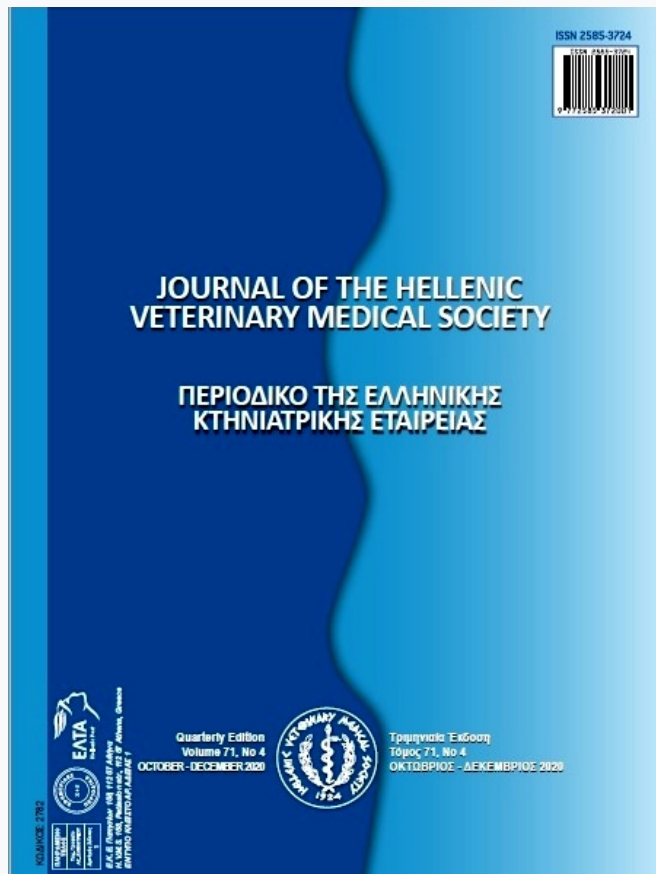


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## Seroprevalence of *Neospora caninum* infection in stray dogs in Chalkidiki, Northern Greece

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**ABSTRACT:** Despite the importance of *N. caninum* in veterinary medicine, knowledge on the prevalence of this parasitosis in dogs is limited in some countries. The aim of this study was to assess the prevalence of *N. caninum* infection in stray dogs in Chalkidiki, Northern Greece. This prospective study was conducted between January 2018 and December 2019 in stray dogs aged  $\geq 6$  months old. Blood samples were collected by venipuncture before the female and male dogs underwent the spay and castration procedures, respectively. The indirect enzyme-linked immunosorbent assay (ELISA) was used to screen dogs for the presence of IgG antibodies against *N. caninum*. Of 511 dogs included in this study, 39 (7.63%) were positive for IgG antibodies against *N. caninum*. Of all the dogs, 221 were males and 290 females, with positive results for IgG antibodies found in 16 (7.24%) males and 23 (7.93%) females. Preventive measures should be developed and implemented to break the domestic cycle between dogs and bovine. We want to highlight the importance of regional reporting of *N. caninum* infection prevalence in dogs and control measures by veterinarians and veterinary authorities to farmers and public, in order to avoid this disease's spread.

**Keywords:** neosporosis, prevalence, dog, Greece

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## INTRODUCTION

Canine neosporosis is caused by *Neospora caninum*, an obligate intracellular parasite of the phylum Apicomplexa, and has a global distribution (Dubey et al., 2007a). Many morphological and biological features of this protozoan are similar to its relative *Toxoplasma gondii* (Dubey and Schares, 2011a) with key differences being observed in their natural host range, antigenicity, virulence factors and pathogenesis (Dubey et al., 2007a).

*N. caninum* has a complex facultative heteroxenous life cycle with canids such as domestic and wild dogs (*Canis familiaris*) (McAllister et al., 1998), grey wolves (*Canis lupus*) (Dubey et al., 2011b), coyotes (*Canis latrans*) (Gondim et al., 2004) and dingoes (*Canis lupus dingo*) (King et al., 2010) confirmed as definitive hosts in which sexual replication the parasite occurs (Donahoe et al., 2015; Dubey and Schares, 2011a). Ruminants, horses, rabbits and mice have been reported as intermediate hosts in which asexual replication takes place. Of these, cattle are the most frequently affected (Donahoe et al., 2015; Dubey et al., 2007b). Interestingly, dogs can also play the role of intermediate host in *N. caninum* life cycle (Dubey and Schares, 2011a; King et al., 2010).

