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RESEARCH ARTICLE

Serostatus of Small Ruminant Toxoplasmosis and Neosporosis Throughout the Southeastern Anatolia Region of Türkiye

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ABSTRACT

Toxoplasma (*T.*) *gondii* and *Neospora* (*N.*) *caninum* are two significant abortifacient apicomplexan protozoa causing severe economic losses in livestock farming. This study was designed to investigate the serological status of *T. gondii* and *N. caninum* in sheep and goats across the Southeastern Anatolia Region of Türkiye, including some provinces bordering Syria and Iraq. Totally, 906 serum samples collected from female sheep (n=510) and goats (n=396) were investigated for specific antibodies against these protozoa by recombinant *Tg*SAG2 and *Nc*SAG1 protein-based indirect ELISA (iELISA). The individual seroprevalence of *T. gondii* and *N. caninum* in sheep was determined as 32.6 and 3.1%, respectively, while the seroprevalence of infection with both species was 33.1%. On the other hand, *T. gondii* and *N. caninum* seroprevalence in goats were 32.1 and 20.2%, respectively, while the total seroprevalence of infection with both species was 39.4%. The highest seroprevalence values for *T. gondii* ($P<0.05$) and *N. caninum* ($P>0.05$) were determined in sheep from Diyarbakır and Mardin provinces, respectively, while in goats, higher seroprevalence values for both protozoa were determined in Diyarbakır province compared to other provinces ($P<0.05$). Statistical analyses revealed that seroprevalence values varying between provinces were significant ($P<0.05$), except for ovine neosporosis ($P>0.05$) and when seroprevalence values between animal species were compared, caprine neosporosis was statistically significantly higher ($P<0.05$). The fact that 35.9% of the small ruminants enrolled in the study had been exposed to abortive apicomplexan protozoa during their lives is a serious concern, given the zoonotic aspects of these protozoa and the economic damage they can cause.

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INTRODUCTION

Toxoplasmosis and neosporosis are two important parasitic diseases that can have a profound impact on the health and economic well-being of small ruminant farming communities. *T. gondii* and *N. caninum*, which differ antigenically, have genetic, structural, and immunological relationships (Dubey, 2003; Reid *et al*., 2012). Toxoplasmosis is a primary cause of abortion, stillbirth, and neonatal mortality in small ruminants (Kaltungo and Musa, 2013). Similarly, neosporosis, caused by *N. caninum*, is a leading cause of reproductive losses in cattle and is increasingly recognized as an important disease in sheep and goats (Dubey and Lindsay, 1996). The pathogenesis of these parasitic infections is complex and not fully understood.

T. gondii, is a widespread zoonotic parasite that has a wide host range, including a variety of warm-blooded animals and humans. Infection in small ruminants can occur through the ingestion of oocysts shed by infected cats or the consumption of contaminated feed and water. In pregnant animals, toxoplasmosis can lead to parasitemia and invasion of the placenta and fetus, resulting in abortion or congenital infection (Kaltungo and Musa, 2013). *N. caninum* follows a similar pattern, with parasitemia followed by placental and fetal invasion, leading to the birth of congenitally infected offspring or abortion; on the other hand, it is a primary cause of bovine abortion cases, but, recent studies have shown that *N. caninum* can also cause abortion in small ruminants (Dubey *et al*., 2017; Sánchez-Sánchez *et al*., 2021). Unlike *T. gondii*, the

zoonotic potential of *N. caninum* has not yet been clarified (Dubey, 1999; Dubey *et al*., 2006).

Small ruminant toxoplasmosis and neosporosis can adversely affect the economy through reduced milk production, decreased fertility, and livestock losses due to abortion, and neonatal mortality can have significant financial consequences for individual farmers and the broader agricultural sector (Kaltungo and Musa, 2013; Schneider *et al*., 2013). Moreover, the zoonotic potential of these parasites poses a public health concern. While the risk of human infection from sheep and goats is not as well-documented as for other livestock, the consumption of undercooked meat or inadvertent exposure to oocysts in the environment can lead to human toxoplasmosis and potentially neosporosis (Dubey and Lindsay, 1996).

