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Original article

Investigation of neosporosis in cattle in the Artvin region by ELISA method

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This study is based on the first author's Master's thesis.

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Abstract

This study aimed to determine the seroprevalence of *Neospora caninum* infection and to evaluate possible risk factors in cattle from Artvin province, Northeastern Turkey. A total of 180 blood samples were collected from 47 herds across nine districts between December 2023 and June 2024. Serum samples were analyzed using a commercial ELISA kit. Overall, 46 (25.55%) cattle were seropositive for *N. caninum*, and seropositive animals were found in 25 out of 47 herds (53.2%), indicating widespread herd-level infection. Although no significant associations were found between seropositivity and age, sex, or breed ($p>0.05$), a statistically significant association ($p\leq0.05$) was observed between *N. caninum* seropositivity and farms with a recorded history of abortion. The high proportion of farms keeping dogs (70.21%) and the infection pattern suggest that both vertical transmission and environmental contamination play important roles in maintaining infection. This study provides the first available serological data on *N. caninum* infection in cattle from Artvin province. Continuous monitoring and preventive strategies are recommended to minimize the reproductive losses and economic impact associated with bovine neosporosis in the region.

Keywords: abortion, Artvin, cattle, ELISA, *Neospora caninum*

Introduction

Abortion is a major economic concern for farmers (Yang et al. 2022). *Neospora caninum*, an apicomplexan protozoan, is a leading cause of abortion in cattle worldwide, including in Turkey (Akca et al. 2005, Dubey and Schares 2011, Reichel et al. 2013, Kose et al. 2021, Metwally et al. 2023). The primary clinical sign of bovine neosporosis is abortion (Dubey 2003, Reichel et al. 2013). Serological studies show that the prevalence rates of *N. caninum* vary between 0.5% and 97.2% worldwide and between 0% and 66.6% in Turkey (Guedes et al. 2008, Ocal et al., 2014, Bartova et al. 2015, Erol et al. 2019). Infection rates vary significantly across and within countries, leading to substantial economic losses in the livestock industry (Clothier and Anderson 2016, Demir et al. 2020). The lack of an effective treatment or vaccine remains a major challenge (Manca et al. 2022).

Neospora caninum belongs to the phylum Apicomplexa, family Sarcocystidae, and is closely related to *Toxoplasma gondii*, another important protozoan causing reproductive losses in animals. This taxonomic classification highlights their genetic and biological similarities, particularly in tissue cyst formation and intracellular parasitism. *N. caninum* exhibits a facultatively heteroxenous life cycle, involving both definitive (canid) and intermediate (cattle and other mammals) hosts. Transmission may occur horizontally through ingestion of oocysts shed by infected dogs, or vertically from dam to fetus via the placenta. Vertical transmission plays a crucial role in maintaining infection within herds, while horizontal spread contributes to environmental contamination and inter-herd dissemination (Dubey 1999, Dijkstra et al. 2001, Schares et al. 2001, Gondim et al. 2005).

To our knowledge, there is no previously published study investigating the seroprevalence of *N. caninum* in cattle in Artvin province, located on the northeastern border of Turkey. This region, characterized by high forest density, mixed animal husbandry practices, and frequent interactions between cattle and stray or feral dogs, presents a unique ecological environment for the potential spread of neosporosis. Therefore, this study aims to determine the presence of *N. caninum*, an important abortive in cattle, in Artvin province using the ELISA method, and to evaluate the epidemiological status and potential regional impact of the disease.

Materials and Methods

Ethical statement

The necessary permissions for this study were obtained from the Republic of Turkey Ministry of Agriculture and Forestry (Artvin Provincial Directorate of Agriculture and Forestry) with the official letter dated 23.06.2023 and numbered E-32342694-325.99-10352805, as well as from the Kafkas University Animal Experiments Local Ethics Committee with the official letter dated 20.06.2023 and numbered KAU-HADYEK/2023-080.

Study area

This study was conducted between December 2023 and June 2024 in the central district and eight counties of Artvin province (Ardanuc, Arhavi, Borcka, Hopa, Kemalpasa, Murgul, Savsat and Yusufeli) (Fig. 1). A total of 180 blood samples were collected from cattle, with 20 samples obtained from each district. During the sampling process, the animals were categorized into two age groups: under 2 years and over 2 years. The categorization of animals into two age groups, under 2 years and over 2 years, was done to differentiate between the potential impacts of age on the incidence of the condition being studied. Younger animals may have different susceptibility or health outcomes compared to older ones, and this age classification allows for a more accurate analysis of the data in relation to age-related factors.