Canids can acquire infection by ingestion of the infected tissues from the intermediate hosts or of the sporulated oocysts from the environment, and/or by vertical transmission (Dijkstra et al., 2001; Gondim et al., 2002; Schares et al., 2001). Dogs have been shown to shed oocysts into the environment after ingestion of infected offal or placental membranes from infected cows (Donahoe et al., 2015; King et al., 2010), maintaining *N. caninum* life cycle. Neosporosis has emerged as a serious disease in cattle and dogs worldwide (Dubey and Schares, 2011a; Dubey et al., 2007b).

Presence of the infected dogs in cattle farms is considered a great risk factor for bovines. Despite the importance of *N. caninum* in veterinary medicine, knowledge on the prevalence of this parasitosis in dogs is limited in some countries, including Greece.

The aim of this study was to assess the prevalence of canine *N. caninum* infection in dogs in Chalkidiki, Northern Greece. This is the first report on the seroprevalence of *N. caninum* in stray dogs in this region.

## MATERIALS AND METHODS

### Study design and size of the analyzed population

This was a prospective study conducted between

January 2018 and December 2019 in Chalkidiki, Northern Greece including 511 stray dogs that were part of the municipal neutering program. All dogs were aged  $\geq 6$  months old, but the exact age was not assessed and recorded.

Blood samples were collected by venipuncture before the female and male dogs underwent the spay and castration procedures, respectively. The samples were separated, and serum was collected. The storage was performed in individual plastic tubes at  $-20^{\circ}\text{C}$ , pending testing examination for the presence of *N. caninum* antibodies.

### *N. caninum* antibody detection

To determine canine's serological status for *N. caninum*, the blood samples were centrifugated at 2000 rpm for 15 minutes and serum samples were obtained for further examination. The indirect enzyme-linked immunosorbent assay (ELISA) was used to screen dogs for the presence of IgG antibodies against *N. caninum*.

The commercial kit ID Screen® *Neospora caninum* In Direct Multi-species ELISA (IDVet®, Montpellier, France) was used and manufacturer's instructions were followed. The same assay was used in other studies (Enăchescu et al., 2012; Sharma et al., 2015; Villagra-Blanco et al., 2018).

### Statistical analysis

Statistical analyses were performed using the IBM SPSS statistics 26.0. Data were tabulated as categorical numbers and percentages. Results interpretation were descriptive. A statistical analysis was also performed using the chi-square ( $\chi^2$ ) test, with a statistical significance level of  $p < 0.05$ .

## RESULTS

Of 511 dogs included in this study, 39 (7.63%) were positive for IgG antibodies against *N. caninum*. Of all the dogs, 221 were males and 290 females, with positive results for IgG antibodies found in 16 (7.24%) males and 23 (7.93%) females (Table 1.) No differences of significant importance were recorded between the male and female groups of infected dogs ( $p > 0.05$ ).

## DISCUSSION

Current information regarding to the prevalence of *N. caninum* infection in dogs suggests that neosporosis is spread in many areas worldwide. We recorded a prevalence of 7.22% in stray dogs from the region of Chalkidiki, Northern Greece. Compared to other stud-

ies, our findings suggest a lower prevalence in this part of Greece compared to North West Italy (36.4%) (Ferroglio et al., 2007), Romania (32.7%) (Gavrea et al., 2012), Czech Republic (19.2% in canine shelter dogs) (Vaclavek et al., 2007), Serbia (17.2%) (Kuruca et al., 2013), south-eastern Poland (16.4%) (Ploneczka and Mazurkiewicz, 2008), rural areas of central Poland (21.7%) (Gozdzik et al., 2011), Spain (12.2%) (Ortuno et al., 2002) and China (20%) (Gao and Wang, 2019).

