Research in Veterinary Science 192 (2025) 105729

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Nationwide serological survey and risk factors for *Toxoplasma gondii* and *Neospora caninum* infections in alpacas (*Vicugna pacos*) and llamas (*Lama glama*) in Italy

Elisa Castaldo [a], Walter Basso [b,*,1], Gastón Moré [b], Alessia Ciaramelli [c], Nicola D'Alessio [d], Michele Capasso [a], Giovanni Sgroi [d], Sara Tonon [e], Farwa Humak [a], Alessia Gazzonis [f], Gereon Schares [g], Vincenzo Veneziano [a,**,1]

[a] *Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples 80137, Italy*
[b] *Institute of Parasitology, Vetsuisse-Faculty, University of Bern, Bern 3012, Switzerland*
[c] *Veterinary Practitioner, Viterbo 01100, Italy*
[d] *Department of Animal Health, Experimental Zooprophylactic Institute of southern Italy, Portici 80055, Italy*
[e] *Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Rome 00178, Italy*
[f] *Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi 26900, Italy*
[g] *Friedrich-Loeffler Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald-Insel Riems 17493, Germany*

ARTICLE INFO

Keywords:
Toxoplasmosis
Neosporosis
South American camelids
Seroprevalence
ELISA
Western blot

ABSTRACT

Toxoplasma gondii and *Neospora caninum* are worldwide distributed protozoa, recognized as major causes of economic losses in livestock production due to reproductive failure. Camelids can also serve as intermediate hosts for these protozoa. Research on these parasites in South American Camelids (SAC) in Europe is scarce. Therefore, this study aimed to estimate the seroprevalence and distribution of *T. gondii* and *N. caninum* infections in SAC in Italy and investigate risk factors for infection applying a structured online questionnaire filled in by owners. A total of 506 SAC sera (486 alpacas, *Vicugna pacos,* and 20 llamas, *Lama glama*) were tested to detect antibodies against *T. gondii* and *N. caninum* using enzyme-linked immunosorbent assays (ELISA), and results were confirmed with immunoblot. Seroprevalences of 34.8 % (176/506) for *T. gondii,* with 33.1 % (161/486) for alpacas and 75.0 % (15/20) for llamas, and 5.7 % (29/506) for *N. caninum,* with 5.6 % (27/486) for alpacas and 10.0 % (2/20) for llamas were found. Simultaneous presence of antibodies against *T. gondii* and *N. caninum* was detected in 3.6 % (18/506) of the samples. The variables older age and llama species were identified as risk factors ($p < 0.05$) for *T. gondii*, while the presence of dogs and fertility disorders were associated with *N. caninum* seropositivity. This study provides the first large-scale serological evidence of *T. gondii* and *N. caninum* infections in alpacas and llamas in Italy, highlighting their widespread distribution and contributing valuable data to the national epidemiological scenario. Further studies are needed to evaluate the role of these parasites as cause of abortion.

1. Introduction

The apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum* are obligate intracellular protozoa with a worldwide distribution. Felids, both wild and domestic species, represent the definitive hosts for *T. gondii* (Dubey et al., 2020a), while dogs and certain wild canids (i.e., coyotes, wolves, and dingoes) are definitive hosts for *N. caninum* (Dubey and Schares, 2011). Both parasites have heteroxenous life cycles, with the sexually reproductive stages occurring in the small intestine of the definitive host and asexual stages in extraintestinal tissues (e.g., hearth, skeletal muscles, brain, liver, kidneys) of the intermediate hosts (Dubey, 1998, 2009; Dubey et al., 2004). Definitive hosts are responsible for oocyst shedding, playing a key role in contamination of the environment (Dubey, 2021). Furthermore, suitable environmental conditions (e.g.,

* Correspondence to: W. Basso, Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland.
** Correspondence to: V. Veneziano, Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples 80137, Italy.
E-mail addresses: walter.basso@unibe.ch (W. Basso), vinvene@unina.it (V. Veneziano).
[1] Equally contributing senior authors

https://doi.org/10.1016/j.rvsc.2025.105729
Received 20 March 2025; Received in revised form 16 May 2025; Accepted 27 May 2025
Available online 29 May 2025

moisture and warmth) promote oocyst sporulation, which could remain infectious for several months to years, both in aquatic and terrestrial environments, serving as infectious source for a wide range of potential hosts (Dubey, 1998; Silva and Machado, 2016). Warm-blooded species, including humans, can act as intermediate hosts for *T. gondii*, whereas cattle, small and wild ruminants are the main intermediate hosts for *N. caninum* (Dubey et al., 2017, 2020a). Both protozoa are recognized as major causes of economic losses in livestock production due to reproductive failure (Webster and Dubey, 2010; Dubey et al., 2017). *T. gondii* infection represents an important cause of abortion in sheep and goats, whereas it has negligible significance in cattle (Webster and Dubey, 2010). On the other hand, *N. caninum* is a major cause of abortion and neonatal mortality in cattle but has also been described as an abortifacient and cause of perinatal mortality in sheep, goats, and wild ruminants (Basso et al., 2014; Basso et al., 2022; González-Warleta et al., 2018). In herbivores, oocysts represent the primary source of infection for *T. gondii* and *N. caninum,* mainly acquired from a contaminated environment through the consumption of food and/or water. In carnivorous and/or omnivorous species, the infection could also result from the consumption of tissue cysts in raw or undercooked meat of infected animals. Furthermore, vertical transmission is an additional route of *T. gondii* and *N. caninum* infection in mammals (Dubey and Schares, 2011; Almeria and Dubey, 2021).

Camelids could also potentially act as intermediate hosts both for *T. gondii* and *N. caninum* and these infections may occasionally cause abortions and fatal systemic infection (Serrano-Martínez et al., 2004, 2007; Dubey et al., 2014a, 2014b). Domesticated South American Camelids (SAC), referring to llamas (*Lama glama*) and alpacas (*Vicugna pacos*), are native to south American countries where these animals are raised for meat, fibre, and hide production, playing a key role in the economic system (Fowler, 2010). In camelids, the global abortion rates caused by infectious diseases range from 10 % to over 70 % depending on region and facility, and toxoplasmosis has been recognized as one of the main causes of abortion in llamas and alpacas (Tibary et al., 2006). Since reproductive problems may impair breeding profitability (Serrano-Martínez et al., 2007), these protozoa have been mainly investigated in South American countries (i.e. Peru, Argentina and Chile), where prevalences for *T. gondii* ranged from 8.6 % to 44.2 % and from 3.0 % to 44.5 % in llamas and alpacas, respectively, while *N. caninum* prevalences ranged from 1.0 % to 23.3 % in llamas and from 0.3 % to 42.4 % in alpacas (Chávez-Velásquez et al., 2005; Wolf et al., 2005; Moré et al., 2008; Basso et al., 2020). In recent years, the breeding of alpacas and llamas has expanded beyond South America and has been introduced into Europe as exotic species (De La Lama et al., 2022), including Italy, where these species are kept mainly for fibre production. However, little is known about the prevalence of these parasitic infections in SAC in Europe, except for a large survey from Switzerland (Basso et al., 2020), and some reports on a restricted number of animals (fewer than 35) from Germany (Wolf et al., 2005), Czech Republic (Bártová et al., 2017), and Italy (Marková et al., 2018).

This study aimed to estimate the seroprevalence and distribution of *T. gondii* and *N. caninum* infections in a large population of SAC in Italy and investigate the risk factors for infection, shedding light on the epidemiology of these parasitic infections in SAC in the European scenario.

2. Materials and methods

2.1. Study animals and sampling

The sampling was performed from September 2023 to March 2024 on 506 SAC (i.e., 486 alpacas and 20 llamas) kept in 38 sampling sites (i.e., 24 farms, 8 zoos, and 6 private gardens in which these species were kept as companion animals). The sampling sites were distributed in three different Italian macro-areas, being: 22/38 (57.9 %) located in northern Italy, including a total of 200 animals (39.5 %), 12/38 (31.6 %) in central Italy with a total of 281 animals (55.5 %), and 4/38 (10.5 %) in southern Italy with a total of 25 animals (4.9 %).