Animals were selected from 47 herds distributed across the nine districts. To ensure representativeness, 3-5 animals were randomly chosen from each herd based on herd size and accessibility. This proportional sampling method allowed both small and medium-sized family farms to be equally represented.

Sample collection

The serological study was conducted in the central district and eight counties of Artvin province. Based on a 95% confidence level and an expected prevalence of 10% (+/- 5% margin of error), 20 cattle were randomly selected from each district. Blood samples from a total of 180 cattle were collected through farm visits. Farms with a history of abortions were prioritized, and from December 2023 to June 2024, 5-10 ml of blood was drawn from the jugular vein and stored in sterile vacuum tubes without anticoagulant (Table 1).

During the sampling process, information regarding the animals' age, sex, abortion history and reproductive health was obtained from the farmers and recorded. Additionally, a five-question survey was administered

Fig. 1. The settlements where blood samples were collected (map generated using ArcGIS).

Table 1. Distribution of sampled cattle and farms across settlement, along with abortion history (n).

Settlement	Total Number of Farms Included in the Sampling	Number of Animals Sampled	Abortion Status/History Distribution (n)
Ardanuc	1	20	20
Arhavi	10	20	20
Borcka	9	20	5
Hopa	12	20	12
Kemalpasa	5	20	10
Central district	3	20	12
Murgul	3	20	15
Savsat	3	20	20
Yusufeli	1	20	20
Total	47	180	134

n – Number of animals with a history of abortion on the farm

Table 2. Questions presented to livestock owners regarding the infection status of dogs.

1	Are there dogs in your vicinity? a) Yes b) No
2	Do you feed your dogs with household leftovers/food scraps? a) Yes b) No
3	Do you feed your dogs raw meat? a) Yes b) No
4	Have you ever observed any neurological symptoms in your dogs? a) Yes b) No
5	Do you take any precautions to protect your dogs from neosporosis? a) Yes b) No

to livestock owners to gather information about the potential infection status in dogs (Table 2).

The blood samples were labeled with protocol numbers and transported under cold chain conditions. After being centrifuged at 3000 rpm for 15 minutes, the resulting sera were transferred into Eppendorf tubes. The samples were stored at -20°C until the analyses were completed.

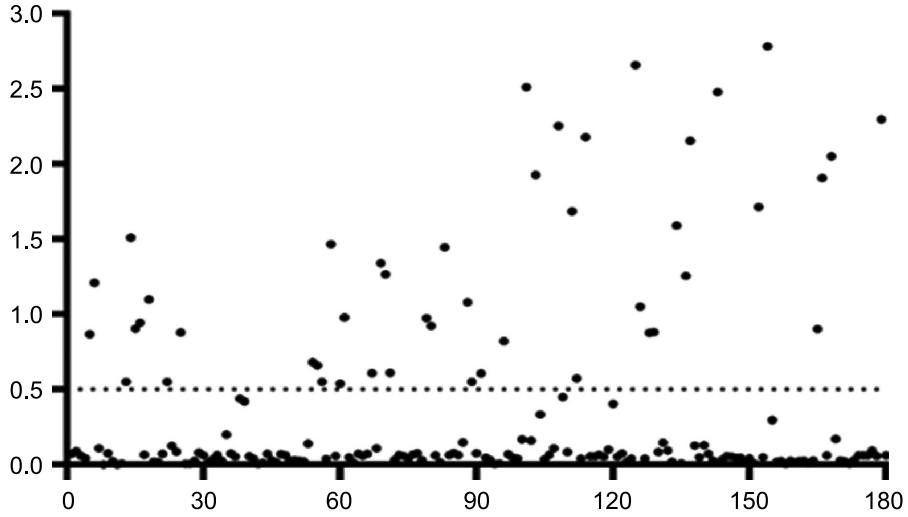

Fig. 2. Distribution of S/P ratios in cattle tested for *Neospora caninum* infection by ELISA. The scatter plot illustrates the sample-to--positive (S/P) ratio values of individual bovine serum samples (n = 180) assessed using a commercial ELISA kit. Each dot corresponds to a single animal. The horizontal dotted line represents the diagnostic cut-off value (S/P = 0.5), above which samples are classified as seropositive. This distribution highlights the coexistence of seronegative and seropositive animals in the tested population, suggesting varying levels of exposure to *N. caninum* within the study area.