Contrary to our findings, in some of the northern European countries, the prevalence of *N. caninum* infection were notably lower: Sweden with a prevalence of 0.5% (Bjorkman et al., 1994), Germany with 4% (Klein and Müller, 2001) and Austria with 3.6% (Wanha et al., 2005). In Korea (Nguyen et al., 2012) and Grenada, West Indies (Sharma et al., 2015) were also recorded lower prevalences (3.6% and 1.6%, respectively) compared to Chalkidiki (7.2%).

Even though the prevalence of *N. caninum* infection may be high in some areas, the clinical signs are rare in adult dogs. Bitches that have given birth to puppies congenitally infected with this parasite do not present any clinical signs (Dubey et al., 2007a; Villagra-Blanco et al., 2018). Nevertheless, naturally acquired *N. caninum* infection by transmission to offspring in succeeding generations can occur (Barber and Trees, 1998; Crookshanks et al., 2007). The study conducted by Barber and Trees (1998) has showed that the frequency of vertical transmission is variable, as long as 80% of puppies born to seropositive mothers were not infected (Barber and Trees, 1998).

Even if clinical canine neosporosis is rare, studies on the prevalence and epidemiology of this disease can contribute to a better organization of the preventive measures in individual areas in order to minimize both canine and intermediate hosts infection, especially in cattle.

In case of clinical neosporosis in dogs, neuromuscular signs including ataxia, ascending paralysis and other general nervous clinical symptoms are present (Lindsay and Dubey, 2000). Other manifestations include myocardial, pulmonary, dermatological and reproductive disorders (Barber and Trees, 1998; Dubey et al., 2011b; Dubey et al., 2007b).

As definitive hosts, *N. caninum*-infected dogs shed oocysts into the environment for long periods of time, contributing to the spread and maintenance of this parasite in the environment (Basso et al., 2001; Dubey et al., 2007b). Oocysts are the key factor in the

epidemiology of neosporosis. Even if they are shed in an un-sporulated form, they can sporulate outside the host within 24 hours (Dubey et al., 2007b).

Regarding gender, in our study the percentages of *N. caninum* seropositive females and male dogs were similar with no statistically significant association (7.93% [N=23] versus 7.24% [N=16], respectively). This finding is in line with previous studies that have reported gender as not a risk factor for seropositivity (Cheadle et al., 1999; Collantes-Fernández et al., 2008; Ferroglio et al., 2007). On the contrast, Nazir et al. (2014) referred a significantly higher prevalence in male stray dogs. Regarding age, dogs of any age could be infected, with an increased prevalence reported in older dogs compared to younger ones (Basso et al., 2001; Capelli et al., 2004).

Reports on neosporosis prevalence in different areas worldwide could be of a great help for identification of regions at risk. The presence of stray dogs and their potential exposure to *N. caninum* should be considered a risk factor for neosporosis spread in canine and bovine populations.

*N. caninum* causes abortions from month 3 of gestation onwards in both dairy and beef cattle (Bartels et al., 1999; Dubey et al., 2007b; Reiterová et al., 2009). This parasite can also cause fetal viability disorders or neurological birth defects in newborn calves (Lassen et al., 2012; Malaguti et al., 2012) and those younger than 2 months of age (Dubey, 2003).

Because of their free ranging, stray adult dogs, in addition to ingestion of sporulated oocysts from the environment, they can have direct access to ingest tissue cysts originating from miscarriage products of cattle abortions or consumption of raw meat of other intermediate hosts. Preventive measures should be developed and implemented to break the domestic cycle between dogs and bovine. This could lead to a better control of bovine neosporosis and reduction of the economic impact of this parasitosis.

To note, a worldwide general strategy to control neosporosis is not applicable due to the regional differences in the epidemiology of bovine neosporosis, reason why the regional epidemiology of neosporosis should be assessed before elaborating on a control program (Dubey et al., 2007b).

## CONCLUSIONS

*N. caninum* was found prevalent in adult stray dogs from Chalkidiki, Greece. 39 out of 511 dogs (7.63%)

were positive for IgG antibodies against *N. caninum*. This recommends an important prevalence that cannot be neglected. We would like to highlight the importance of regional reporting of *N. caninum* infection prevalence in stray dogs and control measures by

veterinarians and veterinary authorities to farmers and public, in order to avoid this disease's spread.