Numerous epidemiological studies regarding toxoplasmosis and neosporosis have been carried out in various regions of the world (Wanha *et al*., 2005; Ayub *et al*., 2024; Diakoua *et al*., 2013; Stelzer *et al*., 2019, Rafique *et al*., 2022; Irshad *et al*., 2024). However, epidemiological information on the regional seroprevalence of *T. gondii* and *N. caninum* in small ruminants in Türkiye is limited and some provincial epidemiological studies were found (Öncel and Vural, 2006; Zhou *et al*., 2016; Ekşi *et al*., 2018; Ütük and Ekşi, 2019; Celik *et al*., 2020). Therefore, this study aimed to determine *T. gondii* and *N. caninum* seroprevalence in sheep and goats across the Southeastern Anatolian Region of Türkiye.

MATERIALS AND METHODS

Sampling site and study design: Serum samples were collected from small ruminants reared in the Southeastern Anatolia Region of Türkiye, which consists of nine provinces, some of which are neighboring Syria and Iraq. The Southeastern Anatolia Region has a hot and dry climate with hot and dry summers and its common vegetation is steppe. The region has a surface area of 76,192 km^2 and a human population of 8,576,391. The economy of the region is based on agriculture and animal husbandry, except for Gaziantep province, which is developed in terms of industry. According to the data of TUIK (TUIK, 2023), Türkiye is one of the leading countries in small ruminant breeding in Europe with 42,060,470 sheep and 10,302,940 goats, and the Southeastern Anatolia Region has a significant share in the country's animal husbandry with 7,368,715 sheep and 2,542,476 goats. Especially in terms of goat breeding, the Southeastern Anatolia Region accounts for almost a quarter of the country's goat population. The number of animals was determined for each animal species according to the formula $n=[(1.96)^2.P_{exp}(1-P_{exp})]/d^2$ described by Thrusfield (2007). *P*, *d*, and *n* represent the estimated seroprevalence, the estimated precision, and the estimated sample size, respectively. In the absence of data regarding the *T. gondii* and *N. caninum* seroprevalence in the study population, the expected prevalence (P_{exp}) was set at 50% and the estimated precision (*d*) at 5%, and the minimum number of animals that could be used in the study for each species was set at 384. However, considering the provincial animal numbers, more samples were collected depending on the provinces. Therefore, a total of 906 apparently healthy small ruminants, 510 sheep and 396 goats, all female and over 1 year old, were included in the study. Detailed information on the sampled regions and animal numbers are presented in Figure 1.

Serum samples: The sheep were individually bled through the jugular vein, and a 5 mL blood sample was collected into serum tubes using sterile needles for each animal. All collected blood samples were held in a cooler box and then centrifuged (2000 rpm for 20 minutes) to yield serum in the laboratory. After separation, serum samples were refrigerated and kept at -20°C until used in a recombinant *Tg*SAG2 and *Nc*SAG1 based iELISA.

Indirect Enzyme-linked Immunosorbent Assay: To detect the presence of specific antibodies in the ovine and caprine serum samples, an iELISA using recombinant *T. gondii* SAG2 (*Tg*SAG2) and *N. caninum* SAG1 (*Nc*SAG1) proteins as antigens was used as described by Zhou *et al*. (2016). In the indirect ELISA test, *Tg*SAG2, *Nc*SAG1, and GST proteins were reconstituted with carbonate-bicarbonate buffer (pH 9.6, 0.05 M) at 2 µg/ml concentration. The ELISA plate wells were coated with 100 µl of antigen and incubated at 4°C overnight. Following pouring the coating solution and washing with PBST, the plates were blocked for 1 hour at 37°C with 3% skimmed milk solution containing 1X PBS. The microplate wells were rewashed using PBST, and the 1:100 diluted serum samples (with 3% skim milk solution) were added and incubated for 1 hour at 37°C. Washing processes were performed with PBST six times after this step. Binding antibodies were visualized with horseradish peroxidase-conjugated anti-sheep IgG secondary antibody (Bethyl, Montgomery, AL, USA), anti-goat IgG secondary antibody (1:4000) and ABTS [2,2'-azinobis (3-etilbenztiazolinsülfonik asit)] substrate (Sigma, ABD, Louis, MO, ABD). The color formation was observed at room temperature, and 2 M sulfuric acid as a stop solution was added to inactivate the horseradish peroxidase enzyme. Optical density (OD) was measured with an ELISA microplate reader (Rayto Microplate Reader, Model: RT-2100C) at 415 nm.