Laboratory study

Detection of antibodies Against *Neospora caninum*

In this study, a commercial ELISA kit (Idexx Neospora X2®, Switzerland) was used to detect *Neospora caninum* antibodies. Laboratory analyses were conducted at the Parasitology Laboratory of Kafkas University Faculty of Veterinary Medicine in accordance with the manufacturer's protocol.

The mean optical density values of positive and negative controls were calculated, confirming compliance with validation criteria ($PC\bar{x}$ = 1.191; $NC\bar{x}$ = 0.167). The S/P values of the samples were determined using the standard formula, and test results were evaluated based on the following criteria: Negative: S/P<0.50, Positive: S/P≥0.50.

Statistical analysis

The data obtained in the study (age, breed, sex, abortion history, location, disease status, and responses to questions directed at animal owners regarding dogs) were analyzed using the Pearson Chi-Square Test in SPSS 20.0 software.

Results

As part of the study, a total of 180 cattle samples were analyzed, and 46 (25.55%) were found to be seropositive (Fig. 2). *Neospora caninum* seropositive animals were detected in 25 out of 47 herds (53.2%), indicating that infection is widely distributed at the herd level in Artvin province. Seropositive animals were detected in all surveyed districts, with the highest seropositivity rate observed (Fig. 3) in Arhavi (17.39%; 8/46) and the lowest in Ardanuc (4.35%; 2/46).

During the field study, the questionnaire presented in Table 2 was administered to farm owners. According to the results, 33 out of 47 farms (70.21%) reported the presence or ownership of dogs on or around the premises. It was stated that all of these dogs were fed household leftovers, and none had exhibited any neurological symptoms. Additionally, it was determined that none of the farm owners had taken any preventive measures against neosporosis. When the survey responses were evaluated based on the presence of dogs at the farms, the practice of feeding dogs raw meat, and the frequency of veterinary check-ups, no statistically significant difference was found regarding the raw meat feeding variable (p≥0.05).

When seropositivity rates were evaluated based on individual factors such as age, sex and breed across the foci (settlements), no statistically significant differences were observed (p≥0.05). However, a statistically significant association was detected in foci where a history of abortion had been reported on the farm (p≤0.05). This finding suggests that the abortion cases may be related to *N. caninum* infection and indicates a potential dissemination of the pathogen within these areas (see Table 3). These analyses were performed to better understand the transmission dynamics of the infection and to identify possible risk factors.