## CONFLICT OF INTEREST

None declared.

## REFERENCES

- Barber JS, Trees AJ (1998) Naturally occurring vertical transmission of *Neospora caninum* in dogs. *Int J Parasitol* 28:57-64.
- Bartels C, Wouda W, Schukken Y (1999) Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). *Theriogenology* 52:247-257.
- Basso W, Venturini L, Venturini M, Hill D, Kwok O, Shen S, Dubey J (2001) First isolation of *Neospora caninum* from the feces of a naturally infected dog. *J Parasitol* 87:612-618.
- Bjorkman, Lundén A, Uggla A (1994) Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in Swedish dogs. *Acta Vet Scand* 35:445-447.
- Capelli G, Nardelli S, di Regalbono AF, Scala A, Pietrobelli M (2004) Seroprevalence survey of *Neospora caninum* infection in dogs in north-eastern Italy. *Vet parasitol* 123:143-148.
- Cheadle MA, Lindsay DS, Rowe S, Dykstra C, Williams M, Spencer J, Toivio-Kinnucan M, Lenz S, Newton J, Rolsma M (1999) Prevalence of antibodies to *Neospora Caninum* in dogs. *Int J Parasitol* 29:1537-1543.
- Collantes-Fernández E, Gómez-Bautista M, Miró G, Álvarez-García G, Pereira-Bueno J, Frisuelos C, Ortega-Mora L (2008) Seroprevalence and risk factors associated with *Neospora caninum* infection in different dog populations in Spain. *Vet Parasitol* 152:148-151.
- Crookshanks JL, Taylor SM, Haines DM, Shelton GD (2007) Treatment of canine pediatric *Neospora caninum* myositis following immunohistochemical identification of tachyzoites in muscle biopsies. *Can Vet J* 48:506-508.
- Dijkstra T, Eysker M, Schares G, Conraths F, Wouda W, Barkema H (2001) Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *Int J Parasitol* 31:747-752.
- Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Slapeta J (2015) A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. *Int J Parasitol Parasites Wildl* 4:216-238.
- Dubey J, Schares G (2011a) Neosporosis in animals—the last five years. *Vet parasitol* 180:90-108.
- Dubey JP (2003) Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol* 41:1-16.
- Dubey JP, Jenkins MC, Rajendran C, Miska K, Ferreira LR, Martins J, Kwok OC, Choudhary S (2011b) Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. *Vet Parasitol* 181:382-387.
- Dubey JP, Schares G, Ortega-Mora LM (2007b) Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev* 20:323-367.
- Dubey JP, Vianna MC, Kwok OC, Hill DE, Miska KB, Tuo W, Velmurugan GV, Conors M, Jenkins MC (2007a) Neosporosis in Beagle dogs: clinical signs, diagnosis, treatment, isolation and genetic characterization of *Neospora caninum*. *Vet Parasitol* 149:158-166.
- Enăchescu V, Ioniță M, Mitrea IL (2012) Preliminary data in comparative serodiagnostic of *Neospora caninum* in dogs. *Scientific Works. C Series. Vet Med* 58:128-135.
- Ferroglio E, Pasino M, Ronco F, Bena A, Trisciuglio A (2007) Seroprevalence of antibodies to *Neospora caninum* in urban and rural dogs in north-west Italy. *Zoonoses Public Health* 54:135-139.
- Gao X, Wang H (2019) Seroprevalence and risk factors for *Neospora caninum* infection in dogs in rural northeastern mainland China. *Parasite* 26:32.
- Gavrea R, Mircean V, Pastiu A, Cozma V (2012) Epidemiological survey of *Neospora caninum* infection in dogs from Romania. *Vet Parasitol* 188:382-385.
- Gondim LF, McAllister MM, Pitt WC, Zemlicka DE (2004) Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int J Parasitol* 34:159-161.
- Gondim LFP, Gao L, McAllister M (2002) Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro isolation from oocysts. *J Parasitol* 88:1159-1163.
