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ORIGINAL ARTICLE

Toxoplasma gondii in small ruminants in Northern Italy – prevalence and risk factors

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Abstract

Objective. The aim of the survey was to evaluate *Toxoplasma gondii* seroprevalence in small ruminants and possible risk factors associated with the infection.

Materials and methods. Sera from 474 goats and 502 sheep reared on 42 farms in northern Italy were collected and tested for IgG antibodies to *T. gondii* by IFAT (indirect immunofluorescence antibody test). To identify risk factors, a binary logistic regression analysis of the variables was performed. An audit form about farm management was used.

Results. Antibodies to *T. gondii* were found in 96.6% of goat farms and in 87.5% of sheep farms; 41.7% goats and 59.3% sheep resulted positive. Seroprevalence was significantly higher in sheep than in goats. Seroprevalence values were similar in goats from eastern and western areas, whereas goats from the southern area were at lower risk of infection. Saanen goats presented the lowest seroprevalence (30.7 %), whereas cross-breed exhibited the highest rate (48.7%). Goats from farms housing both sheep and goats had an infection risk 1.39 times higher than goats from farms that did not house sheep. Animals bred on intensive farms showed lower prevalence (22.1%) in comparison with those from extensive (45.6%) or semi-intensive farms (60%). Sampling area was one of the strongest predictors of *T. gondii* infection in sheep flocks. Transhumant flocks showed a higher risk of infection by *T. gondii* compared with semi-intensive farms (66.8% *vs.* 38.4%).

Conclusions. The highest *T. gondii* seroprevalence values were registered in transhumant flocks of sheep and in family businesses rearing goats. As these traditional activities represent an important resource for the conservation of the territory and its economy, management practices for a better control of the disease should be improved.

Key words

Toxoplasma gondii, goat, sheep, Italy, IFAT, risk factors

INTRODUCTION

Toxoplasma gondii is an important protozoan parasite found worldwide that potentially infects all warm-blooded vertebrates, including mammals, birds and humans [1]. The infection is a major public health concern since it affects one-third of the human population [2]. Although usually asymptomatic in immunocompetent patients, toxoplasmosis can appear as encephalitis, pneumonia and myocarditis in immunocompromised hosts affected by chronic infection due to a reactivation of *T. gondii* [3]. Moreover, it causes abortion and congenital infections in approximately 0.1–1‰ of newborns in Europe who may suffer from severe ocular diseases (4–27%), general health problems (1–2%) or even die [2].

Consumption of raw or undercooked meat is considered to be one of the most important sources of infection for humans, even if distribution and number of tissue cysts vary among intermediate host species [4]. In particular, a large number of cysts were demonstrated in meat or cured meat products from ovine or caprine hosts, these species representing a significant way of infection mainly in those regions or countries where mutton and goat meat is routinely consumed [2, 4]. An additional way of transmission, through the consumption of unpasteurized goat or sheep dairy products, has also been suggested [2, 5]. From a veterinary viewpoint, *T. gondii* has been recognized worldwide as one of major causes of infectious reproductive failure in small ruminants [6, 7]. Regarding these hosts, in Europe, a wide range of seroprevalence values (sheep: 27.8–89%; goats: 18.5–52.8%) have been reported [8, 9, 10, 11, 12, 13, 14]. In Italy, *Toxoplasma* infection is largely spread among humans and animals [15, 16]. Considering *T. gondii* seroprevalence in small ruminants, the lowest values were registered in Sardinia, both in goats (12.3%) and sheep (28.4%) [17]. In other regions, values varied from 28.5% – 78% in sheep, and 11.7% – 60.6% in goats [18, 19]. In northern Italy, only a few data on the infection of small ruminants are available, not updated, or just limited to small areas [20]; moreover, data on goats are missing. Though mainly diffused in the insular, central or southern regions of Italy, small ruminant breeding, in particular goat farming, is important from the zootechnical and economic standpoint in northern regions. In fact, in the last 35 years, the total number of goats has increased by 3.3% in these areas and the proportion of caprine milk yield from the northern regions represented 37.7% of the overall production in the country [21]. Besides, some traditional forms of breeding, such as sheep transhumance or summer pasture on mountainous areas for goats, still persist. Nevertheless, this sector has significantly been scarcely supported when compared with other zootechnical

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activities. In particular, the monitoring of parasitic diseases, toxoplasmosis included, cannot be considered proportionate to the expansion of small ruminants in northern Italy.