Differences in sampling between macro-areas were due to the different SAC farms distribution along the Italian territory at the beginning of the study (67.5 % in northern area, 23.5 % in central area, 10.0 % in southern areas; (Vet Info, 2024).

Veterinary practitioners were involved in opportunistic blood sampling during routine clinical examinations by puncture from the *vena jugularis* using disposable syringes and placed into screw capped sterile 10 ml tubes without anticoagulant (APTACA®, Asti, Italy), slowly, avoiding haemolysis. Samples were labelled and stored in a portable icebox during transportation to the Department of Veterinary Medicine and Animal Productions of the University of Naples "Federico II". The samples were centrifuged at 3000 x*g,* 10 min and extracted sera were placed into 1.5 ml Eppendorf tubes and stored at −20 °C until serological analysis performed at the Institute of Parasitology, Vetsuisse Faculty Bern (IPB).

At sampling time, data regarding animal identification number, sex, age, body condition score (BCS), and breed were recorded from each animal. The animals were classified into four age-classes: crias (≤ six months), weaners (> six months < twelve months), tuis (≥ one year < two years), and adults (≥ two years) according to Love (2017). Body condition score was determined using the specific alpaca and llama chart developed by Van Saun (2013). Briefly, a scale from 1 to 5 with half scores was used (1 = emaciated, 1.5 = poor, 2 = thin, 2.5 = borderline, 3 = moderate, 3.5 = high moderate, 4 = excess, 4.5 = fat, 5 = grossly obese).

Of the study animals, 64.2 % (325/506) were females, 31.6 % (160/506) were intact males, and 4.2 % (21/506) were castrated males; whereas 4.2 % (21/506) were crias, 4.0 % (20/506) were weaners, 12.8 % (65/506) were tuis, and 79.1 % (400/506) were adults. The mean age of animals ± standard deviation was 4.8 ± 3.9 years, with range from 1 month to 23 years.

2.2. Enzyme-linked immunosorbent assay (ELISA)

According to previous studies in SAC (Bártová et al., 2017; Basso et al., 2020), sera were analysed with a commercial indirect enzyme-linked immunosorbent assay (ELISA) for the detection of anti-*T. gondii* antibodies ("TOXO-MS") (ID Screen® Toxoplasmosis Indirect Multi-Species, ID.vet, Montpellier, France), and by a competitive ELISA for the detection of *N. caninum* antibodies ("NCC") (ID Screen® *Neospora caninum* Competition, ID.vet, Montpellier, France). Serological analyses were performed according to the manufacturer's instructions using positive and negative control sera provided by the kit. In addition, known internal *N. caninum* or *T. gondii* positive and negative control SAC sera, previously tested at the IPB (Basso et al., 2020), were included on each plate.

For each tested serum by TOXO-MS, a sample-to-positive ratio (S/P ratio) was calculated based on the optical density (OD) of the sample and the positive and negative controls of the kit. Absorbance was measured spectrophotometrically at 450 nm (Tecan Group Ltd., Männedorf, Switzerland) and values interpreted applying the formula supplied in the kit: S/P% = (OD sample − OD negative control)/ (OD positive control − OD negative control) × 100. According to the manufacturer, tested samples which produced S/P% ≤ 40 % were considered negative, doubtful if 40 % < S/P% > 50 % and positive if S/P% ≥ 50 %. However, this test is not validated by the manufacturer for its use on SAC. Therefore, a previously published cut-off, which was optimized for these species by comparing ELISA results with those obtained by immunoblot was considered i.e. S/P 36.2 %, with a reported relative diagnostic sensitivity of 94.5 % and a relative diagnostic specificity of 97.9 % (Basso et al., 2020).

For *N. caninum*, a competition percentage (S/N%) was calculated for each sample according to the formula: S/N% = (OD sample/ OD negative control) x 100. Animals with S/N% ≤ 50 % were considered

positive, doubtful if S/N% was from 50 % to ≤60 %, and negative if S/N % was >60 %.

2.3. Immunoblot

To confirm the optimized SAC cut-off suggested by Basso et al. (2020), all samples showing ≥24 % S/P ≤ 70 % results in TOXO-MS, as well as all samples showing ≥30 % S/N ≤ 80 % results in NCC, including samples with doubtful results, were tested by *in-house T. gondii* tachyzoite surface antigen TgSAG1 (P30)-based and *N. caninum* tachyzoite-based immunoblots following the protocols described by Basso et al. (2020).

In addition, known *N. caninum* and *T. gondii* positive and negative internal controls, previously used in ELISA, as well as 3 sera with the highest and 3 sera with the lowest ELISA values were included in the immunoblot assays.

To exclude the possibility of a false positive reaction in ELISA due to the co-presence of antibodies against *T. gondii* and *N. caninum*, all samples showing positive results for both *T. gondii* and *N. caninum* in ELISA were also tested by immunoblot.

To evaluate the agreement between ELISA cut-off suggested by the manufacturer and the optimized SAC cut-off proposed by Basso et al. (2020), an inter-rater agreement (kappa) was further calculated using GraphPad Software and kappa values (κ) were classified as follows: slight agreement (κ = 0–0.20), fair agreement (κ = 0.21–0.40), moderate agreement (κ = 0.41–0.60), substantial agreement (κ = 0.61–0.80), or almost perfect agreement (κ = 0.81–1.00).

2.4. Questionnaire

In order to address questions related to *T. gondii* and *N. caninum* infections a questionnaire was developed and filled by SAC owners using an online programme, Microsoft Forms® (Microsoft Corporation, Redmond, WA, USA). The structured questionnaire consisted of 89 questions: 61 close-ended multiple-choice questions, 15 yes/no questions, and 13 open questions. The questions were assigned to a section, for a total of four key areas: *1*) animals owner data (e.g. farming experience, education level and main occupation), *2*) farm veterinarian's data, *3*) farm structure and management (e.g. herd size (small ≤20 animals, medium >20 and ≤ 50, large >50 animals)), primary use of animals, country of animal origin, access to pasture, co-grazing with ruminants, co-living with cats/dogs), *4*) animal health management and clinical history with a specific part inquiring on reproductive disorders (e.g., occurrence of abortions, stillbirths, dystocia, stunted growth, and malformation in crias) (**Supplementary material S1**).

2.5. Statistical analysis

The identification of risk factors associated with seropositivity for *T. gondii* and *N. caninum* was performed by multilevel-modelling, using the information on farms and individual study animals. According to ELISA results and confirmation by immunoblot, the animal serostatus (i.e., seropositive or seronegative) for *T. gondii* or *N. caninum* was considered as the dependent variable, and the potential risk factors as independent or explanatory variables. Given that individual animals clustered in farms, the variable farm was included as a random effect variable in modelling seropositivity for both protozoa. A total of 203 explanatory variables were tested in association with the serostatus of individual animals for both protozoa.

Multilevel-modelling at animal level was performed using function *glmer* (package "lme4" with Laplace method as default approximation), binomial distribution, binary response variable "animal serostatus" with seropositive or seronegative values, random factor "farm name", and effect modifier "age in years" (only for *T. gondii*). Explanatory variables significant at p < 0.05 in the bivariable (*T. gondii*) or univariable (*N. caninum*) models were retained for the multivariable analysis. Variables, for which it was assumed to be confounders (extraneous variables that influence the studied variables leading to a distort relationship between them) were excluded from multivariable modelling. By backward and forward selection, only statistically significant variables were retained in the final multivariable models. The null hypothesis (H0) aimed to prove the independence between the infection status and the corresponding risk factors (categorical variables). Statistical modelling was made in R 4.4.2 (http://www.R-proje ct.org). The modelling strategy applied here-in was previously used by Lücht et al. (2019), Basso et al. (2020); Basso et al. (2022)) and Gliga et al. (2022) and adapted for this study. The Akaike information criterion (AIC) was used to characterize the relative model quality.