Evaluation of the ELISA findings: The OD_{415} value of the GST protein was subtracted from the OD_{415} value of r*Tg*SAG2 and r*Nc*SAG1 for each sample to evaluate the ELISA results. Cut-off values were calculated by adding three-fold the standard deviation to the mean OD_{415} value of negative sheep sera. If the OD_{415} value of the sample was higher than the cut-off value, the sample was considered positive. In this study, *T. gondii* and *N. caninum* positive and negative sheep and goat serum samples were obtained from our previous study (Zhou *et al*., 2016). Recombinant *Tg*SA2 and r*Nc*SAG1 proteins were obtained from the Obihiro University of Agriculture and Veterinary Medicine, National Research Center for Protozoan Diseases, Obihiro, Japan. The cut off values for *T. gondii* were 0.269 and 0.289 in sheep and goats, respectively, and 0.331 and 0.347 for *N. caninum.*

Statistical analysis: The statistical program SPSS version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

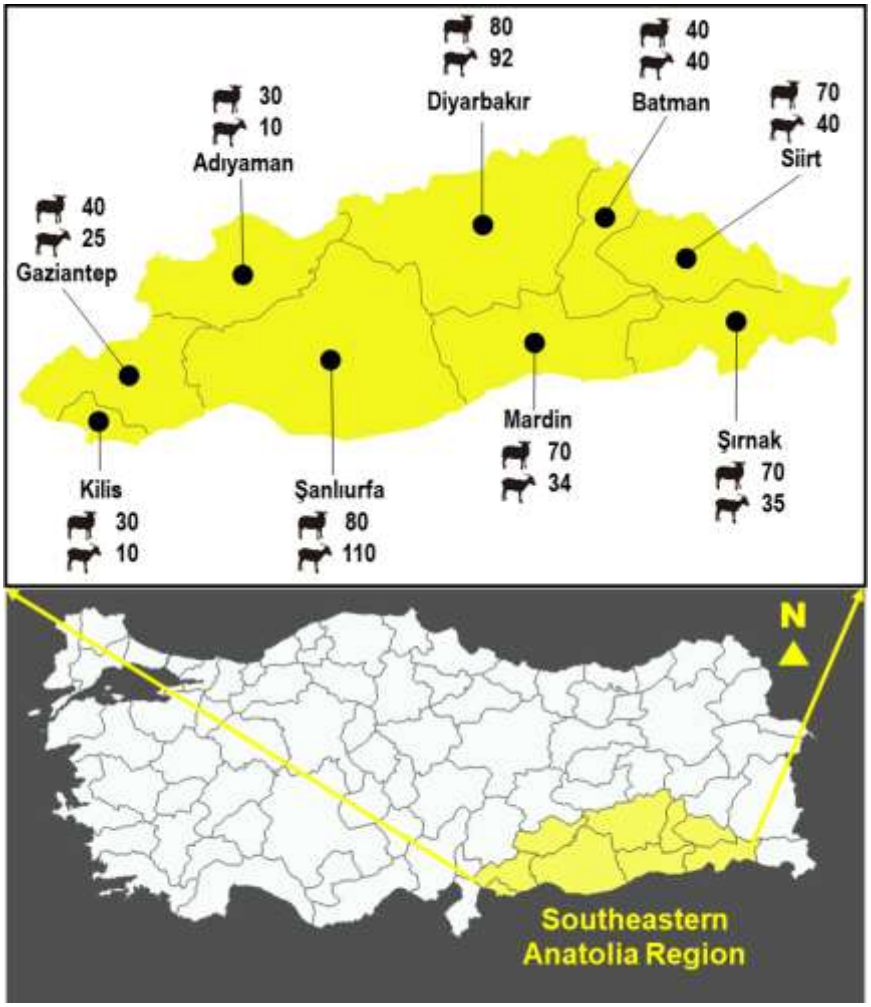

Fig. 1: The sampling area and the number of animals according to provinces.

was used to analyze all data. Statistical comparison of *T. gondii* and *N. caninum* seroprevalence values between different animal species and provinces was calculated by Fisher's Exact Test (Fisher's chi-square test). Statistically significancy and insignificancy were interpreted by using *P* and chi-squared (χ_2) values.