OBJECTIVE

The presented study aimed to update information on the seroprevalence of *T. gondii* infection in small ruminants from Lombardy, a region of northern Italy, where these animals – goats in particular – are of relevant importance. The study also evaluates possible risk factors associated with the infection.

MATERIALS AND METHOD

Area description. The survey was carried out in some areas of Lombardy in northern Italy (45°40'N, 9°30'E), the most suitable for goat and sheep breeding, i.e. in the southern province of Milan, east of Bergamo and west of Varese. They were selected considering their wide sheep and goat population, their varied animal management systems and different landscapes and climate (http://www.scia.sinanet.apat.it/sciaweb/scia_mappe.html). The province of Milan (1,575 km^2) is mainly flat and the altitude of sampled farms ranges from 80 – 220 m a.s.l. The area has considerable farming activity mainly characterized by large intensive goat farms focused only on milk production. The territories of Bergamo (2,745 km^2) and Varese (1,199 km^2) show flatland (95 and 194 m a.s.l., respectively), hills and mountains of the Lombard Alps (1,508 and 896 m a.s.l., respectively). In these provinces, the farms are smaller and produce traditional cheese. In Lombardy, sheep transhumance is still practiced; in the winter, the sheep are moved from alpine pastures to Milan's lowlands, following the main routes (north to south) passing through Bergamo province, down to the plain areas of River Po. Goats raised in extensive farms usually graze from March or May to October or November during the day (or at night in the hottest months), and are kept in a fold at night (or during the day in the hottest months), depending on the area.

Study population and sample collection. Data obtained by ISTAT [21] showed that in the province of Milan (southern area) there were 7,153 small ruminants (27 sheep and 47 goat farms), 49,218 (89 sheep and 365 goat farms) in Bergamo (eastern area), and 9,238 (109 sheep and 186 goat farms) in Varese (western area). A minimum sample size was determined by using the programme Winepiscope 2.0 (http://www.clive.ed.ac.uk/winepiscope/0) to exclude (if all samples are negative) a *T. gondii* seroprevalence ≤15% within the animals in the sampled herds at a confidence level (CI) of 95%. Overall, 502 sheep and 474 goat blood samples from 45 farms were collected between October 2012 – May 2013. Ten farms (4 goat, 3 sheep and 3 mixed farms; mean 120 animals, min-max 20–500) were located in the southern area, 22 (17 goat, 4 sheep and 1 mixed farm; mean 27, min-max 10–200) in the western area and 13 (3 sheep and 10 mixed farms; mean 1,100, min-max 250–1,600) in the eastern area. GPS (Global Positioning System) coordinates of each farm were gathered to map its location. Within each selected flock/herd, animals (aged 4–159 months) were sampled by systematic random selection, proportionally to the total number of adults present on the farm. Regarding sampled goat breeds, two were cosmopolite (Alpine and Saanen), one autochthonous (Nera di Verzasca) and the others crossbreed. As for sheep breeds, one was cosmopolite (Merinos), one local (Bergamasca), and the others crossbreed. Blood samples were collected from the jugular vein and preserved in tubes without anticoagulants. Sera were separated by centrifugation (15 min; 2,120 g) and stored at -20 °C until serological testing.

Questionnaire data collection. A questionnaire about farm management was submitted to and filled-out by farmers and veterinarian practitioners at sampling time. It included questions on rearing system (extensive, intensive or semi-intensive for goats; extensive, semi-intensive or transhumant for sheep), species bred (only sheep or goats or mixed), farm size (number of animals on a farm), possibility to graze, nutrition (only grazing or supplementation with feeding concentrate), water source (stagnant water source or municipal water), purchase of spare breeding animals, presence of other species on the farm or sharing grazing (bovine, equine, wild ungulates), or domestic animals on the farm (dogs, swine or poultry), and presence of resident and/or stray cats on the property. Besides, individual data on animals were collected (sex, age, breed) and used as explanatory variables.