3. Results

3.1. ELISA results

According to TOXO-MS cut-off proposed by the kit's manufacturer, 171/506 (33.8 %) animals showed positive results for *T. gondii*, 332/506 (65.6 %) negative, and 3/506 (0.6 %) doubtful. Based on TOXO-MS re-adjusted cut-off for SAC, the 3 doubtful samples and 2 previously negative samples were reclassified as positive, resulting in a general *T. gondii* seroprevalence of 34.8 % (176/506, 95 % CI 30.6–38.9), which corresponded to seroprevalences of 33.1 % (161/486, 95 % CI 28.9–37.3) in alpacas and 75.0 % (15/20, 95 % CI 56.0–94.0) in llamas. Furthermore, the seroprevalence at the farm level was 89.5 % (34/38, 95 % CI 79.7–99.2). Among the positive animals, 45.0 % (90/200, 95 % CI 38.1–51.9), 26.3 % (74/281, 95 % CI 21.2–31.5), and 48.0 % (12/25, 95 % CI 28.4–67.6) were from north, central and south Italy, respectively.

For *N. caninum*, an overall seroprevalence of 5.7 % (29/506, 95 % CI 3.7–7.8) was found at the animal level considering the NCC manufacturer's thresholds. This was 5.6 % (27/486, 95 % CI 3.5–7.6) for alpacas and 10.0 % (2/20, 95 % CI 0–23.1) for llamas. Among the farms, 26.3 % (10/38, 95 % CI 12.3–40.3) had at least one *N. caninum* seropositive animal. According to these results, 7.0 % (14/200, 95 % CI 3.5–10.5) animals were from northern Italy, and 5.3 % (15/281, 95 % CI 2.7–8.0) from central Italy. No seropositive animals were found from southern Italy (Fig. 1).

Simultaneous presence of antibodies against *T. gondii* and *N. caninum* was detected in 18/506 (3.6 %, 95 % CI 1.9–5.2) of the samples.

3.2. Immunoblot results

Based on TOXO-MS results, a total of 44 sera were tested by immunoblot for the detection of *T. gondii*-specific antibodies. In detail, negative samples showing ≥24 % S/P ≤ 40 %, doubtful samples, and positive samples showing ≥50 % S/P ≤ 70 %, as well as samples with the highest S/P values and lower S/P values were included, as well as the negative and positive *T. gondii* internal controls used in ELISA. In addition, samples showing positive results for both *T. gondii* and *N. caninum* were tested (n = 18) (**Supplementary Fig. S1**).

The comparison between ELISA kit and immunoblot results showed a substantial inter-rater agreement (κ = 0.634; SE of κ = 0.108; 95 % CI 0.422–0.846; Weighted κ = 0.681) (**Supplementary Table S1**) being higher in case of optimized SAC cut-off (κ = 0.790, SE of κ = 0.100, 95 % CI, 0.595–0.986, Weighted κ = 0.790) (**Supplementary Table S2**).

With regards to *N. caninum*, negative samples showing S/N > 60 %, and positive samples showing S/N ≤ 50 %, as well as samples with the highest S/N values and samples with the lower S/N values were tested by immunoblot. Moreover, samples positive for both *T. gondii* and *N. caninum* were included, as well as positive and negative *N. caninum* internal controls sera previously used in ELISA, for a total of 29 samples (**Supplementary Fig. S2**).

The inter-rater agreement of ELISA manufacturer cut-off with immunoblot was almost perfect (κ = 0.901, SE of κ = 0.097, 95 % CI

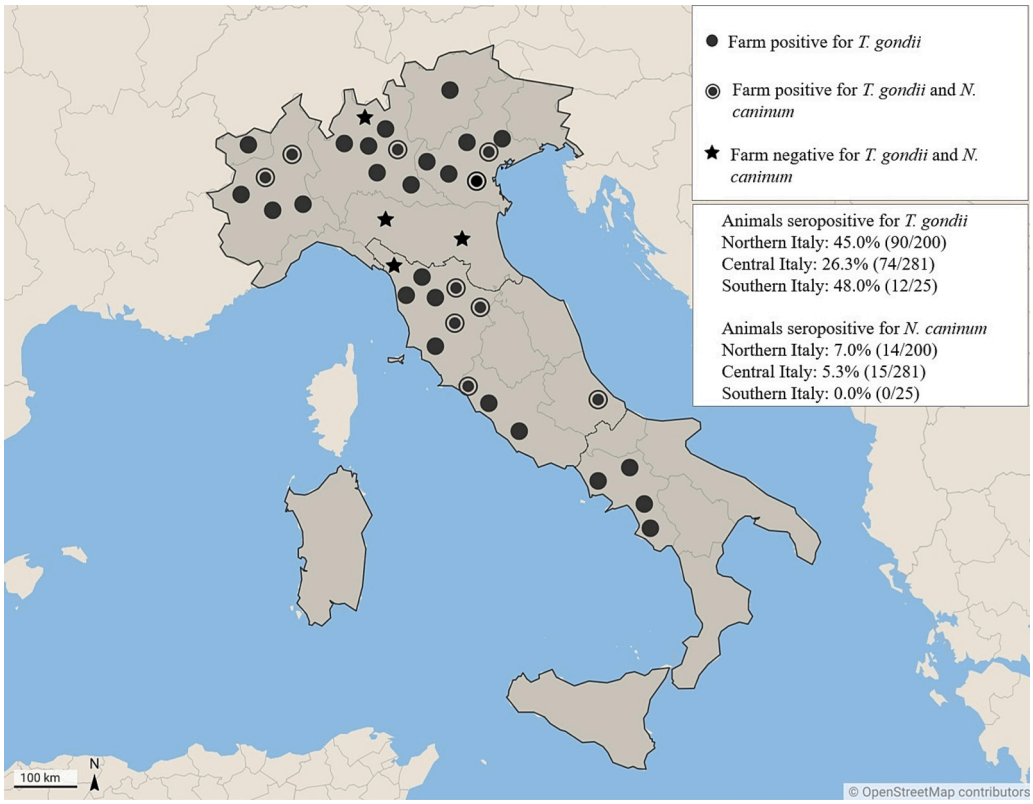

Fig. 1. Map of Italy showing the distribution of farms ($n = 38$) where the presence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in South American Camelids was investigated.

0.711–1.000 Weighted $\kappa = 0.901$) (**Supplementary Table S3)**.

All the samples, which tested positive for both *T. gondii* and *N. caninum* in ELISA test ($n = 16$ alpacas and $n = 2$ llamas) were confirmed as seropositive by immunoblot for both pathogens (**Supplementary Table S4**).

3.3. Questionnaire

A total of 38 questionnaires were collected during the survey (100 % return rate). The average SAC farming experience of respondents was 10.0 years (minimum 1, maximum 27 years). The majority of respondents had a secondary grade level of education (47.4 %, 18/38), followed by university degree (34.2 %, 13/38), while the remaining (18.4 %, 7/38) had lower qualifications. Among the interviewees, 28.9 % (11/38) stated that keeping of SAC was their main occupation, while 71.1 % (27/38) declared to be engaged in different working areas and kept animals as secondary activity. Details on the results of the questionnaire survey including answers on farm structure, husbandry practices and clinical history are shown in Table 1.

3.4. Statistical analysis

The bivariable model showed that *T. gondii* seropositivity increased with age (OR = 1.36, 95 % CI: 1.27–1.47, $p < 2e\text{-}16$), thus the variable age was included into each of the calculated models as an effect-modifying explanatory variable. The risk factor analysis showed that the presence of hens on the farm (OR = 1.21, 95 % CI: 1.00–1.45, $p = 0.0467$) and the practice of pasture sharing with sheep (OR = 7.36, 95 % CI: 1.01–53.70, $p = 0.0489$) were associated with *T. gondii* infection. Additionally, llamas had a higher risk to become infected with *T. gondii* than alpacas (OR = 4.33, 95 % CI: 1.11–16.92, $p = 0.0349$). Besides, management practices, such as keeping animals in paddock with sand soil (compared to other soil types, such as concrete, hay, straw, rubber, wood shaving, or ground), and performing daily cleaning of environments to remove faeces (compared with less frequent or absence of cleaning) were identified as protective factors in both bivariable (**Supplementary Table S5**) and multivariable models (Table 2). Other variables, such as keeping animals for commercial purpose (i.e. selling breeding stock and crias), and getting information on SAC breeding from scientific journals were identified as protective factors but considered as potential confounders (**Supplementary Table S5**).