Ethical statement: All experimental procedures followed the ethical guidelines of the Veterinary Faculty of Selcuk University, Experimental Animals Production and Research Center Ethics Committee (SUVDAMEK: 2024/126).

RESULTS

In this study, r*Tg*SAG2 and r*Nc*SAG1 based iELISA was used to determine the serological status of *T. gondii* and *N. caninum* in sheep and goats in 9 provinces of Southeastern Anatolia Region of Türkiye. It was found that 33.1% (169/510) of the sheep in the region were infected with at least one pathogen. In the study where the seroprevalence of ovine toxoplasmosis was determined as 32.5% (166/510), the seroprevalence of *N. caninum* was determined to be as low as 3.1% (16/510). It is noteworthy that Diyarbakır was the province with the highest seroprevalence of both *T. gondii* (56.3%) and *N. caninum* (8.8%) in sheep, while anti-*T. gondii* specific antibodies were not detected in Gaziantep and anti-*N. caninum* specific antibodies were not detected in Adıyaman, Batman, Gaziantep and Kilis. Detailed findings obtained from sheep in the study are given in Figure 2 and Table 1.

The total seroprevalence value in goats was higher than the seroprevalence value in sheep. It was determined that 39.4% (156/396) of the goats in the region were infected with at least one pathogen. In the study where the seroprevalence of caprine toxoplasmosis was determined as 32.1% (127/396), it was determined that the seroprevalence value of *N. caninum* was 20.2% (80/396), which was considerably higher than the seroprevalence of ovine neosporosis. Diyarbakır had the highest *T. gondii* seroprevalence (65.2%) and Mardin had the highest *N. caninum* seroprevalence (41.2%), while anti-*T. gondii*-specific antibodies were not detected in Gaziantep and Kilis, and anti-*N. caninum*-specific antibodies were not detected in Adıyaman and Kilis. The detailed findings obtained from goats in the study are given in Figure 2 and Table 2.

In the study, it was determined that the total seroprevalence values of *T. gondii* and *N. caninum* in sheep and goats did not show a statistically significant difference (P=0.082, χ^2=3.101). When the prevalence of *T. gondii* and *N. caninum* infections were analyzed separately, it was found that *T. gondii* seroprevalence was not statistically different between sheep and goats (P=0.886), but *N. caninum* seroprevalence was statistically significantly higher in goats (P=0.000, χ^2=68.523).

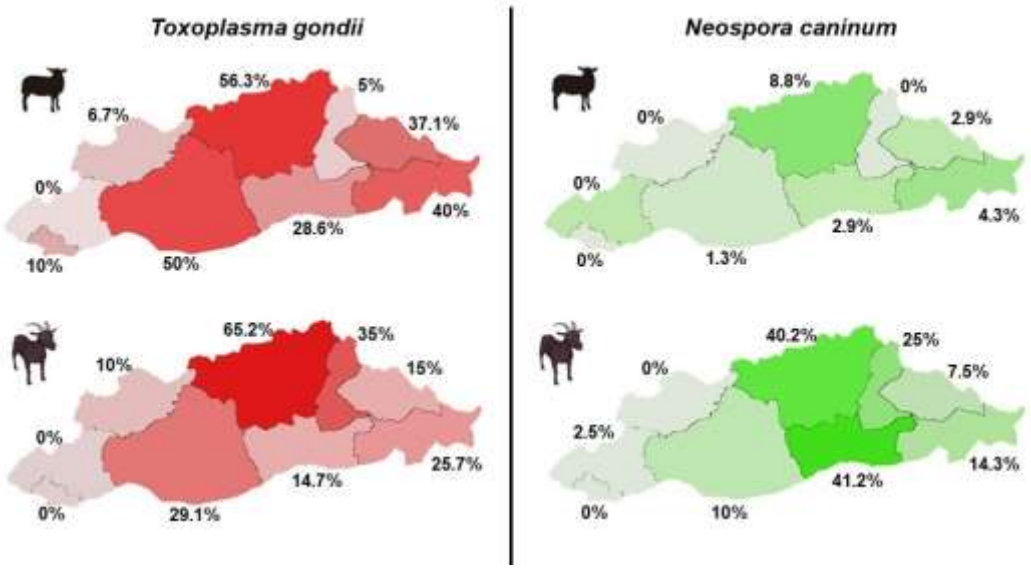

Fig. 2: Provincial serostatus of *Toxoplasma gondii* and *Neospora caninum*.