Serology. Serum samples were analyzed using a commercial indirect immunofluorescence antibody assay (IFAT) to determine the presence of IgG antibodies against T. *gondii*. The serological test was performed according to the method described by Camargo [22] using slides spotted with whole RH strain tachyzoites (Mega CorDiagnostik, Horbranz, Austria, Austria) as antigens and fluorescein isothiocyanate-labelled rabbit anti-sheep IgG (whole molecule, Sigma-Aldrich, St. Louis, MO, USA) diluted 1:100 in PBS plus 0.01% Evans blue as conjugate for sheep. For goats, fluorescein isothiocyanate-labelled rabbit anti-goats IgG (whole molecule, Sigma-Aldrich, St. Louis, MO, USA) diluted 1:200 in PBS plus 0.01% Evans blue were used as conjugate. Sera were screened considering 1:64 dilution as the cut-off and those testing positive were serially two-fold diluted to determine the end-point titre [23, 24]. Positive and negative controls were included in each assay and the slides were examined under a fluorescence microscope (Axioscope 2, Zeiss) at 400 or 1,000× magnification. Only a bright, linear, peripheral fluorescence extending to the whole e body of the tachyzoites was considered a positive reaction.

Statistical analysis. The seroprevalence at individual and farm level was computed with the associated 95% confidence interval. A farm was considered positive if at least one seropositive animal was found. Pearson's chi-square was used to test for the difference between species. Univariate binary logistic regression analysis was performed to determine factors that could be considered predictors of seropositivity to *T. gondii*.

All the answers from the questionnaire were included in the statistical analysis as independent variables. Further, all variables were entered in a multivariate model, developed by backward elimination until all remaining variables were significant ($p<0.05$). Statistical analysis was performed with SPSS (version 19.0; SPSS, Chicago, IL, USA).