For *N. caninum* the seropositivity did not show an increase with the age of the animals; consequently, the variable age was considered as a further explanatory variable, and the models were calculated as univariable.

The presence of dogs on the farm was identified as risk factor (OR = 7.46, 95 % CI: 1.85–30.17, $p = 0.00479$), and an association with the occurrence of fertility disorders (OR = 7.60, 95 % CI: 2.33–24.82, $p = 0.000777$) was detected (**Supplementary Table S6**) and confirmed by multivariable modelling (Table 3).

Seroprevalence for *T. gondii* and *N. caninum* according to the main variables and risk factors are reported in Table 4.

4. Discussion

To the authors' best knowledge, the present study provides the first serological evidence of *T. gondii* and *N. caninum* infections, as well as an analysis of associated risk factors in a large-scale population of SAC in Italy.

In this study Multi-Species ELISAs were employed, and the kappa values obtained between ELISA and immunoblot support their use in these animals. However, diagnostic limitations when using a multi-species kit cannot be completely ruled out, therefore the use of immunoblot as a confirmatory test for ELISA results was performed to ensure diagnostic reliability.

The inter-rater agreement (**Supplementary Table S1**) confirmed the

Table 1
Main results of the questionnaire survey conducted among South American Camelid owners in Italy ($n = 38$), focusing on farm structure, husbandry practices and occurrence of reproductive disorders.

Question/Answer	Percentage (n/total)
Herd size	
Small	76.3 % (29/38)
Medium	15.8 % (6/38)
Large	7.9 % (3/38)
Main purpose of keeping animals	
Fibre production	43.0 % (16/38)
Trekking activity	35.0 % (13/38)
Companion animals	32.0 % (12/38)
Breeding	27.0 % (10/38)
Zoos	19.0 % (7/38)
Pet therapy	8.0 % (3/38)
Country of animal origin	
Italy	55.3 % (21/38)
Italy and foreign countries	44.7 % (17/38)
Europe	**94.1 % (16/17)**
Germany	52.9 % (8/17)
Switzerland	41.2 % (7/17)
Belgium, France, United Kingdom	17.6 % (3/17)
Austria, the Netherlands	5.9 % (1/17)
Oceania	**17.6 % (3/17)**
New Zealand	11.8 % (2/17)
Australia	5.9 % (1/17)
America	**17.6 % (3/17)**
Chile	11.8 % (2/17)
Peru, USA	5.9 % (1/17)
Domestic pets on the farm	
Owned cats on the farm in the last 2 years	**37.1 % (13/38)**
Presence of kittens on the farm in the last 2 years	23.7 % (9/38)
Owned cats access to the stables and/or pasture	31.6 % (12/38)
Access of non-owned cats to the stable and or/pasture	**50.0 % (19/38)**
Unknown	31.6 % (12/38)
Owned dogs on the farm in the last 2 years	**65.8 % (25/38)**
Presence of puppies on the farm in the last 2 years	28.9 % (11/38)
Owned dogs access to the stables and/or pasture	44.7 % (17/38)
Access of non-owned dogs to the stable and or/pasture	**18.4 % (7/38)**
Livestock species on the farm	
Ruminants	**52.6 % (20/38)**
Goats	85.0 % (17/20)
Sheep	75.0 % (15/20)
Cattle	30.0 % (6/20)
Buffaloes	20.0 % (4/20)
Co-grazing between SAC and ruminants	40.0 % (8/20)
Hens	**29.0 % (11/38)**
Pasture	
Access to pasture	**81.6 % (31/38)**
Permanent	67.7 % (21/31)
Seasonal	32.3 % (10/31)
Faeces removal	**74.2 % (23/31)**
Daily	39.1 % (9/23)
Weekly	26.0 % (6/23)
Monthly	13.0 % (3/23)
Once a year	4.3 % (1/23)
Twice a year	17.4 % (4/23)
Clinical history	
Occurrence of reproductive disorders	**36.8 % (14/38)**
Abortion	18.4 % (7/38)
Fertility disorders	15.8 % (6/38)
Stunted growth	13.2 % (5/38)
Dystocia	10.5 % (5/38)
Stillbirth	7.9 % (4/38)
Malformation in crias	7.9 % (4/38)
Lack of milk in lactating females	7.9 % (4/38)

optimized cut-off for TOXO-MS ELISA proposed by Basso et al. (2020) (S/P 36.2 %). Accordingly, a general seroprevalence of *T. gondii* of 34.8 % at animal level (i.e., 33.1 % in alpacas and 75.0 % in llamas) and 89.5 % at farm level was herein recorded suggesting a widespread environmental contamination with oocysts with a nearly equal distribution across macro-areas.

Italy ranks the third position among European countries in cat population, with 10,2 million animals in 2023 (Statista, 2024). Despite the high number of cats, the seroprevalence found in SAC in Italy appears to be lower than that reported in Germany (43.8 %; Wolf et al., 2005), Switzerland (83.2 %; Basso et al., 2020), and Czech Republic (88.9 %; Bártová et al., 2017), although in some of these studies only a limited number of animals was tested. Nevertheless, the seroprevalence reported here is consistent with a previous report from southern Italy, which showed a seropositivity of 33.3 % (Marková et al., 2018), although only 4 alpacas and 5 llamas kept in different zoos were analysed. Potentially, the density of definitive hosts and consequent environment contamination with oocysts in the different areas could influence the infection patterns. Seroprevalence rates could be related to the proximity to definitive hosts but also to husbandry management practices, weather condition and soil type (Dubey and Beattie, 1988), as well as to differences in diagnostic methods, which can vary in sensitivity and specificity (Maspi et al., 2021).

The variable age was identified as a risk factor for *T. gondii* infection, with older animals being more frequently infected, likely due to a longer exposure to *T. gondii* oocysts, which increases the chance of infection over time (Abdallah et al., 2020; Stelzer et al., 2019). This suggests that horizontal infection may have a primary role in the epidemiology of toxoplasmosis in SAC. In contrast, the presence of cats was not associated with an increased risk of seropositivity for *T. gondii*. This could be

Table 2
Final optimized generalized linear mixed multivariable model showing the identified risk factors for *Toxoplasma gondii* seropositivity in South American camelids in Italy including the variable "farm" as a random effect variable and the variable "age" as an effect modifying explanatory variable.

Variable	Odds ratio (95 % CI)	z-value	*p*-value
(Intercept)	0.04 (0.02–0.08)	−10.383	< 2e-16***
Age	1.41 (1.30–1.52)	8.434	< 2e-16***
Species			
Alpaca (Ref.)	–	–	–
Llama	7.33 (1.99–27.00)	2.995	0.002748**
Hens			
No (Ref.)	–	–	–
Yes	1.14 (1.01–1.30)	2.11	0.034853*
Sand			
No (Ref.)	–	–	–
Yes	0.12 (0.03–0.59)	−2.629	0.008566**
Daily (Ref.)	–	–	–
Weekly	2.96 (1.56–5.60)	3.327	0.000877***
Monthly	11.44 (3.95–33.11)	4.496	0.00000691***
Once a year	9.60 (1.52–60.76)	2.402	0.016312*
Twice a year	11.26 (3.43–36.95)	3.993	0.0000652***
Never	2.78 (1.30–5.94)	2.638	0.008331**

AIC = Akaike Information Index 443.8, CI = Confidence interval, Ref. = reference, e = exponential, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 3
Final optimized generalized linear mixed multivariable models to determine potential risk factors for *Neospora caninum* seropositivity in South American camelids in Italy including the variable "farm" as a random effect variable.

Variable	Odds ratio (95 % CI)	z-value	*p*-value
(Intercept)	3.59e-02 (0.02–0.06)	−12.472	< 2e-16***
Animal species on pasture			
No (Ref.)	–	–	–
Dogs	3.10 (1.10–8.68)	2.15	0.03157*
Goats	0.72 (0.08–6.22)	−0.297	0.7666
Goats and sheep	9.95e-13 (0-Inf)	0	0.99999
Sheep	1.36e-12 (0-Inf)	0	0.99998
Fertility disorders			
No (Ref.)	–	–	–
Yes	4.72 (1.61–13.86)	2.823	0.00476**

Abbreviations: AIC = Akaike Information Index 207.4, CI = Confidence interval, Ref. = reference, e = exponential, Inf = infinite, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 4
Seroprevalence for *Toxoplasma gondii* and *Neospora caninum* according to the main variables and risk factors.