Table 1: *Toxoplasma gondii* and *Neospora caninum* seroprevalence values in sheep by province.

Province	n	*Toxoplasma gondii*		*Neospora caninum*		Co-infection	Seropositivity at least one of the investigated protozoa	
		+	-	+	-		+	-
Adıyaman	30	2	28	0	30	0	2	28
Batman	40	2	38	0	40	0	2	38
Diyarbakır	80	45	35	7	73	6	46	34
Gaziantep	40	0	40	1	39	0	1	39
Kilis	30	3	27	0	30	0	3	27
Mardin	70	20	50	2	68	1	21	49
Siirt	70	26	44	2	68	2	26	44
Şanlıurfa	80	40	40	1	79	1	40	40
Şırnak	70	28	42	3	67	3	28	42
Total (%)	510	166 (32.5%)	344 (67.5%)	16 (3.1%)	494 (96.9%)	13 (2.5%)	169 (33.1%)	341 (66.9%)

Table 2: *Toxoplasma gondii* and *Neospora caninum* seroprevalence values in goats by province.

Province	n	*Toxoplasma gondii*		*Neospora caninum*		Co-infection	Seropositivity at least one of the investigated protozoa	
		+	-	+	-		+	-
Adıyaman	10	1	9	0	10	0	1	9
Batman	40	14	26	10	30	4	20	20
Diyarbakır	92	60	32	37	55	32	65	27
Gaziantep	25	0	25	0	25	0	0	25
Kilis	10	0	10	0	10	0	0	10
Mardin	34	5	29	14	20	2	17	17
Siirt	40	6	34	3	37	1	8	32
Şanlıurfa	110	32	78	11	99	9	34	76
Şırnak	35	9	26	5	30	3	11	24
Total (%)	396	127 (32.1%)	269 (67.9%)	80 (20.2%)	316 (79.8%)	51 (12.9%)	156 (39.4%)	240 (60.6%)

Analysis by species showed that total seroprevalence and *T. gondii* seroprevalence values for sheep showed a statistically significant difference ($P<0.05$), while *N. caninum* seroprevalence values did not show a statistically significant difference between provinces ($P=0.101$, $\chi^2=13.333$). On the other hand, both the total seroprevalence and the seroprevalence of *T. gondii* and *N. caninum* in goats showed statistically significant differences between provinces ($P=0.000$).

DISCUSSION

Toxoplasmosis and neosporosis are significant concerns in livestock industries, as they can lead to abortion, stillbirth, and other reproductive issues. Toxoplasmosis, is considered a major public health concern because it can be transmitted from animals to humans, posing a threat to human health and well-being (Abdallah *et al*., 2019). Neosporosis, which causes abortion, neonatal mortality and neurological disorders, has also become a major threat to the health and productivity of sheep and goats worldwide. However, it has less frequently been detected in livestock animals than toxoplasmosis (Kaltungo and Musa, 2013; Sánchez-Sánchez *et al*., 2021). Although *N. caninum* is not considered a zoonotic agent, recent investigations have generated awareness of its human infectivity (Gharekhani *et al*., 2021). Given the genetic and biological similarities between *T. gondii* and *N. caninum*, the potential for *N. caninum* to pose a zoonotic threat cannot be completely excluded (Dubey *et al*., 2007). For all the reasons mentioned above, this study was conducted to investigate the prevalence of small ruminant toxoplasmosis and neosporosis infections in the Southeastern Anatolia Region, where preventive veterinary medicine practices are inadequately applied compared to many regions of