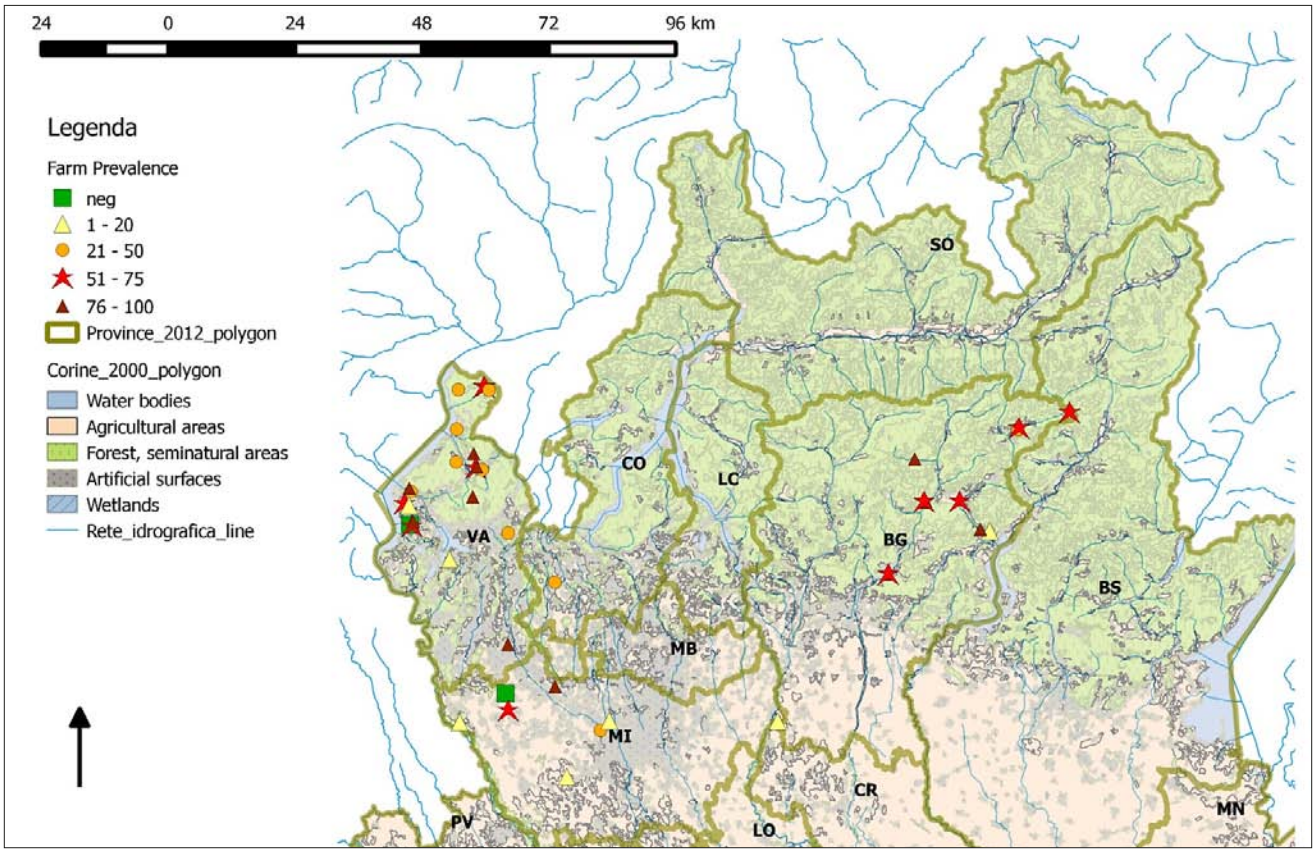

Figure 1. Map of location of sampled farms in three surveyed areas of northern Italy (VA= western area, MI= southern area and BG= eastern area). Different markers represent different seroprevalence values

RESULTS

Antibodies to *T. gondii* were found in 28 (96.6%; 90–100%, 95% CI) from 29 goat farms, whereas 21 (87.5%; 74.3–100%, 95% CI) from 24 sheep farms showed at least one seropositive animal. The spatial distribution of positive farms is represented in Figure 1. At the individual level, 41.7% (198/474) of goats and 59.3% (298/502) of sheep proved positive. Most of the small ruminants were seropositive with titres of 1:64; higher antibody titres (1:512) were found in 3.6% and 4.2% of goats and sheep, respectively (Tab. 1). According to host species, the distribution of infected animals varied among farms. As for goats, on 8 farms (27.6%) all tested animals had antibodies to *T. gondii*; on 6 farms (20.7%) more than 60% were seropositive and on 5 farms (17.24%) ≥50% or <60% were seropositive (Fig. 2A). As for sheep, seronegative animals were found on all farms; on 11 farms (45.8%) more than 60% were seropositive and only 1 farm had a percentage of ≥50% or <60% of seropositive sheep (Fig. 2B). According to age, in goats the highest percentage of infection was found in 4-year-old animals, whereas in older

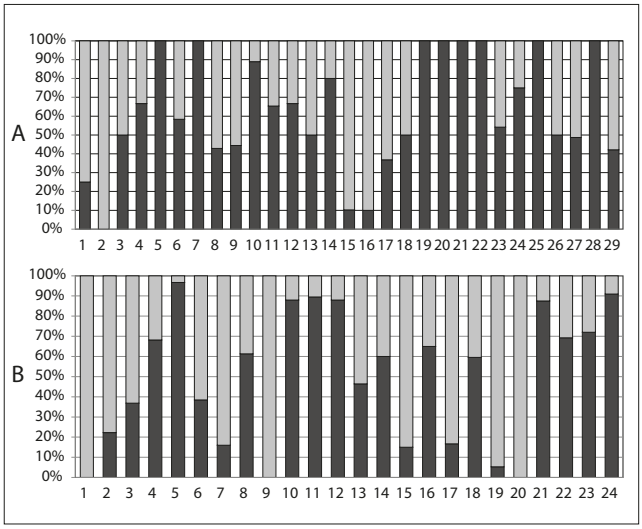

Figure 2. Proportion of *T. gondii*-seropositive (black) and negative (grey) animals in 29 goat farms (A) and 24 sheep farms (B) in northern Italy

Table 1. Rates of infection with *Toxoplasma gondii* in studied population according to animal species and antibody titer