Variables	*Toxoplasma gondii*		*Neospora caninum*	
	Positive animals (%, n)	95 % CI	Positive animals (%, n)	95 % CI
Sex				
Female	36.0 % (117/325)	30.78–41.22	5.5 % (18/325)	3.05–8.03
Intact male	29.4 (47/160)	22.32–36.43	6.3 % (10/160)	2.50–10.00
Castrated male	57.1 % (12/21)	35.98–78.31	4.8 % (1/21)	0–13.87
Age				
Crias	14.3 % (3/21)	0–29.25	9.5 % (2/21)	0–22.08
Weaners	0.0 % (0/20)	0	5.0 % (1/20)	0–14.55
Tuis	16.9 % (11/65)	7.81–26.04	1.5 % (1/65)	0–4.53
Adults	40.5 % (162/400)	35.69–45.31	6.3 % (25/400)	3.88–8.6
Area				
Northern	45.0 % (90/200)	38.11–51.89	7.0 % (14/200)	3.46–10.54
Central	26.3 % (74/281)	21.18–31.48	5.3 % (15/281)	2.71–7.97
Southern	48.0 % (12/25)	28.42–67.58	0.0 % (0/25)	0
Herd size				
Small	43.7 % (80/183)	36.53–50.90	3.8 % (7/183)	1.05–6.60
Medium	48.4 % (45/93)	38.23–58.54	16.1 % (15/93)	8.65–23.60
Large	22.2 % (51/230)	16.81–27.54	3.0 % (7/230)	0.82–5.26
Species				
Alpaca	33.1 % (161/486)	28.94–37.31	5.6 % (27/486)	3.52–7.59
Llama	75.0 % (15/20)	56.02–93.98	10.0 % (2/20)	0–23.15
Main purposes of keeping animals				
Fibre production	28.8 % (105/364)	24.19–33.50	6.0 % (22/364)	3.60–8.49
Trekking activity	28.0 % (93/332)	23.18–32.84	3.9 % (13/332)	1.83–6.00
Companion animals	28.0 % (83/296)	22.92–33.16	3.0 % (9/296)	1.08–5.00
Breeding	22.1 % (73/330)	17.64–26.60	4.2 % (14/330)	2.07–6.42
Zoo	58.6 % (17/29)	40.70–76.55	3.4 % (1/29)	0–10.09
Pet-therapy	28.6 % (6/21)	9.25–47.89	4.8 % (1/21)	0–13.87
Co-living with livestock species				
Goats	47.2 % (42/89)	36.82–57.56	2.2 % (2/89)	0–5.33
Sheep	46.2 % (30/65)	34.03–58.27	3.1 % (2/65)	0–7.28
Cattle	68.8 % (11/16)	46.04–91.46	6.3 % (1/16)	0–18.11
Buffaloes	72.7 % (8/11)	46.41–99.05	9.1 % (1/11)	0–26.08
Hens	47.8 % (55/115)	38.70–56.96	10.4 % (12/115)	4.85–16.02
Domestic animals				
Owned cats	27.1 % (88/325)	22.25–31.91	4.0 % (13/325)	1.87–6.13
Kittens	26.6 % (71/267)	21.29–31.89	3.7 % (10/267)	1.47–6.02
Non-owned cats	31.2 % (119/382)	26.51–35.80	7.1 % (27/382)	4.50–9.64
Owned dogs	26.3 % (80/304)	21.37–31.27	4.3 % (13/304)	2.00–6.55
Puppies	29.8 % (99/332)	24.90–34.74	6.6 % (22/332)	3.95–9.30
Non-owned dogs	22.8 % (58/254)	17.67–28.00	4.3 % (11/254)	1.83–6.83
Type of bedding				
Concrete	44.8 % (60/134)	36.36–53.20	13.4 % (18/134)	7–66–19.21
Hay	30.6 % (94/307)	25.46–35.77	3.3 % (10/307)	1.27–5.24
Straw	40.3 % (27/67)	28.55–52.04	4.5 % (3/67)	0–9.43
Wood shavings	37.5 % (15/40)	22.50–52.50	7.5 % (3/40)	0–15.66
Wooden floor	33.3 % (2/6)	0–71.05	0.0 % (0/6)	0
Sand	17.1 % (7/41)	5.56–28.59	9.8 % (4/41)	0.67–18.84
Ground	43.0 % (49/114)	33.89–52.07	4.4 % (5/114)	0.63–8.15
Frequency of faeces removal				
Never	38.8 % (19/49)	25.13–52.42	4.1 % (2/49)	0–9.62
Daily	23.4 % (64/274)	18.35–28.37	5.1 % (14/274)	2.50–7.72
Weekly	44.2 % (38/86)	33.69–54.68	3.5 % (3/86)	0–7.37
Monthly	63.2 % (12/19)	41.47–84.85	0.0 % (0/19)	0
Once a year	60.0 % (3/5)	17.06–100.0	0.0 % (0/5)	0
Twice a year	82.1 % (23/28)	67.96–96.33	32.1 % (9/28)	14.84–49.44
Fertility disorders				
Abortion	29.7 % (78/263)	24.14–35.18	6.8 % (18/263)	3.79–9.90
Fertility disorders	72.5 % (29/40)	58.66–86.34	27.5 % (11/40)	13.66–41.34
Stunted growth	57.6 % (19/33)	40.71–74.44	6.1 % (2/33)	0–14.20
Dystocia	22.1 % (54/244)	16.92–27.34	4.5 % (11/244)	1.90–7.11
Stillbirth	14.6 % (29/198)	9.72–19.57	5.1 % (11/217)	2.15–7.99
Malformation in crias	29.4 % (69/235)	23.54–35.18	6.8 % (16/235)	3.59–10.03
Lack of milk in females	50.0 % (10/20)	28.09–71.91	10.0 % (2/20)	3.15–23.15

explained by the limited presence of kittens on the investigated farms, as they represent the primary responsible for oocyst contamination (Dubey and Beattie, 1988), or by the possibility of infection being acquired not only directly through the faeces of infected on-farm cats but also from further sources, like contaminated water or fodder (Dubey, 2009). Interestingly, the co-housing with hens and shared environments between SAC and sheep were detected as risk factors for *T. gondii* infection. This could be related to differences in management practices that indirectly favour *T. gondii* transmission (i.e., backyard farming, a higher likelihood of cats' presence, less hygienic conditions). Indeed, although chickens and sheep do not represent a direct infection source for SAC, they can serve as source for cats, which may feed on infected tissues and thereafter shedding millions of oocysts (Dubey, 2010; Selim et al., 2023). Furthermore, chicken serve as sentinel species for soil contamination with oocysts due to their behaviour to peck the ground to feed. In this way they may also feed on transport hosts (i.e. earthworms and insects) that could mechanically carry or be contaminated with oocysts, as well as being frequently infected (Stelzer et al., 2019; Dubey et al., 2020b; Andreopoulou et al., 2023). It could be hypothesized that in

farms where materials from sheep abortions due to toxoplasmosis are not properly disposed the likelihood of exposure to the infection is higher since cats can easily ingest such materials from aborted foetuses and become infected (Dahmane et al., 2024).

Unlike sheep and goats, for which evidence of abortion due to *T. gondii* infection has been reported (Nayeri et al., 2021), only limited evidence exists for camelids. In this study no positive association was found between *T. gondii* infection and occurrence of abortion. Dubey et al. (2014a) provided the first documented case of *T. gondii* associated abortion in alpacas by detecting *T. gondii* cysts in the brain and kidney of an aborted foetus suggesting that congenital transmission may occur. In addition, Rüfli et al. (2021) detected presence of *T. gondii* antibodies in one aborted foetus and one stillborn cria in Switzerland, giving more support to this observation. However, the vertical transmission is *vexata quaestio* as also supported by a recent meta-analysis (Djeddou-Benabid et al., 2025) and further data on aborted foetuses should be obtained.