Türkiye. There is an ongoing need for serological screening to determine the current status of infection in each region and to take action to prevent the spread of infection based on the results. Li *et al*. (2021) reported the superiority of the recombinant *Tg*SAG2 protein-based indirect ELISA for the detection of anti-*T. gondii* specific antibodies in the serodiagnosis of *T. gondii* and that iELISA is very practical in studies where a large number of samples are tested. Therefore, the seroprevalence of *T. gondii* and *N. caninum* in the region of Southeastern Anatolia, where sheep farming is intensive, was determined using recombinant *Tg*SAG2- and *Nc*SAG1-based iELISA, respectively in this study. The findings of the study show that seroprevalence of *T. gondii* is high in both sheep (32.6%) and goats (32.1%), while *N. caninum* infections are higher in goats (20.2%) compared to sheep (3.1%). In this study in which total seroprevalence was 35.9%, the seroprevalence value of both parasite species in goats (39.4%) was higher than that in sheep (33.1%). Although both species are susceptible to these infections, available evidence suggests that goats tend to have a higher incidence compared to sheep (Puije *et al*., 2000). While the exact reasons for the disparities in toxoplasmosis and neosporosis infections between goats and sheep remain to be fully elucidated, the available evidence points to a multifactorial interplay between factors such as grazing behavior, feeding habits, and species-specific susceptibility to these parasites. Goats are generally more curious and adventurous, often browsing on a variety of plants, including those that may be contaminated with the oocysts of these parasites. In contrast, sheep tend to be more selective in their grazing, potentially reducing their exposure to the infective stages of these parasites (Abdallah *et al*., 2019).

Kolören and Dubey (2020) reviewed epidemiological studies on the prevalence of *T. gondii* in Türkiye and reported that the seroprevalence of ovine toxoplasmosis was 9.5-98.92%, while caprine toxoplasmosis had a seroprevalence value of 12.9-95.24% with various serological tests including SFDT, LAT, IHA, IFA and ELISA. An iELISA based on r*Tg*SAG2, which is frequently preferred in epidemiological studies and serological screening, was preferred in this study. It has been reported that iELISA combined with recombinant *Tg*SAG2 or other recombinant *T. gondii* proteins can detect specific antibodies raised against the parasite with high sensitivity in both acute and chronic *T. gondii* infections (Li *et al*., 2021). Only one study carried out in Türkiye to date has investigated the seroprevalence of toxoplasmosis in small ruminants using the r*Tg*SA2 iELISA technique, and it revealed the seroprevalence of ovine toxoplasmosis in the provinces of Karaman, Konya, and Zonguldak as 20%, and the seroprevalence in goats in the provinces of Karaman and Konya was 12.9% (Zhou *et al*., 2016). Although many small-scale studies using different techniques have been carried out, it is thought that regional studies on the prevalence of *T. gondii* in small ruminants in Türkiye are insufficient. This is the first serological study to reveal the prevalence of *T. gondii* in the Southeastern Anatolia Region, which has an important position in terms of sheep and goat breeding in Türkiye. The seroprevalence value in sheep with r*Tg*SAG2 iELISA was determined as 32.5% and in goats as 32.1% in the present study. These seroprevalence levels are quite high and worrying, as *T. gondii* causes abortion in animals, and there is a risk of transmission to humans through tissue cysts in animals (Steltzer *et al*., 2019; Kolören and Dubey, 2020).

Toxoplasma gondii seroprevalence showed statistically significant differences at provincial level in the study. *T. gondii* seroprevalence was highest in Diyarbakır province in both sheep (56.3%) and goats (65.2%), while no specific antibodies were found in sera from Gaziantep province in sheep and Gaziantep and Kilis provinces in goats. This difference and the high seroprevalence of *T. gondii* in sheep and goats in the south-eastern Anatolian region of Türkiye is likely due to a combination of factors, including the region's climate, farming practices, and the presence of definitive hosts that can shed oocysts and contaminate the environment (Yentur Doni *et al*., 2015; Masombuka *et al*., 2024). Moreover, Dubey (2010) noted that the durability of oocysts to withstand outdoor conditions may vary depending on different climatic characteristics, and this may affect the prevalence of infection. Considering the zoonotic aspect of *T. gondii*, this high seroprevalence is a serious risk, especially for the human population living in the region. *T. gondii* is known to cause significant disease in humans and has been associated with miscarriages and stillbirths in pregnant women (Abdallah *et al*., 2019). In a study conducted in Türkiye, Demiray *et al*. (2022) reported the anti-*Toxoplasma* IgG seroprevalence in pregnant women as 36.76% and reported that the region with the highest seroprevalence value was the Southeastern Anatolia Region with a rate of 59.43%. Given this situation, it is inevitable to establish a link between the high seroprevalence values detected in sheep and goats in the study and the transmission routes of this protozoon. It is thought that the red meat consumption habits arising from the traditions of the people of the region also contribute to the high seroprevalence value in humans.