	Sampled animals	Titre									
		1:64		1:128		1:256		1:512		Total	
	n	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI
Goats	474	88 (18.6)	15.1–22.1	39 (8.2)	5.7–10.6	54 (11.4)	8.54–14.2	17 (3.6)	1.9–5.2	198 (41.7)	37.3–46.2
Sheep	502	152 (30.3)	26.2–34.3	50 (10.0)	7.3–12.6	75 (14.9)	11.7–18	21 (4.2)	2.4–5.9	298 (59.3)	53.7–64.8

n: number of animals; %: seroprevalence; 95% CI: 95% Confidence Interval

Table 2. Potential risk factors for *Toxoplasma gondii* seropositivity in goats by univariate analysis

Variable	Category	n	Prevalence (%)	OR§	95% CI*	p-value
Sampling area	Eastern (reference)	16	50.0			
	Southern	61	29.2	0.412	0.194–0.876	**0.021**
	Western	121	51.9	1.080	0.516–2.262	0.838
Altitude	Continuous variable	474		1.000	1.000–1.001	0.103
Breed	Nera di Verzasca (reference)	56	44.4			
	Alpine	37	38.9	0.797	0.464–1.371	0.413
	Crossbreed	74	48.7	1.186	0.738–1.905	0.481
	Saanen	31	30.7	0.554	0.319–0.959	**0.035**
Gender	Male (reference)	7	53.8			
	Female	191	41.4	0.606	0.201–1.833	0.375
Age	Continuous variable	474		1.011	1.004–1.017	**0.001**
Species on farm	Only goats (reference)	160	39.4			
	Goats+Sheep	38	55.9	1.396	1.077–1.808	**0.012**
Number of animals on farm	Continuous variable	474		0.998	0.997–0.999	**<0.0001**
Rearing system	Semi-intensive (reference)	93	60.0			
	Extensive	67	45.6	0.558	0.354–0.882	**0.012**
	Intensive	38	22.1	0.189	0.117–0.306	**<0.0001**
Grazing	No (reference)	74	31.0			
	Yes	124	52.8	2.491	1.712–3.625	**<0.0001**
Feeding concentrate	No (reference)	97	36.9			
	Yes	101	47.9	1.571	1.087–2.271	**0.016**
Water source	Municipal water (reference)	74	31.0			
	River	124	52.8	2.491	1.712–3.625	**<0.0001**
Presence of other species	No (reference)	81	35.1			
	Yes	117	48.1	1.705	1.178–2.467	**0.005**
Presence of cats	No (reference)	16	50.0			
	Yes	64	48.1	0.928	0.429–2.007	0.849
Purchase of spare breeding animals	No (reference)	52	52.5			
	Yes	47	43.9	0.708	0.409–1.226	0.218

Statistically significant variables are indicated by bold typing
§OR = Odds ratio
*95%CI: 95% Confidence Interval

animals (>6 years) the percentage of seropositive animals decreased. In sheep, seroprevalence directly increased with increasing age, and the highest seroprevalence values were registered in animals >6-years-old. Seroprevalence was significantly higher in sheep than in goats (Pearson's chi-square, p<0.0001); therefore, analysis of the potential risk factors was conducted separately for the two species.

Results obtained from risk factor univariate analysis for goats are given in Table 2. For goats, animals sampled in eastern and western areas showed similar seroprevalence values; goats from the southern area had a lower probability of become infected (OR= 0.412; 95% CI: 0.194–0.876). Considering breeds, Saanen goats presented the lowest seroprevalence (30.7%) whereas crossbreeds exhibited the highest level (48.7%). The risk for a Saanen being infected was 0.554 times less than for a Nera di Verzasca (OR= 0.554; 95% CI: 0.319–0.959%). Goat age was one of the strongest predictors of *T. gondii* infection; the odds for a goat being infected were 1.011 times greater for every 1 month increase in age. The risk factor 'number of animals on the farm' was highly significant: increasing the number of animals on a farm of one unity, their risk of becoming seropositive was 0.998 lower. Goats from farms breeding both sheep and goats had a risk of being infected 1.396 times higher than goats from farms housing no sheep. Regarding the variable 'rearing system', animals bred on intensive farms showed lower prevalence (22.1%) in comparison with those bred in extensive (45.6%) or semi-intensive farms (60%); the risk of infection increased from intensive farms (OR= 0.189; 95% CI: 0.117–0.306) to extensive farms (OR= 0.558; 95% CI: 0.354–0.882). Other significant risk factors resulted from the possibility to graze, type of feeding, water source, and presence of other animal species (Tab. 2). In the final multivariable model, only three variables and two interactions were entered (Tab. 3).