According to previous investigations (Wolf et al., 2005; Basso et al., 2020), alpacas showed lower *T. gondii* seroprevalence than llamas. In this study there were no significant differences in general management practices between llamas and alpacas that could justify the higher seropositivity in llamas. However, only a limited number of llamas was tested, and results should be carefully interpreted.

In the current study, animal sex was not a risk factor for exposure to *T. gondii*. This result is consistent with some previous research (Ramirez et al., 2005; Li et al., 2020); however, it differs from results reported by others where female alpacas, llamas and camels were showing higher seroprevalences than males (Basso et al., 2020; Selim et al., 2023).

Besides, keeping SAC in paddock with sandy soil was identified as a protective factor for *T. gondii* infection. Biological, chemical and physical properties of soil can differ depending on soil type and the season, influencing the viability of *T. gondii* oocysts (Lélu et al., 2011). Gao et al. (2016) proved that although oocysts can be detected in any soil types, gravel and sand are among the soil types with the least contamination. This could be due to the rapid drying of sand, which has a poor water-holding capacity, thereby reducing retained moisture. In addition, sand tends to heat up more quickly and reach higher temperatures than soil when exposed to sunlight, due to its lower moisture content and distinct thermal properties, potentially impairing oocyst survival (Gao et al., 2016; Omar and Alsharaeh, 2024).

Daily cleaning of environments, either through mechanical or manual removal of faeces, had a protective role against *T. gondii* infection. Thus, implementing general measures of hygiene and regular cleaning regimes in farm environments can help reduce cat faecal contamination within the facilities (Stelzer et al., 2019). Such measures, in turn, may lower oocyst contamination and minimize the exposure of intermediate hosts.

Alpaca and llama meat demand is increasing not only in South America, where 39.6 % of total meat production is processed for jerky and sausages, but also in North America, Europe and Oceania (De La Lama et al., 2022). In Italy SAC meat is not currently marketed; however the potential for this sector to emerge in the future cannot be excluded. Thus, to mitigate the risk of infection from untreated SAC meat to humans, proper cooking and/or freezing measures of the meat are strongly recommended (Almeria and Dubey, 2021).

With regards to *N. caninum* the inter-rater agreement applying the NCC manufacturer's thresholds was high (**Supplementary Table S3**), and a seroprevalence of 5.7 % at animal level (i.e., 5.6 % in alpacas and 10.0 % in llamas) and 26.3 % at farm level were observed. The seroprevalence was similar in northern and central Italy, whereas it seemed to be lower in the southern area; however, this needs further investigation due to the low number of tested samples from this area. In Europe the epidemiology of *N. caninum* in SAC has been very scarcely investigated and data were reported only from Switzerland (3.5 % out of 374 alpacas and 2.5 % out of 197 llamas; Basso et al., 2020), Czech Republic (1 out of 12 SAC; Bártová et al., 2017), Germany (0 out of 32 SAC; Wolf et al., 2005) and Italy (0 out of 9 SAC; Marková et al., 2018).

Dogs are well known to play a key role in the spread and maintenance of *N. caninum* (Lindsay and Dubey, 2020). Accordingly, the results of the statistical analysis revealed that the presence of dogs on the farm significantly increases the risk for *N. caninum* infection in SAC. In Italy, the seroprevalence of infected dogs living in rural areas has been estimated to be as high as 36.4 % (Ferroglio et al., 2007); nevertheless, the prevalence of infected dogs naturally shedding oocyst in faeces is most likely notably lower, around 0.03 % (Schares et al., 2005), thus, a general lower environmental contamination with oocysts may be assumed (Basso et al., 2022). These data may explain why, on a global scale, the environmental exposure to *N. caninum* infection seems to be lower compared to *T. gondii*. However, results for *T. gondii* and *N. caninum* from different studies need to be interpreted with care as serological results are affected by the diagnostic methods, study designs, and sample sizes (Dubey and Schares, 2011).

Besides dogs, wolves were also confirmed as definitive host of *N. caninum* (Dubey and Schares, 2011). A recent study reported that the estimated wolf population is increasing across the Italian Apennines, reaching about 2557 individuals (Gervasi et al., 2024); nevertheless, national data on neosporosis and oocyst shedding in these animals are not available. In the present survey most farms owned dogs, and it was hypothesized that the presence of farm dogs may discourage wild canids from entering the premises (Dubey et al., 2007). Additionally, almost all farms used netting systems to limit the presence of wildlife. This result could indicate a marginal implication of wild canids in the sylvatic cycle of *N. caninum* as previous reported in vicuñas (Wolf et al., 2005); however, it is still worthy of further investigations.

In cattle, vertical transmission is recognized as the primary route of *N. caninum* infection, enabling the perpetuation of the infection across herd generations even without the presence of the definitive hosts (Moré et al., 2009). In SAC little is known about transplacental transmission. However, the detection of *N. caninum* DNA in the brain of aborted alpaca and llama foetuses suggested that this way of transmission may also occur in SAC, providing presumptive evidence that *N. caninum* was a more likely causative agent of abortion than *T. gondii* (Serrano-Martínez et al., 2007). In the current study, a strong association between the presence of *N. caninum* antibodies and fertility disorders among the studied SAC population was observed. *Neospora caninum*-associated abortion primarily occurred during early and mid-pregnancy in SAC (Serrano-Martínez et al., 2007). However, it is uncertain whether farmers had pregnancy diagnosis performed by veterinarians. Consequently, early embryonic loses may have occurred on farms without being detected. Similarly, abortions may have gone unnoticed, as expelled foetuses could have been scavenged by domestic animals, such as dogs and cats, searching for food (Serrano-Martínez et al., 2007). The failure to detect these events may have contributed to the perception of "infertility disorders" by farmers. Nevertheless, the observed association could point that irrespective of the low *N. caninum* seroprevalence in SACs, neosporosis may have some involvement as cause of reproductive problems in these animal species.

5. Conclusion

This survey provides the first nationwide seroepidemiological data for *T. gondii* and *N. caninum* in alpacas and llamas in Italy. The study showed that these protozoan infections in SAC are widespread throughout the country, providing a valuable assessment of risk factors in the Italian breeding context. The results suggest environmental contamination and show that farm management practices may play a key role in the epidemiology of these parasites. However, data on the clinical impact of toxoplasmosis and neosporosis in SAC and the role of these protozoa in causing abortion in SAC in Italy are still lacking and further clinical studies are needed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2025.105729.

CRediT authorship contribution statement

Elisa Castaldo: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Walter Basso:** Writing – review & editing, Methodology, Conceptualization. **Gastón Moré:** Writing – review & editing. **Alessia Ciaramelli:** Methodology. **Nicola D'Alessio:** Funding acquisition. **Michele Capasso:** Methodology. **Giovanni Sgroi:** Methodology, Funding acquisition. **Sara Tonon:** Project administration, Funding acquisition. **Farwa Humak:** Methodology. **Alessia Gazzonis:** Methodology. **Gereon Schares:** Writing – review & editing, Formal analysis, Data curation. **Vincenzo Veneziano:** Writing – review & editing, Project administration, Conceptualization.

Funding

The study received partial support from a Grant from the Ministry of Health of the Italian Republic (IZSLT 11/24 RC) and internal funding from the Institute of Parasitology of the University of Bern, Switzerland.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the freelance veterinarians and camelid breeders who contributed to this research.

References

Abdallah, M.C., Kamel, M., Karima, B., Samir, A., Mohammed Hocine, B., Djamel, K., Rachid, K., Khatima, A.O., 2020. First report of *Toxoplasma gondii* infection and associated risk factors in the dromedary camel (*Camelus dromedarius*) population in south East Algeria. Vet. Parasitol. Reg. Stud. Reports. 22, 100475. https://doi.org/10.1016/j.vprsr.2020.100475.

Almeria, S., Dubey, J.P., 2021. Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. Res. Vet. Sci. 135, 371–385. https://doi.org/10.1016/j.rvsc.2020.10.019.