It is noteworthy that *N. caninum*, one of the primary infectious protozoan pathogens of bovine abortion cases, is frequently associated with abortions in sheep and goats (Sánchez-Sánchez *et al*., 2021; Nayeri *et al*., 2022). Although there are a limited number of provincial or district-scale studies on the seroepidemiology of *N. caninum* in small ruminants in Türkiye, there are no comprehensive epidemiological data on a regional or country-wide basis. Small-scale studies conducted in Türkiye show that the seroprevalence of ovine neosporosis is between 2.1% and 12.4% (Gökçe *et al*., 2015; Zhou *et al*., 2016; Eşki *et al*., 2018; Karatepe and Karatepe, 2020), while the seroprevalence of caprine neosporosis is between 0.27% and 25.9% (Cayvaz and Karatepe, 2011; Zhou *et al*., 2016; Utuk and Eski, 2019; Özdamar *et al*., 2021; Atelge *et al*., 2022; Toy and Oğuz, 2023). This study presents current data on the serological status of ovine and caprine neosporosis in the Southeastern Anatolia Region, where modern livestock management practices are inadequate compared to the western regions of Türkiye (Aydemir and Pıçak, 2007). In the study, anti-*N. caninum* antibodies were detected in 3.1% and 20.2% of sheep and goats, respectively. It is seen that the seroprevalence of *N. caninum* in both animal species is compatible with the findings of previous studies conducted in Türkiye. Cayvaz and Karatepe (2011) determined the prevalence of caprine

neosporosis as 25.9% in goats, including aborted goats in Niğde province. In addition, in a study conducted by our group, a 50.4% seroprevalence of ovine neosporosis in a sheep flock with a history of abortion demonstrates the seriousness of the situation for both sheep and goats (Unpublished). Although this study revealed a slightly lower seroprevalence due to the fact that apparently healthy goats were investigated for anti-*N. caninum* antibodies, this study differs positively from many other studies due to the number of animals investigated, the size of the sampled area, and the use of the iELISA method based on *N. caninum* rSAG1 protein, which is reliably preferred in serological diagnosis (Chahan *et al*., 2003). Statistical analyses revealed that the seroprevalence of ovine neosporosis did not show a significant difference among the provinces of the Southeastern Anatolia Region, while the highest seroprevalence in sheep was detected in Diyarbakır province (8.8%). The seroprevalence of caprine neosporosis showed statistically significant differences between provinces, with the highest seroprevalence in Mardin province (41.2%). Vanderburg *et al*. (2014) observed that the seroprevalence of *N. caninum* varies significantly even between close geographical regions. This emphasizes the importance of understanding localized risk factors that may contribute to these differences. The underlying drivers of these provincial differences in seroprevalence likely involve a complex interplay of factors, such as variations in animal husbandry practices, environmental contamination, and even sociocultural influences on herd management.

Conclusions: In conclusion, the high seroprevalence across the region underscores the significant economic and public health implications of these parasitic infections. The consistent findings of elevated seropositivity rates in these small ruminants emphasize the need for comprehensive control strategies to mitigate the impact on livestock production and potential zoonotic transmission. The widespread distribution of these infections, as demonstrated by studies spanning different ecological zones, highlights the autochthonous nature of these parasites and the ubiquitous risk they pose to small ruminant populations. Given the pivotal role of sheep and goats as major sources of infection for both humans and other animals, the implementation of effective surveillance, prevention, and management programs is crucial. Overall, the high seroprevalence findings emphasize the imperative for a One Health approach that integrates veterinary, public health, and environmental considerations to address the multifaceted challenges posed by *T. gondii* and *N. caninum* in sheep and goat populations. In this direction, one of the first things to be done is to reveal the prevalence of the diseases serologically. Obtaining data representing the country on toxoplasmosis and neosporosis by conducting epidemiological studies on a larger scale will pave the way for steps to be taken regarding the measures to be taken.

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Authors contributions: C.C., F.S., and O.C. conceptualized the study. C.C., and O.C. collected the study materials. C.C., F.S., and O.C. conducted laboratory analysis. C.C. wrote the original draft of the manuscript. All authors have agreed to this final version of the manuscript and give their consent for its publication.

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