

# Seroprevalence of Neospora caninum in Goats in Gevas District of Van

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*Neospora caninum* is recognized worldwide as one of the most important abortive pathogens in cattle. Although abortion cases are also recorded in sheep and goats, information and epidemiological data on neosporosis in goats are insufficient. In Türkiye, neosporosis in goats has been serologically detected in Adana, Antalya, Ankara, Burdur, Elazig, Erzurum, Kirsehir, Kirikkale, Kilis, Konya, Nigde, Sanliurfa, Ordu and Osmaniye provinces. To the best of our knowledge, there are no studies detecting *N. caninum* in goats living in Van province. For this reason, in this study, it was aimed to determine the seroprevalence of *N. caninum* in goats of different ages and sexes from 15 villages of Gevas. A commercially cELISA was used to detect specific anti-*N. caninum* antibodies in the serum samples. Only one of the examined goats (0.27%) was seropositive for neosporosis. It was determined that the positive sample was over 4 years old and had abortion history. In conclusion, the presence and seroprevalence of *N. caninum* infection in the goat population in the Gevas region of Van province, a region where this protozoon has not been adequately investigated, was revealed for the first report with this study.

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# INTRODUCTION

*Neospora caninum* is a worldwide obligate intracellular cyst-forming coccidian parasite. *N. caninum* causes severe neuromuscular disease in dogs and is considered one of the primary reasons of abortions in cattle. The disease is responsible for severe economic losses in the beef and dairy industries, mainly associated with poor reproductive performance of affected cattle, but also associated with reduced milk production, early slaughter and increased weight loss (Dubey 2003; Dubey and Schares 2011; Reichel et al. 2013). Until now, only domestic dogs (*Canis familiais*), Australian dingoes (*Canis lupus dingo*), coyotes (*Canis latrans*), and gray wolves (*Canis lupus*) are considered definitive hosts of the parasite (McAllister et al. 1998; Gondim et al. 2004; King et al. 2010; Dubey et al. 2011). Although serological positivity against *N. caninum* has been reported in several animal species, only a few are considered intermediate hosts (ruminants animals), including some mammalian and avian species (McAllister et al. 1998; Gondim et al. 2011; Dubey and Schares 2011; de Barros et al. 2018).

In humans, the presence of anti-*N. caninum* antibodies has been detected; but its zoonotic potential is unknown (Tranas et al. 1999). Although *N. caninum* infection in small ruminants is thought to be less important than in cattle, it is known to cause reproductive disorders and clinical diseases (Dubey and Schares 2011). It has been showed by fetal fluid serology that *N. caninum* causes transplacental infection in goats (Unzaga et al. 2014). However, *N. caninum* DNA has been found in the brains of aborted goat fetuses in Spain (Moreno et al. 2012).

Epidemiological data on neosporosis in goats in Türkiye are lacking and limited to geographical regions. As a result of serological researches conducted in the Central Anatolia Region (Ankara, Kirikkale, Kirsehir, Konya and Nigde), the average neosprosis rate in goats was found to be 10.4%. The highest prevalence was reported in the Eastern Anatolia Region (Elazig and Erzurum) with an average of 13.79%. The lowest average seropositivity rate of 5.43% was found in the Mediterranean Region (Adana, Antalya, Burdur and Osmaniye). For the Black Sea Region, an infection rate of 8.69% was reported only in the study conducted in Ordu province. It was detected at a rate of 8.45% in the South East Anatolian region (Kilis and Sanliurfa) (Sevgili et al. 2003; Cayvaz and Karatepe 2011; Utuk et al. 2011; Okur 2015; Zhou et al. 2016; Utuk and Eski 2017; Utuk and Eski 2019; Eski 2020; Özdamar et al. 2021; Çubukçu and Gökpınar 2021; Gökpınar 2022).

Goats are a valuable meat and milk source in several countries, including Türkiye, and for this reason, they are the most preferred animal species by their animal farming. Low animal productivity is often due to a lack of knowledge about disease control, and in this sense there is a serious lack of knowledge about neosporosis in goats. ELISA and IFAT tests are the most common methods used to diagnose neosporosis in goats. Accurate and early diagnosis is also important in struggling with disease the disease. In the present study, we aimed to sero-epidemiological data by revealing the presence and prevalence of anti-*Neospora caninum* antibodies in goats bred in the Gevas district of Van province by ELISA method.