Andreopoulou, M., Schares, G., Koethe, M., Chaligiannis, I., Maksimov, P., Joeres, M., Cardron, G., Goroll, T., Sotiraki, S., Daugschies, A., Bangoura, B., 2023. Prevalence and molecular characterization of *Toxoplasma gondii* in different types of poultry in Greece, associated risk factors and co-existence with *Eimeria* spp. Parasitol. Res. 122 (1), 97–111. https://doi.org/10.1007/s00436-022-07701-6.

Bártová, E., Kobédová, K., Lamka, J., Kotrba, R., Vodička, R., Sedlák, K., 2017. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in exotic ruminants and camelids in the Czech Republic. Parasitol. Res. 116 (7), 1925–1929. https://doi.org/10.1007/s00436-017-5470-6.

Basso, W., Moré, G., Quiroga, M.A., Balducchi, D., Schares, G., Venturini, M.C., 2014. *Neospora caninum* is a cause of perinatal mortality in axis deer (*Axis axis*). Vet. Parasitol. 199 (3–4), 255–258. https://doi.org/10.1016/j.vetpar.2013.10.020.

Basso, W., Sollberger, E., Schares, G., Küker, S., Ardüser, F., Moore-Jones, G., Zanolari, P., 2020. *Toxoplasma gondii* and *Neospora caninum* infections in South American camelids in Switzerland and assessment of serological tests for diagnosis. Parasit. Vectors 13 (1), 256. https://doi.org/10.1186/s13071-020-04128-9.

Gliga, D.S., Basso, W., Ardüser, F., Moore-Jones, G., Schares, G., Zanolari, P., Frey, C.F., 2022. Switzerland-wide *Neospora caninum* seroprevalence in female cattle and identification of risk factors for infection. Front. Vet. Sci. 9, 1059697. https://doi.org/10.3389/fvets.2022.1059697.

Basso, W., Holenweger, F., Schares, G., Müller, N., Campero, L.M., Ardüser, F., Moore-Jones, G., Frey, C.F., Zanolari, P., 2022. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted foetuses. Food Waterborne Parasitol. 28, e00176. https://doi.org/10.1016/j.fawpar.2022.e00176.

Chávez-Velásquez, A., Alvarez-García, G., Gómez-Bautista, M., Casas-Astos, E., Serrano-Martínez, E., Ortega-Mora, L.M., 2005. *Toxoplasma gondii* infection in adult llamas (*Lama glama*) and vicunas (*Vicugna vicugna*) in the Peruvian Andean region. Vet. Parasitol. 130 (1–2), 93–97. https://doi.org/10.1016/j.vetpar.2005.03.023.

Dahmane, A., Almeida, D., Reghaissia, N., Baroudi, D., Samari, H., Abdelli, A., Laatamna, A., Mesquita, J.R., 2024. Seroprevalence assessment and risk factor analysis of *Toxoplasma gondii* infection in goats from northeastern Algeria. Animals 14 (6), 883. https://doi.org/10.3390/ani14060883.

De La Lama, G.C.M., Mamani Linares, W., Estévez-Moreno, L.X., 2022. Species destined for non-traditional meat production: goats and South American domestic camelids. In: Faucitano, L. (Ed.), Preslaughter Handling and Slaughter of Meat Animals, the Netherlands, pp. 349–389.

Djeddou-Benabid, A., Houssou, H., Djeddi, K., Ouachtati, D., 2025. Toxoplasmosis in camelids: the last 20 years. BJAER 8 (1), e76696. https://doi.org/10.34188/bjaerv8n1-025.

Dubey, J.P., 1998. *Toxoplasma gondii* oocyst survival under defined temperatures. J. Parasitol. 84, 862–865. https://doi.org/10.2307/3284606.

Dubey, J.P., 2009. History of the discovery of the life cycle of *Toxoplasma gondii*. Int. J. Parasitol. 39 (8), 877–882. https://doi.org/10.1016/j.ijpara.2009.01.005.

Dubey, J.P., 2010. *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health 57 (1), 60–73. https://doi.org/10.1111/j.1863-2378.2009.01274.x.

Dubey, J.P., 2021. Toxoplasmosis of Animals and Humans. CRC Press, Boca Raton, Florida, USA, p. 542.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Ratón, Florida, USA, p. 220.

Dubey, J.P., Schares, G., 2011. Neosporosis in animals–the last five years. Vet. Parasitol. 180 (1–2), 90–108. https://doi.org/10.1016/j.vetpar.2011.05.031.

Dubey, J.P., Sreekumar, C., Knickman, E., Miska, K.B., Vianna, M.C., Kwok, O.C., Hill, D. E., Jenkins, M.C., Lindsay, D.S., Greene, C.E., 2004. Biologic, morphologic, and molecular characterisation of *Neospora caninum* isolates from littermate dogs. Int. J. Parasitol. 34 (10), 1157–1167. https://doi.org/10.1016/j.ijpara.2004.07.005.

Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemiology and control of neosporosis and *Neospora caninum*. Clin. Microbiol. Rev. 20 (2), 323–367. https://doi.org/10.1128/CMR.00031-06.

Dubey, J.P., Johnson, J.E., Hanson, M.A., Pierce, V., 2014a. Toxoplasmosis-associated abortion in an alpaca (*Vicugna pacos*) fetus. J. Zoo Wildl. Med. 45 (2), 461–464. https://doi.org/10.1638/2014-0006R.1.

Dubey, J.P., Newell, T.K., Verma, S.K., Calero-Bernal, R., Stevens, E.L., 2014b. *Toxoplasma gondii* infection in llama (*Lama glama*): acute visceral disseminated lesions, diagnosis, and development of tissue cysts. J. Parasitol. 100 (3), 288–294. https://doi.org/10.1645/13-427.1.

Dubey, J.P., Hemphill, A., Calero Bernal, R., Schares, G., 2017. Neosporosis in Animals. CRC Press, Boca Raton, Florida, USA, p. 529.

Dubey, J.P., Cerqueira-Cezar, C.K., Murata, F.H.A., Kwok, O.C.H., Yang, Y.R., Su, C., 2020a. All about toxoplasmosis in cats: the last decade. Vet. Parasitol. 283, 109145. https://doi.org/10.1016/j.vetpar.2020.109145.

Dubey, J.P., Pena, H.F.J., Cerqueira-Cézar, C.K., Murata, F.H.A., Kwok, O.C.H., Yang, Y. R., Gennari, S.M., Su, C., 2020b. Epidemiologic significance of *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): the past decade. Parasitology 147 (12), 1263–1289. https://doi.org/10.1017/S0031182020001134.

Ferroglio, E., Pasino, M., Ronco, F., Benà, A., Trisciuoglio, A., 2007. Seroprevalence of antibodies to *Neospora caninum* in urban and rural dogs in north-West Italy. Zoonoses Public Health 54 (3–4), 135–139. https://doi.org/10.1111/j.1863-2378.2007.01033.x.

Fowler, M.E., 2010. General biology and evolution. In: Fowler, M.E. (Ed.), Medicine and Surgery of South American Camelids: Llama, Alpaca, vicuña, guanaco. Wiley Blackwell, Ames, Iowa State, pp. 3–16.

Gao, X., Wang, H., Wang, H., Qin, H., Xiao, J., 2016. Land use and soil contamination with *Toxoplasma gondii* oocysts in urban areas. Sci. Total Environ. 568, 1086–1091. https://doi.org/10.1016/j.scitotenv.2016.06.165.

Gervasi, V., Aragno, P., Salvatori, V., Caniglia, R., De Angelis, D., Fabbri, E., La Morgia, V., Marucco, F., Velli, E., Genovesi, P., 2024. Estimating distribution and abundance of wide-ranging species with integrated spatial models: opportunities revealed by the first wolf assessment in south-Central Italy. Ecol. Evol. 14 (5), e11285. https://doi.org/10.1002/ece3.11285.

