serological studies in goats where both protozoans were investigated [16, 17, 18, 19]. These studies show *T. gondii* individual seroprevalences greater than 53%, whereas *N. caninum* values do not exceed 16%. In this way, the within-herd seroprevalence is considerably lower in *N. caninum* than in *T. gondii*, despite the percentage of positive herds being moderately high.

A limited number of animals showed mixed infections, but 91.1% of *N. caninum* seropositive goats were also positive to *T. gondii*. Similarly, Bartova and Sedlak [16], using the same cELISA for detecting anti-*N. caninum* antibodies, observed that all goats testing positive to *N. caninum* were also positive to *T. gondii*. In addition, González-Warleta et al. [11] found that dairy cattle infected with *T. gondii* showed a higher seroprevalence by *N. caninum*. In this regard, Álvarez-García et al. [20] found a low specificity (65.1–66.5%) for the VMRD cELISA using the manufacturer's cut-off value, suggesting that cross-reactions with other closely-related protozoans, such as *Sarcocystis* spp., may be responsible for these false positive results. This may be the reason for identification of *T. gondii* seropositivity as a risk factor for *N. caninum* seropositivity and *vice versa*. In contrast, Čobádiová et al. [17] observed a better specificity of the cELISA-VMRD using the manufacturer's cut-off value.

Mixed-logistic regression results showed that goats from central-coastal areas presented a significantly higher probability of being seropositive to *T. gondii* than those from the mountain area, as reported in sheep from the same region [9, 12]. The constant level of humidity and the higher mean annual temperature of the central-coastal area favour the sporulation, viability and spread in the environment of *T. gondii* oocysts [21]. In addition, goats from the mountain area usually spend long periods in large low-density grazing areas, away from both residential areas and goat facilities, where contact with the definitive host, and therefore the risk of infection, is less common [22].

Goats reared with sheep showed higher seroprevalence of *T. gondii* than those belonging to pure flocks. Those results are consistent with the findings of Gazzonis et al. [23], who observed an infection risk 1.39 times higher in goats from mixed farms. In mixed farms, a higher *T. gondii* seroprevalence has been found in sheep than in goats, which may be due to their foraging behaviour and diet selection [24]; while goats tend to eat from higher bushes and shrubs, sheep tend to graze close to the soil, being more likely to ingest oocysts on the pasture. All those data suggest that sheep act as an indirect facilitator of *T. gondii* exposure to caprine livestock. Further research to unravel the relationships between these two ruminant species in the epidemiology of *T. gondii* is needed to improve understanding of the behavior of toxoplasmosis on mixed farms.

The 'Cabra galega' autochthonous pure breed showed a significantly lower seroprevalence to *N. caninum* (1.7%) than cross-breed goats (9.1%). Although previous investigations are not very conclusive concerning the role of breed on infection by *N. caninum*, some authors have reported lower prevalence values in rustic and local breed goats than in cross-breeds [25]. In this sense, Pérez-Creo et al. [26] reported that the 'Cabra Galega' breed also showed significantly lower seroprevalence values for *Fasciola hepatica* than cross-breed animals.

CONCLUSIONS

The presented results reveal that the seroprevalence values of *T. gondii* are very high in caprine livestock from north-western Spain, thus representing a noticeable zoonotic risk. The data obtained show that the rearing of goats, while avoiding the presence of sheep in the herd, is a useful practice for reducing the seroprevalence of *T. gondii* and, consequently, the risks to other hosts. The use of locally adapted breeds may also be important for reducing the presence of *N. caninum* in goat populations, although further studies are needed to elucide the real role of caprine livestock on the epidemiology of this protozoan. Consideration of the risk factors identified in this study should allow the application of more efficient measures when designing *T. gondii* and *N. caninum* control

programmes, avoiding the appearance of outbreaks of reproductive disorders by both protozoans in goats.

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