## MATERIALS AND METHODS

Serum samples of 368 healthy adult goats were collected in the period April-June 2022. The goats originated from 15 different villages of Gevas (Abali (n = 33), Altinsac (n = 33), Anakoy (n = 31), Baglama (n = 17), Daldere (n = 50), Dokuzagac (n = 17), Gundogan (n = 14), Hasbey (n = 50), Ikizler (n = 14), Inkoy (n = 34), Kayalar (n = 19), Kurultu (n = 16), Timar (n = 11), Toreli (n = 15), and Yanikcay (n = 14). Blood was taken from the vena jugular of goats into sterile vacuum tubes in accordance with the technique. Goat sera was separated in the laboratory and stored at -20 °C until serological analysis.

ELISA test was performed in Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, and Parasitology Laboratory. Sera were screened for antibodies to *N. caninum* by commercial competitive ELISA (ID Screen® *Neospora caninum* ELISA Kit, France). Blood serums with an S/P ratio above or equal to 50% were considered positive, and serum samples with a S/P ratio less than or equal to 40% were considered negative. Samples between 40% and 50% were considered suspicious. This diagnostic kit is designed to detect antibodies directed against *Neospora caninum* in bovine, ovine or caprine serum, plasma, or milk samples.

### RESULTS

Out of the 368 samples analyzed, only one was seropositive for *N. caninum* by means of cELISA, thus corresponding to a prevalence of 0.27% distributed in different locations (Fig. 1).



Figure 1. % S/P values determined by ELISA in neosporosis positive and negative goats

The presence of dogs, which act as the final host in the biology of the parasite, has been detected in the environment of the goats. Since only one positive data set was formed in our study, statistical power was not found to be sufficient and appropriate. As a result of the examination of the samples taken from Daldere (1/50), seropositivity of *N. caninum* was detected in 1 (2%) while *N. caninum* seroprevalence was not observed in other regions (Table 1). In terms of age groups, seropositivity was detected in 1 (1.75%) of 57 goats over 4 years of age, while seropositivity was not observed in other age group goats (Table 2). Aborted goats were determined among the examined goats when blood samples were taken, and they are given in Table 3. Accordingly, seropositivity was found in 1 (0.32%) of 310 goats that had aborted, while seropositivity was not found in any of the 58 goats that did not abort. While seropositivity was detected in 1 out of 340 female goats examined, no seropositivity was found in any of the 18 male goats (Table 4).

Study area	Number of goats tested	Number of goats positive	Seropositive %
Kayalar (n/19)	_		
Gundogan (n/14)	42	0	0
Timar $(n/11)$			
Inkoy (n/34)			
Altinsac (n/33)			
Kurultu (n/16)			

**Table 1.** Seropositivity of Neospora caninum in goat population from different regions of Gevas.

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Ikizler (n/14)						
Hasbey (n/50)						
Daldere (n/50)*	247	1	0.4			
Anakoy (n/31)						
Toreli (n/15)						
Abali (n/33)						
Yanikcay (n/14)						
Dokuzagaç (n/17)	79	0	0			
Baglama (n/17)						
Total	368	1	0.27			

Level Martin and A T : C.

\* Seropositive region

**Table 2.** Seropositivity of Neospora caninum infection in goats according to age

Age (year)	Number of goats tested	Number of goats positive	Seropositive %
0-2	16	0	0
2-3	42	0	0
3-4	253	0	0
> 4	57	1	1.75
Total	368	1	0.27

Table 3.	Seropositivi	v of Neosp	ora caninum	infection in	goats accord	ing to d	abortion
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Abortion	Number of tested	goats Number o positive	f goats	Seropositive %
Yes	310	1		0.32
No	58	0		0
Total	368	1		0.27

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I abic 4.	seroposu	ινιι  Ο	neospore	і санінит	injection	in a	gouis accoraing to sex

Sex	Number of goats tested	Number of goats positive	Seropositive %
Female	340	1	0.29
Male	18	0	0
Total	368	1	0.27

