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Research article
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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 ≥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 ≥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 °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 (χ^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 row 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 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.

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