- Gozdzik K, Wrzesien R, Wielgosz-Ostolska A, Bien J, Kozak-Ljunggren M, Cabaj W (2011) Prevalence of antibodies against *Neospora caninum* in dogs from urban areas in Central Poland. *Parasitol Res* 108:991-996.
- King JS, Slapeta J, Jenkins DJ, Al-Qassab SE, Ellis JT, Windsor PA (2010) Australian dingoes are definitive hosts of *Neospora caninum*. *Int J Parasitol* 40:945-950.
- Klein BU, Müller E (2001) Seroprevalence of antibodies to *Neospora caninum* in dogs with and without clinical suspicion for neosporosis in Germany [Seroprävalenz von Antikörpern gegen *Neospora caninum* bei Hunden mit und ohne klinischem Neosporoseverdacht in Deutschland]. *Praktische Tierarzt* 82:437-440.
- Kuruca L, Spasojevic-Kosic L, Simin S, Savovic M, Laus S, Lalosevic V (2013) *Neospora caninum* antibodies in dairy cows and domestic dogs from Vojvodina, Serbia. *Parasite* 20:40.
- Lassen B, Orro T, Alekseev A, Raaperi K, Jarvis T, Viltrop A (2012) *Neospora caninum* in Estonian dairy herds in relation to herd size, reproduction parameters, bovine virus diarrhoea virus, and bovine herpes virus 1. *Vet Parasitol* 190:43-50.
- Lindsay D, Dubey J (2000) Canine neosporosis. *Vet Parasitol* 14:1-11.
- Malaguti JMA, Cabral AD, Abdalla RP, Salgueiro YO, Galletti NTC, Okuda LH, Cunha EMS, Pituco EM, Fava CD (2012) *Neospora caninum* as causative agent of bovine encephalitis in Brazil. *Rev Bras Parasitol Vet* 21:48-54.
- McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM (1998) Dogs are definitive hosts of *Neospora caninum*. *Int J Parasitol* 28:1473-1478.
- Nazir MM, Maqbool A, Akhtar M, Ayaz M, Ahmad AN, Ashraf K, Ali A, Alam MA, Ali MA, Khalid AR, Lindsay DS (2014) *Neospora caninum* prevalence in dogs raised under different living conditions. *Vet Parasitol* 204:364-368.
- Nguyen TT, Choe SE, Byun JW, Koh HB, Lee HS, Kang SW (2012) Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dogs from Korea. *Acta Parasitol* 57:7-12.
- Ortuno A, Castella J, Almeria S (2002) Seroprevalence of antibodies to *Neospora caninum* in dogs from Spain. *J Parasitol* 88:1263-1266.
- Ploneczka K, Mazurkiewicz M (2008) Seroprevalence of *Neospora caninum* in dogs in south-western Poland. *Vet Parasitol* 153:168-171.
- Reiterová K, Špilovská S, Antolová D, Dubinský P (2009) *Neospora caninum*, potential cause of abortions in dairy cows: the current serological follow-up in Slovakia. *Vet Parasitol* 159:1-6.
- Schares G, Heydorn A, Cüppers A, Conraths F, Mehlhorn H (2001) *Hammondia heydorni*-like oocysts shed by a naturally infected dog and *Neospora caninum* NC-1 cannot be distinguished. *Parasitol Res* 87:808-816.
- Sharma R, Kimmitt T, Tiwari K, Chikweto A, Thomas D, Lanza Perea M, Bhaiyat MI (2015) Serological evidence of antibodies to *Neospora caninum* in stray and owned Grenadian dogs. *Trop Biomed* 32:286-290.
- Vaclavek P, Sedlak K, Hurkova L, Vodrazka P, Sebesta R, Koudela B (2007) Serological survey of *Neospora caninum* in dogs in the Czech Republic and a long-term study of dynamics of antibodies. *Vet Parasitol* 143:35-41.
- Villagra-Blanco R, Angelova L, Conze T, Schares G, Bärwald A, Taubert A, Hermosilla C, Wehrend A (2018) Seroprevalence of *Neospora caninum*-specific antibodies in German breeding bitches. *Parasite Vector* 11:96.
- Wanha K, Edelhofer R, Gabler-Eduardo C, Prosl H (2005) Prevalence of antibodies against *Neospora caninum* and *Toxoplasma gondii* in dogs and foxes in Austria. *Vet Parasitol* 128:189-193.