## DISCUSSION

Abortions are among the most important causes of infertility in small ruminants with herd animals. While there are animals in which individual abortion and infertility can be tolerated, when considered as a herd, the abortion problem causes significant economic losses and threatens human health in some zoonotic infections. The causes of abortion cases that can be seen at any stage of pregnancy in small ruminants are examined under two main headings as non-infectious and infectious. Studies show that 90% of the cases are caused by infection. We can collect infection-related abortions in goats under four headings as bacterial, viral, parasitic and fungal agents. N. caninum, Sarcocystis spp. and T. gondii protozoa, which are located in the apicomplexa phylum, constitute the parasitic abortion agents. Among them, N. caninum has been shown to be the most important cause of cattle abortions in many parts of the world. Recent studies have shown that N. caninum causes abortions, fetal deaths and stillbirths in goats as well as in cattle (Gazzonis et al. 2019; Rodrigues et al. 2021).

The prevalence of neosporosis in goats in the world is 19% in Taiwan (Chiang et al. 2020), 16.7% in Thailand (Udonsom et al. 2020), 6% in Spain (Diaz et al. 2016), 26.11% in Brazil in (Braz et al., 2018), 6.2% in Iran (Gharekhani et al. 2016), 8.6% in Pakistan (Nasir et al. 2012), 0.48% in Germany (Villagra-Blanco et al. 2017), 5.5% in Argentina (Gos et al. 2017), 12% in Jordan (Abo-Shehada and Abu-Halaweh 2010), 6% in Czech Republic (Bartova and Sedlak 2012), 9% in Poland (Czopowicz et al. 2011), 15.5% in Slovakia (Čobádiová et al. 2013), 5.7% in Italy (Gazzonis et al. 2016), 5.6% in Iraq (Ghattof and Faraj 2015), 3.8% in Mexico (Huerta-Peña

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et al. 2011), 2.3% in Romania (Iovu et al. 2012), 0.9% in Korea (Jung et al. 2014), 7.23% in China (Liu et al. 2015), 0.7% in Sri Lanka (Naguleswaran et al. 2004), and 5.8% in the West Indies (Sharma et al. 2015) rates by serological methods. Epidemiological data on neosporosis in goats in Türkiye are lacking and limited to geographical regions. As a result of the studies carried out with serological methods, the lowest (1.9%) seroprevalence was found in Osmaniye (Eski 2020) and the highest (25.9%) seroprevalence in Niğde province (Cayvaz ve Karatepe 2011). In this study, *N. caninum* seropositivity was determined in 1 (0.27%) of 368 goats, which were examined serologically by ELISA method and sampled from several locations of Gevaş region. The prevalence rates determined in the research region have been found in some other studies on goat neosporosis in Türkiye and World (Cayvaz and Karatepe 2011; Utuk and Eski 2017; Braz et al. 2018; Chiang et al. 2020; Udonsom et al. 2020) is lower than the prevalence rates determined. The prevalence of *N. caninum* infection in goats varies considerably between continents and countries worldwide. These variations in seroprevalence may be related to the unique characteristics of each region, such as climatic conditions, differences in animal nutrition and health management, use of different techniques in serological diagnosis, goat populations, and different study design (Rodrigues et al. 2020; Nayeri et al. 2022).

Intensive breeding is often subject to higher hygiene standards and dogs or other canines are not likely to access the placenta of sheep or goats (Gazzonis et al. 2016; Snak et al. 2018; Rodrigues et al. 2021). Traditional farming system (semi-intensive) resulted in a higher risk of *N. caninum* infection in herds compared to intensive. Because, in semi-intensive breeding, supplementing with grain and roughage kept in warehouses where dogs can easily access, especially in winter, is an application that poses a significant risk of infection (Dubey and Schares 2011). Although only one seropositive sample was detected in present study, the goats of this sample were kept with semi-intensive breeding, which is similar to the results of previous studies (Gazzonis et al. 2016; Snak et al. 2018; Utuk and Eski 2019; Rodrigues et al. 2021).