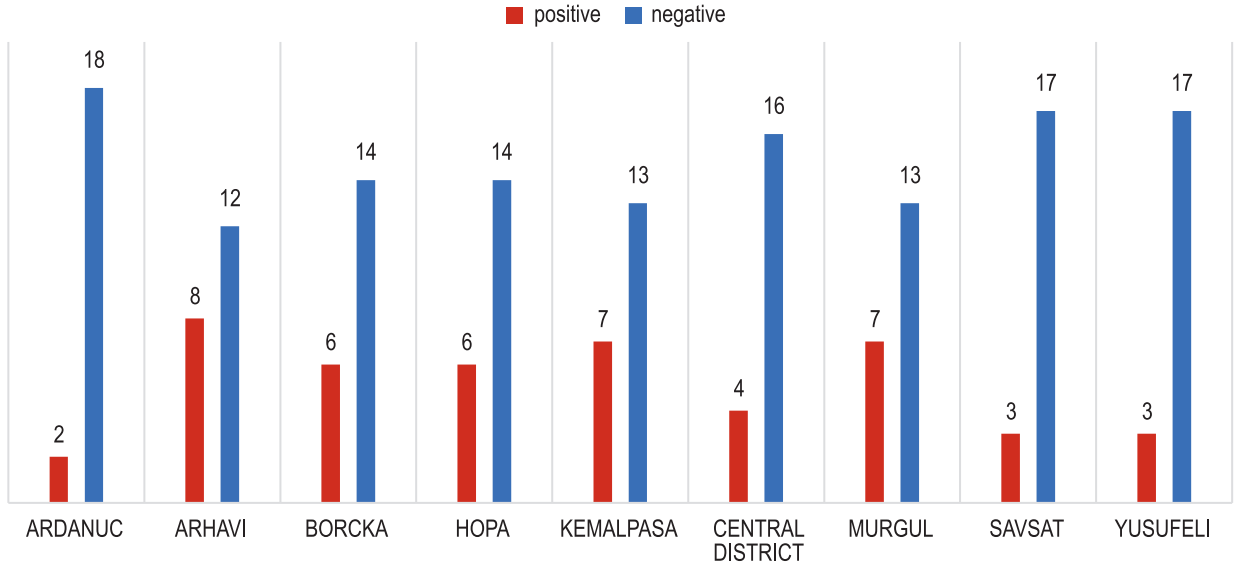

Fig. 3. Distribution of *Neospora caninum* ELISA test results by district. Each bar represents the number of cattle testing positive (red) or negative (blue) for *N. caninum* antibodies in individual districts. The data illustrate regional variation in seropositivity rates, suggesting differences in exposure levels or transmission dynamics. These variations may be influenced by local environmental conditions, farm management practices, or the presence of definitive hosts.

Table 3. ELISA results and rates based on age, sex, breed, and abortion history of sampled serum.

		ARDANUC	ARHAVI	BORCKA	HOPA	KEMALPASA	CENTRAL DISTRICT	MURGUL	SAVSAT	YUSUFELI	X^2	P VALUE
AGE	>2	2	4	3	3	3	2	4	3	3	1.905	0.984
	<2	0	4	3	3	4	2	3	0	0	13.744	0.089
	Total	2	8	6	6	7	4	7	3	3	9.695	0.287
GENDER	Female	2	8	6	4	4	4	5	3	3	10.588	0.226
	Male	0	0	0	2	3	0	2	0	0	0.373	0.830
	Total	2	8	6	6	7	4	7	3	3	12.127	0.146
BREED	Dairy	0	4	0	4	4	0	1	0	0	1.484	0.829
	Combined	2	3	4	2	3	4	5	3	3	7.642	0.469
	Indigenous	0	1	2	0	0	0	1	0	0	1.493	0.828
	Total	2	8	6	6	7	4	7	3	3	9.695	0.287
ABORT HİSTORY	Observed	2	8	1	4	4	0	7	3	3	16.824	0.032
	Not observed	0	0	5	2	3	4	0	0	0	3.806	0.433
	Total	2	8	6	6	7	4	7	3	3	9.695	0.287

Note: A p-value <0.05 was considered statistically significant (Chi-square test). A significant association was found between abortion history and N. caninum seropositivity at the focus level.

Discussion

Neosporosis is recognized as one of the major causes of bovine abortion worldwide, resulting in substantial economic losses due to reproductive failure (Dubey 2003, Reichel et al. 2013). The parasite can be transmitted both vertically from dam to offspring and horizontally, mainly through ingestion of oocysts shed by infected dogs (Dubey 1999, Schares et al. 2001, Gondim et al. 2005). In the present study, the overall seroprevalence of *Neospora caninum* was determined to be 25.55%, which is consistent with values reported

in other regions of Turkey (Ocal et al. 2014, Erol et al. 2019, Demir et al. 2020).

Seropositive animals were detected in 25 out of 47 herds (53.2%), indicating that the infection is widely distributed at the herd level in Artvin province. This suggests that *N. caninum* has become established within the local cattle population, with infection likely maintained through vertical transmission and environmental exposure associated with dogs.

According to data obtained from the Artvin Provincial Directorate of Agriculture and Forestry, the province contains 7,211 cattle farms with approximately 51,956 cattle and 1,490 registered dogs. The reported number of abortion cases varied between years (15 in 2022, 28 in 2023, and 16 in 2024). These annual variations may be associated with changes in farm management practices, environmental conditions, and the dynamics of infectious diseases. Such fluctuations highlight the multifactorial nature of reproductive losses and the potential contribution of *N. caninum* infection.