Table 3. Potential risk factors associated with *T. gondii* seropositivity in goats using multivariate multi-level modeling

Variable	Category	n	Prevalence (%)	OR§	95% CI*	p-value
Breed	Nera di Verzasca (reference)	70	44.4			**<0.0001**
	Alpine	58	38.9	0.797	0.464–1.371	0.413
	Crossbreed	78	48.7	1.204	0.509–2.845	0.673
	Saanen	70	29.6	0.251	0.094–0.669	**0.006**
Rearing system	Semi-intensive (reference)	62	60			**0.019**
	Extensive	80	45.5	0.285	0.119–0.684	**0.005**
	Intensive	134	22	0.669	0.251–1.781	0.421
Age	Continuous variable	474		1.020	1.012–1.028	**<0.0001**
Management * Number of animals on farm	Semi-intensive (reference)	62				**0.002**
	Extensive	80		0.999	0.990–1.009	0.898
	Intensive	134		1.003	0.992–1.015	0.565
Number of animals on farm * breed	Nera di Verzasca (reference)	70				**0.001**
	Alpine	58		.996	0.986–1.005	0.376
	Crossbreed	78		1.001	0.992–1.010	0.820
	Saanen	70		1.003	0.994–1.013	0.478

Statistically significant variables are indicated by bold typing
§OR= Odds ratio
*95%CI: 95% Confidence Interval

For sheep, univariate analysis showed several significant risk factors (Tab. 4). Sampling area was by far one of strongest predictors of *T. gondii* infection in a flock of sheep. The odds on a sheep from the south-eastern area of being diagnosed seropositive to *T. gondii* were 3.256 times higher than for a sheep from the western area (OR= 3.256; 95% CI: 1.985–5.539). Seroprevalence increased to a small extent with the altitude of farms (OR=1.001; 95% CI: 1.001–1.002). Breed was an additional significant risk factor: crossbreeds were more infected than the other two breeds. As for goats, age was a risk factor, where seropositivity increased with the increasing of age (OR= 1.019; 95% CI: 1.011–1.026).

Table 4. Potential risk factors for *Toxoplasma gondii* seropositivity in sheep by univariate analysis.

Variable	Category	n	Prevalence (%)	OR§	95% CI*	p-value
Sampling area	Western (reference)	29	35.4			
	East-southern	269	64.0	3.256	1.985–5.339	**<0.0001**
Altitude	Continuous variable			1.001	1.001–1.002	**<0.0001**
Breed	Bergamasca (reference)	105	52.2			
	Crossbreed	171	71.8	2.333	1.571–3.465	**<0.0001**
	Merinos	22	34.9	0.491	0.273–0.833	**0.017**
Gender	Male (reference)	30	48.4			
	Female	268	60.9	1.662	0.975–2.834	0.062
Age	Continuous variable	502		1.019	1.011–1.026	**<0.0001**
Number of animals on farm	Continuous variable	502		1.001	1.001–1.002	**<0.0001**
Species on farm	Only sheep (reference)	108	55.4			
	Goats+Sheep	190	61.9	1.308	0.909–1.883	0.148
Rearing system	Semi-intensive (reference)	48	38.4			
	Extensive	19	61.3	2.540	1.133–5.696	**0.024**
	Transhumant	231	66.8	3.222	2.108–4.925	**<0.0001**
Presence of other species	No (reference)	72	46.1			
	Yes	226	65.3	2.197	1.495-3.229	**<0.0001**
Presence of cats	No (reference)	190	64.2			
	Yes	36	72.0	1.435	0.740-2.780	0.285

Statistically significant variables are indicated by bold typing
§OR= Odds ratio
*95%CI: 95% Confidence Interval

The higher the number of animals on a farm, the higher their risk of being infected (OR= 1.001; 95% CI: 1.001–1.002). However, transhumant herds, in comparison with semi-intensive ones, appeared to be at higher risk of *T. gondii* infection (66.8% *vs.* 38.4%). Unlike other species, the presence of goats on a farm had no effects on sheep seroprevalence, (OR= 2.197; 95% CI: 1.495–3.229) (Tab. 4). For sheep, variables such as grazing, feeding concentrate and water source were not taken into consideration, the sheep bred under same conditions being monitored. For these animals, the final model included variables such as sampling area, altitude, age, and interaction between rearing system and number of animals on a farm (Tab. 5).