Depends on the previous systematic review and meta-analysis findings, the relationship between the disease and the presence of dogs in the herds was examined, and no significant relationship was found between the occurrence of seropositivity or abortion and the presence of dogs in the farms (Romanelli et al. 2021). However, some studies, on the contrary, claim that the presence of dogs in farms or herds may constitute an important risk factor for *N. caninum* infections (Abo-Shehada and Abu-Halaweh 2010; Liu et al. 2015; Gazzonis et al. 2016). In this study, the presence of shepherd dogs in the herd with a seropositive sample may suggest horizontal transmission. Goats ingesting sporulated *N. caninum* oocysts can cause horizontal transmission.

*Neospora caninum* infection, the relationship between advancing age and the incidence of the disease has been examined by several studies and a high risk of infection has been found. It has been reported that the duration of exposure to the parasite is directly proportional to the risk of infection (Abo-Shehada and Abu-Halaweh 2010; Cayvaz and Karatepe 2011; Tembue et al. 2011; Iovu et al. 2012; Rodrigues et al. 2021). However, age was not found to be associated with infection in different studies conducted in Italy (Gazzonis et al. 2016) and Argentina (Moore et al. 2007). They reported that both vertical and horizontal transmission routes can be prevailing (Gazzonis et al. 2016). In this study, while seropositivity was detected in the age group of goats over 4 years old, seropositivity was not observed in other age group goats. As a general consensus, it is thought that the sex of animals does not affect the prevalence of the disease (Ghattof and Faraj 2015; Arraes-Santos et al. 2016; Gazzonis et al. 2016; Utuk and Eksi 2019). In this study, seropositivity was detected in 1 of 340 females tested by ELISA, while seropositivity was not detected in any of the 18 male goats.

Research on goat neosporosis is less than in sheep. However, goat abortions and neonatal deaths caused by or associated with *N. caninum* have been reported from the USA (Barr et al. 1992; Dubey and Lindsay 1996). Experimental infection of pregnant goats has caused abortions and infected fetuses (Porto et al. 2016). In the present study, most of the animals studied were recorded with a history of abortion and therefore the contribution of *N. caninum* needs to be evaluated. The results obtained showed that there was very low *N. caninum* seroprevalence in goat herds. Getting healthy offspring and continuation of herds is an indispensable element in animal husbandry. The low seroprevalence of *N. caninum*, which is one of the important agents of protozoan abortion etiology, provides an advantage in terms of abortion problems.

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It turns out that many of the remaining animals with *N. caninum* seronegative and a history of health problems such as abortion suffer from miscarriage for an unknown reason. The total cost of *N. caninum* infection has been estimated at US\$710 (438–1043) per dairy cow, and the annual economic cost for Türkiye is US\$40.5 million (24.6–60.3). It has been reported that economic costs are directly related to the value of aborted fetuses (Demir et al. 2020). There is no information about the economic losses caused by *N. caninum* in goats. However, considering the size of the economic losses in cattle, we think that the economic damage caused by reproductive failure in goats should not be ignored.

## CONCLUSIONS

The presence and seroprevalence of *N. caninum* infection in the goat population in the Gevas region of Van province, a region where this protozoan has not been adequately investigated, was revealed for the first report with this study. As a result of the ELISA analysis of blood samples taken from a total of 368 goats in the study, it was determined that 1 (0.27%) was seropositive. In cattle herds with low neosporosis prevalence, test-take-away and selective breeding methods are used (Altbuch et al. 2012; Reichel et al. 2014). It is recommended to try these applications due to the low prevalence in our study. Besides, it is extremely important to apply different strategic approaches (such as biosafety protocols, reduction of dog numbers, and destruction of fetal materials) in the control of this disease.

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### **CONFLICT OF INTEREST**

The author has no conflicts of interest.

### COMPLIANCE WITH ETHICAL STATEMENT

Ethics Committee approval for this research was obtained from the Local Ethics Committee for Animal Experiments of Van Yüzüncü Yıl University, (no. 2023/03-20).

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### **AUTHOR CONTRIBUTIONS**

AT, BO: Idea/Concept; AT, BO: Design; AT: Data Collection and/or Processing; AT, BO: Analysis and/or Interpretation; AT, BO: Literature Review; BO: Writing the Article; BO: Critical Review.

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