Serological studies indicate that Neospora caninum seroprevalence in cattle ranges from 0% to 66.6% in Turkey (Ocal et al. 2014, Erol et al. 2019) and from 0.5% to 97.2% globally (Guedes et al. 2008, Bartova et al. 2015). These variations are influenced by factors such as geographical regions, cattle breeds, management practices, sampling time, herd dynamics, pathogen load, and diagnostic methods (Serrano-Martínez et al. 2019, Gharekhani and Yakhchali 2020, Lindsay and Dubey 2020). Additionally, differences in climatic conditions may affect oocyst survival and sporulation (Rinaldi et al. 2005). The prevalence detected in Artvin province aligns with these findings, suggesting that environmental and management conditions in the northeastern region of Turkey are conducive to the maintenance of *N. caninum* infection.

Neospora caninum is one of the most important etiological agents responsible for abortions in dairy cattle worldwide, including in Turkey (Kul et al. 2009, Dubey and Schares 2011, Acici et al. 2019). Despite the prevalence of abortion and neonatal calf mortality on cattle farms in Artvin province , no prior studies on neosporosis have been conducted in the region. This study, using ELISA for antibody detection, identified a 25.55% seroprevalence, which is consistent with the national and Black Sea Region averages (Erol et al. 2019), highlighting *N. caninum* as a major abortifacient in Artvin cattle.

Artvin, located on the border with Georgia, has no studies on *N. caninum* in Georgia, Rize or Ardahan, although the pathogen has been detected in Erzurum (Balkaya et al. 2012). This suggests potential risks associated with livestock movement in the region. The prevalence of forested areas and the proximity of villages to these areas increase the likelihood of cattle coming into contact with wild animals (such as jackals and wolves), potentially spreading oocysts onto pastures. These factors indicate the region is at risk.

Seroprevalence was highest in Arhavi (17.39%) and lowest in Ardanuc (4.35%). While samples from Ardanuc were collected from a single farm with a history of abortion, those from Arhavi came from 10 different farms. These districts are subject to different climatic influences: Arhavi experiences a humid Black Sea climate, whereas Ardanuc is influenced by both continental and Mediterranean conditions. Although previous studies, such as Rinaldi et al. (2005), have suggested that climatic factors can affect oocyst survival and sporulation, our study did not include specific environmental or climatic data to statistically evaluate this relationship. Therefore, while the observed differences in seroprevalence may suggest a potential influence of climatic and environmental factors, this remains speculative in the absence of supporting data. Further studies integrating detailed climatic, environmental and management data, along with more comprehensive statistical analysis, are needed to clarify the possible role of these variables in *N. caninum* transmission dynamics.

Studies on the epidemiology of neosporosis show varying views on the relationship between age and seroprevalence. While some studies report a correlation between age and seropositivity (Jensen et al. 1999, Eski and Utuk 2018), others found no significant difference (Yildiz et al. 2017). In this study, seroprevalence was examined in cattle younger and older than two years, with no statistically significant difference found. As *N. caninum* can spread via transplacental transmission and contaminated feed and water, it can affect all age groups. The higher seropositivity in animals without an abortion history suggests that horizontal transmission is more prevalent. To detect vertical transmission, blood samples should be taken from newborn calves to investigate the presence of the parasite.

Neospora caninum is a parasite that causes economic losses in dairy operations, and studies examining the relationship between gender and infection are limited. While Eski and Utuk (2018) found no significant difference in infection rates between genders, Metwally et al. (2023) reported a higher seroprevalence in females (29.6%) compared to males (16.9%). In this study, seroprevalence rates of 17.91% in females and 30.43% in males were observed in cattle older than two years, with no significant gender-related difference found. The observed gender differences may be attributed to an imbalance in sample sizes and animal movements.

A study by Van Velsen (2021) indicated that the risk of *N. caninum* transmission through venereal routes in bulls is low, and there is insufficient evidence to suggest

a negative impact on semen quality or production. In the region where this study was conducted, cattle farming is typically practiced in small family-owned operations, with artificial insemination being the preferred method, and breeding bulls are not commonly raised. However, in Artvin, traditional bull wrestling events are held, and wrestling bulls are used for breeding. In a village in Borcka district, where there is a high number of wrestling bulls, a sample taken from this area revealed a seroprevalence of 30%, despite a minimal history of abortion. Although this suggests the possibility of venereal transmission of the infection, further studies are required to confirm this hypothesis.