Table 5. Potential risk factors associated with *T. gondii* seropositivity in sheep using multivariate multi-level modeling

Variable	Category	n	Prevalence (%)	OR§	95% CI*	p-value
Sampling area	Western (reference)	29	35.4			**<0.0001**
	East-southern	269	64.0	7.782	2.139–2.316	**0.002**
Altitude	Continuous variable	502		0.999	0.998–1.000	**0.003**
Age	Continuous variable	502		1.011	1.002–1.021	**0.022**
Management * Number of animals on farm	Semi-intensive (reference)					**<0.0001**
	Extensive	115		1.014	1.007–1.022	**<0.0001**
	Transhumant	77		1.001	1.001–1.002	**<0.0001**

Statistically significant variables are indicated by bold typing
§OR= Odds ratio
*95%CI: 95% Confidence Interval

Both for goats and sheep, variables associated with farm management, such as water source and purchase of spare breeding animals, did not enter the final model.

DISCUSSION

In recent years, increasing attention has been paid to *T. gondii* infection. In fact, the European Food Safety Authority (EFSA) has indicated toxoplasmosis as one of the most important parasitic zoonoses due to its high human incidence, and recently published a scientific opinion clearly stating the need for investigation into its occurrence, both in humans and animals in Europe [5]. Epidemiological data on *T. gondii* infections in animals for human consumption are not regularly collected and the current lack of standardization of diagnostic techniques and protocols should be taken into account when comparing seroprevalence data [25]. The presented study aimed at updating information on *T. gondii* infection in small ruminants in northern Italy, and revealed that the seroprevalence of anti-*Toxoplasma* antibodies was high in both goats and sheep at the individual level (goat= 41.7%, sheep= 59.3%) and at farm level (goat= 96.6%, sheep= 87.5%). Seroprevalence showed higher here than in animals tested in other Italian regions, but in a previous survey similar values were reported in sheep from the same area [20]. High seroprevalence was also found in different European countries, such as the Netherlands, Portugal, France, Switzerland, Romania, Greece, and Spain, and huge variations in the prevalence of *T. gondii* ranging from 18.5% – 52.8% in goats and from 27.8% to 89% in observed sheep [8–14]. The higher seroprevalence recorded in sheep rather than in goats is consistent with values reported in previous studies that considered both species reared in the same areas [12, 13, 14, 17]. Further, in the presented study a large number of sampled animals had a titre of only 1:64 or 1:128, both in goats and in sheep, suggesting that most animals were in the chronic phase of infection.

In both species, seroprevalence was positively correlated to age, as already stated in previous studies [26]. However, a difference in the proportion of seropositive small ruminants was found when the age of animals was separately compared for both species. In sheep, seroprevalence regularly increases

with increasing age, with the highest seroprevalence in animals >6-years-old. Conversely, in goats, the percentage of seropositive animals irregularly varied with age, showing that their antibody response could probably be weaker than in sheep aged from 5 years onward. Such a difference in seroprevalence may be explained by a difference in their immune response. In fact, several studies previously illustrated that both acquisition and expression of immune responses against gastrointestinal nematodes are less efficient in goats than in sheep, although few studies have been published on differences in susceptibility to toxoplasmosis of the two species [27]; besides, other external factors, such as farm management or feeding behavior, could account for this discrepancy [28].

Analysis of risk factors showed that Saanen goats presented the lowest risk of being infected and crossbreeds the highest. Differently, Lopes et al. [14] reported a lower seroprevalence value in crossbreeds compared with defined-breed goats. In northern Italy, noticeably Saanen goats are bred mostly in intensive farms presenting the lowest prevalence (30.7%); thus, the differences reported in the presented survey may be associated with differences in rearing systems and not in breeds.