González-Warleta, M., Castro-Hermida, J.A., Calvo, C., Pérez, V., Gutiérrez-Expósito, D., Regidor-Cerrillo, J., Ortega-Mora, L.M., Mezo, M., 2018. Endogenous transplacental transmission of *Neospora caninum* during successive pregnancies across three generations of naturally infected sheep. Vet. Res. 49 (1), 106. https://doi.org/10.1186/s13567-018-0601-3.

Lélu, M., Gilot-Fromont, E., Aubert, D., Richaume, A., Afonso, E., Dupuis, E., Gotteland, C., Marnef, F., Poulle, M.L., Dumètre, A., Thulliez, P., Dardé, M.L., Villena, I., 2011. Development of a sensitive method for *Toxoplasma gondii* oocyst extraction in soil. Vet. Parasitol. 183 (1–2), 59–67. https://doi.org/10.1016/j.vetpar.2011.06.018.

Li, J., Ma, Y.T., Liang, Q.L., Li, R.L., Zheng, F.G., Liu, Q., Zhu, X.Q., Gao, W.W., 2020. Serological evidence of *Toxoplasma gondii* and *Chlamydia* infection in alpacas (*Vicugna pacos*) in Shanxi Province, northern China. Microb. Pathog. 149, 104399. https://doi.org/10.1016/j.micpath.2020.104399.

Lindsay, D.S., Dubey, J.P., 2020. Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants: An Update. The Veterinary clinics of North America. Food animal practice 36 (1), 205–222. https://doi.org/10.1016/j.cvfa.2019.11.004.

Love, S., 2017. Alpaca Worms - An Overview. https://www.dpi.nsw.gov.au/_data/assets/pdf_file/0004/318217/Alpaca-worms-an-overview.pdf. (Accessed 24 May 2024).

Lücht, M., Stagegaard, J., Conraths, F.J., Schares, G., 2019. *Toxoplasma gondii* in small exotic felids from zoos in Europe and the Middle East: serological prevalence and risk factors. Parasit. Vectors 12 (1), 449. https://doi.org/10.1186/s13071-019-3706-2.

Marková, J., Machačová, T., Bártová, E., Sedlák, K., Budíková, M., Silvestre, P., Laricchiuta, P., Russo, M., Veneziano, V., 2018. *Toxoplasma gondii*, *Neospora caninum* and *Encephalitozoon cuniculi* in animals from captivity (zoo and Circus animals). J. Eukaryot. Microbiol. 66 (3), 442–446. https://doi.org/10.1111/jeu.12688.

Maspi, N., Nayeri, T., Moosazadeh, M., Sarvi, S., Sharif, M., Daryani, A., 2021. Global seroprevalence of *Toxoplasma gondii* in Camelidae: a systematic review and meta-

analysis. Acta Parasitol. 66 (3), 733–744. https://doi.org/10.1007/s11686-020-00333-9.

Moré, G., Pardini, L., Basso, W., Marín, R., Bacigalupe, D., Auad, G., Venturini, L., Venturini, M.C., 2008. Seroprevalence of *Neospora caninum*, *Toxoplasma gondii* and *Sarcocystis* sp. in llamas (*Lama glama*) from Jujuy, Argentina. Vet. Parasitol. 155 (1–2), 158–160. https://doi.org/10.1016/j.vetpar.2008.04.003.

Moré, G., Bacigalupe, D., Basso, W., Rambeaud, M., Beltrame, F., Ramirez, B., Venturini, M.C., Venturini, L., 2009. Frequency of horizontal and vertical transmission for *Sarcocystis cruzi* and *Neospora caninum* in dairy cattle. Vet. Parasitol. 160 (1–2), 51–54. https://doi.org/10.1016/j.vetpar.2008.10.081.

Nayeri, T., Sarvi, S., Moosazadeh, M., Daryani, A., 2021. Global prevalence of *Toxoplasma gondii* infection in the aborted fetuses and ruminants that had an abortion: a systematic review and meta-analysis. Vet. Parasitol. 290, 109370. https://doi.org/10.1016/j.vetpar.2021.109370.

Omar, H., Alsharaeh, E., 2024. Improving water retention in sandy soils with high-performance superabsorbents hydrogel polymer. ACS Omega 9 (22), 23531–23541. https://doi.org/10.1021/acsomega.4c00727.

Ramirez, J.R., Chávez-Velásquez, A., Casas-Astos, E., Rosadio-Alcantara, R., Falcón, P.N., 2005. Seroprevalencia de *Toxoplasma gondii* en alpacas de comunidades de la provincia de Canchis, Cusco. Rev. Inv. Vet. Perú 16, 162–174.

Rüfli, I., Gurtner, C., Basso, W.U., Vidondo, B., Hirsbrunner, G., Zanolari, P., 2021. Causes of abortions in South American camelids in Switzerland-cases and questionnaire. Animals 11 (7), 1956. https://doi.org/10.3390/ani11071956.

Schares, G., Pantchev, N., Barutzki, D., Heydorn, A.O., Bauer, C., Conraths, F.J., 2005. Oocysts of *Neospora caninum*, *Hammondia heydorni*, *Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany. Int. J. Parasitol. 35, 1525–1537. https://doi.org/10.1016/j.ijpara.2005.08.008.

Selim, A., Marawan, M.A., Abdelhady, A., Wakid, M.H., 2023. Seroprevalence and potential risk factors of *Toxoplasma gondii* in dromedary camels. Agricolture 13 (1), 129. https://doi.org/10.3390/agriculture13010129.

Serrano-Martínez, E., Collantes-Fernández, E., Rodríguez-Bertos, A., Casas-Astos, E., Alvarez-García, G., Chávez-Velásquez, A., Ortega-Mora, L.M., 2004. *Neospora* species-associated abortion in alpacas (*Vicugna pacos*) and llamas (*Lama glama*). Vet. Rec. 155 (23), 748–749. https://doi.org/10.1136/vr.155.23.748.

Serrano-Martínez, E., Collantes-Fernández, E., Chávez-Velásquez, A., Rodríguez-Bertos, A., Casas-Astos, E., Risco-Castillo, V., Rosadio-Alcantara, R., Ortega-Mora, L. M., 2007. Evaluation of *Neospora caninum* and *Toxoplasma gondii* infections in alpaca (*Vicugna pacos*) and llama (*Lama glama*) aborted foetuses from Peru. Vet. Parasitol. 150 (1–2), 39–45. https://doi.org/10.1016/j.vetpar.2007.08.048.

Silva, R.C., Machado, G.P., 2016. Canine neosporosis: perspectives on pathogenesis and management. Vet. Med. (Auckland) 7, 59–70. https://doi.org/10.2147/VMRR.S76969.

Statista, 2024. https://www.statista.com/statistics/516000/cat-population-europe-italy/. (Accessed 19 April 2024).

Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L.M., Maksimov, P., Gethmann, J., Conraths, F.J., Schares, G., 2019. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: risk factors and economic impact. Food Waterborne Parasitol. 15, e00037. https://doi.org/10.1016/j.fawpar.2019.e00037.

Tibary, A., Fite, C., Anouassi, A., Sghiri, A., 2006. Infectious causes of reproductive loss in camelids. Theriogenology 66 (3), 633–647. https://doi.org/10.1016/j.theriogenology.2006.04.008.

Van Saun, R., 2013. Body Condition Scoring of Llamas and Alpacas. Penn State Extension. https://extension.psu.edu/body-condition-scoring-of-llamas-and-alpacas (accessed 19 September 2024).

Vet Info, 2024. www.vetinfo.it. (Accessed 29 April 2024).

Webster, J.P., Dubey, J.P., 2010. Toxoplasmosis of animals and humans. Parasit. Vectors 3, 112. https://doi.org/10.1186/1756-3305-3-112.

Wolf, D., Schares, G., Cardenas, O., Huanca, W., Cordero, A., Bärwald, A., Conraths, F.J., Gauly, M., Zahner, H., Bauer, C., 2005. Detection of specific antibodies to *Neospora caninum* and *Toxoplasma gondii* in naturally infected alpacas (*Lama pacos*), llamas (*Lama glama*) and vicunas (*Lama vicugna*) from Peru and Germany. Vet. Parasitol. 130 (1–2), 81–87. https://doi.org/10.1016/j.vetpar.2005.03.024.