The seroprevalence of *N. caninum* appears to be influenced more by environmental factors and management conditions rather than cattle breed (Quintanilla-Gozalo et al. 1999, Akca et al. 2005). Similarly, our study found no statistically significant difference in seroprevalence between breeds, indicating that breed is not a determining factor in the risk of infection. This suggests that, rather than breed differences, factors such as housing conditions, hygiene practices and overall management strategies may play a more significant role in the spread of the infection. Therefore, it can be concluded that more comprehensive approaches that consider environmental factors should be developed to control *N. caninum* infection.

Several global studies have indicated that *N. caninum* is a significant factor in abortion cases, with higher abortion risks observed in seropositive animals (Dubey 2003, Pessoa et al. 2016, Kose et al. 2021, Selim et al. 2023). However, other studies have found no significant difference between abortion history and *N. caninum* seroprevalence (Aktas et al. 2005, Aytekin et al. 2013). Although serological studies face challenges in definitively identifying the cause of abortion due to the latent nature and persistence of the pathogen, it has been suggested that investigating the potential prevalence of *N. caninum*-associated abortions provides reliable results (Cerqueira-Cézar et al. 2017, Tulu et al. 2018). In our study, animals without an abortion history had a higher seropositivity rate, but no statistically significant difference was found between abortion history and seropositivity ($p \geq 0.05$). This outcome may be attributed to a broader definition of abortion history or imbalance in sample sizes. To achieve more accurate results, further studies with similar classifications are needed.

Analysis of the questionnaire data indicated that 70.21% of the surveyed farms had dogs present on or around the premises. Although no statistically significant association was found between feeding dogs raw meat and seropositivity, the coexistence of dogs and seropositive herds supports the hypothesis that dogs may act as potential sources of environmental contamination. A significant correlation ($p \leq 0.05$) was observed between *N. caninum* seropositivity and farms with a history of abortion, suggesting that *N. caninum* infection might contribute to reproductive losses in certain herds.

Previous epidemiological studies have similarly reported that the presence of dogs on farms is a major risk factor associated with increased seroprevalence of *N. caninum* in cattle (Nazir et al. 2014). Direct or indirect contact between dogs and cattle herds, particularly when dogs are allowed to roam freely, has been identified as an important transmission route (Bruhn et al. 2013, Gharekhani et al. 2015). The ingestion of placenta, fetal tissues, uterine debris or offal by dogs plays a crucial role in maintaining the biological cycle of the parasite (Serrano-Martínez et al. 2019, Abdeltif et al. 2022). Although oocyst shedding in dogs is typically transient, infection pressure within herds can persist, particularly when new dogs are introduced or when young puppies become infected (van Velsen 2021).

In the present study, higher seropositivity rates were detected on farms where dogs were present, raw meat was fed, and veterinary supervision was lacking. In traditional family-type farming systems in Turkey, where effective control of definitive hosts is rarely implemented, free-roaming stray dogs and contamination of pastures with oocysts are inevitable. The survey data therefore suggest that the presence of dogs and the practice of feeding raw meat elevate the risk of *N. caninum* infection. Nevertheless, further serological and molecular studies on anti-*N. caninum* antibodies in dogs are warranted to clarify their role in disease transmission dynamics.

These results underline the importance of raising farmer awareness, restricting dogs' access to aborted materials, and improving biosecurity measures. Preventive actions, including control of farm dogs and proper disposal of fetal tissues, are essential to reduce infection risk and prevent within-herd transmission.

In conclusion, this study provides the first available serological data on *N. caninum* infection in cattle from Artvin province, contributing valuable epidemiological information on the regional distribution of the parasite. The results highlight the need for continued surveillance and region-specific control programs to reduce the economic impact of bovine neosporosis.

Conclusion

In this study, *Neospora caninum* infection was detected serologically in cattle from Artvin province, with an overall seroprevalence of 25.55%. Seropositive

animals were identified in 25 out of 47 herds (53.2%), indicating that infection is present at the herd level across the region. A statistically significant association ($p \leq 0.05$) was observed between *N. caninum* seropositivity and farms with a history of abortion, when evaluated based on herd-level data. This finding suggests that *N. caninum* may contribute to reproductive problems in cattle in this area. This study provides the first available serological data on *N. caninum* infection in Artvin province, emphasizing the need for further epidemiological investigations and targeted control strategies. Strengthening herd hygiene, limiting dogs' access to placentas and aborted materials, and improving farmer education are recommended to reduce the spread of neosporosis and minimize related economic losses.

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