In sheep, altitude is a significant risk factor, being positively associated with seroprevalence that increases in hilly areas between 300 – 1,000 m a.s.l where transhumance towards the lowlands in winter is still practiced. Altitude is frequently reported as a risk factor associated with toxoplasmosis in different countries and is related to environmental conditions and different grazing strategies [29, 30]. Moreover, in the south-eastern area where most transhumant sheep herds are, the highest seroprevalence was recorded in these animals.

Rearing system was certainly a very important risk factor associated to the infection, both in goats and sheep: extensive or semi-intensive farms and transhumant herds were the breeding management at higher risk of toxoplasmosis. Intensive farms in northern Italy may have a high level of hygiene preventing *T. gondii* oocysts from spreading throughout their facilities. On the other hand, semi-intensive goat farms, often represented by small family businesses, may have an inadequate hygienic standard and consequent spread of *T. gondii* oocysts among their animals. Extensive farms or transhumant herds may be more exposed to cats in the environment or to contaminated stagnant pools, even though oocysts may be more dispersed in the environment. Nevertheless, a previous survey carried out in Greece recorded the highest infection prevalence on intensive farms [12], highlighting differences in the management of the farms. In intensive farms, the animals could possibly be more exposed to contaminated feed, and farm facilities under intensive or semi-intensive conditions may provide shelter to various hosts of *T. gondii* (such as cats and rodents) which might be involved in the spread of infection. Interestingly, in the presented survey, the presence of cats on farms or on the grazing sites did not represent a meaningful risk factor associated with the infection, which differs from other surveys [19]. According to Tzanidakis et al. [12], farmers might not notice stray cats on their farms or on the grazing sites, which can account for the contamination of pastures or feed or water sources.

Another significant variable was farm size, connected with the rearing system. Regarding goats, the number of seropositive animals was negatively associated with the size of the flock in semi-intensive or extensive farms; therefore, seroprevalence was higher in smaller flocks. Again, small family businesses showed higher prevalence than large intensive farms. In sheep, conversely, seroprevalence was positively correlated with herd size; large transhumant herds possibly contribute to the maintenance of the infection within the animals. Vesco et al. [31] reported a similar situation in sheep reared in Sicily; on the contrary, data reported by Cenci-Goga et al. [19] showed that in sheep reared in Tuscany, seroprevalence is negatively correlated to size farm, corresponding to a major infection in smaller farms, as registered in the goats in the current study. Therefore, different seroprevalence values in both species may correspond to different production systems.

CONCLUSIONS

Toxoplasma infection occurred with a high seroprevalence both in goats and sheep in northern Italy, confirming that the two species could be an important source of *T. gondii* infection in humans in this area. The obtained data provide a further understating of risk factors associated with *T. gondii* infection and of the relationship between the parasite and its small ruminant hosts. Such information can be useful for veterinarians as well as for farmers in order to develop or improve control plans of toxoplasmosis in flocks/herds in the study areas, and/or in those where farm systems are similar to those described in the presented survey. Specific guidelines should be addressed to farmers and particular attention paid to sheep farmers rearing transhumant herds, and to goat farmers running small family businesses, both of which are at higher risk of infection, according to the results of the presented study. The high prevalence recorded in sheep transhumant herds and in small family businesses for goats indicates that these management systems support the spreading and maintenance of infection by *T. gondii.* However, the traditional activities of these farms are important resources for the conservation of a territory and its economy, as they obtain typically cheese directly from raw milk, and many cured meat products that do not have a large distribution. In consideration that goat milk has already been found to cause human infection, and that more recently, *T. gondii* DNA was traced to milk [18], small businesses, being holiday farms and/or based on organic animal husbandry, could represent a relevant risk for humans. Farmers should be aware of the health risks posed by their products and be informed about the main risk factors associated with the infection. In particular, access of cats to their farm premises should be avoided and their population control should be planned. According to the presented results, toxoplasmosis has an important zoonotic impact on the health and production of small ruminants that requires further investigation on these species to improve its understanding and control.

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