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Seroprevalence of *Neospora caninum* in dogs from Khorramabad and Kermanshah, west of Iran

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Abstract

Neosporosis has a global distribution and a great impact on the dairy cow industry. This study was aimed to determine seroprevalence and risk factors of *Neospora caninum* infection in owned and homeless dogs from Khorramabad and Kermanshah areas, west of Iran. A total number of 120 dogs (60 from Khorramabad and 60 from Kermanshah) were randomly selected. Blood samples were taken from cephalic vein and sera were analyzed for detection of anti-*N. caninum* antibodies using *Neospora* modified agglutination test (N-MAT). Of all, 18 (15%) of the examined dogs were infected with *N. caninum*. The seroprevalence of infection was higher in Kermanshah (16.66%) than in Khorramabad (13.33%). There was no significant difference in the seroprevalence among household dogs (27.27%), farm dogs (16.66%), and homeless dogs (12.65%). It is critical in these areas to implement the necessary measures to control the parasite and manage the population of dogs with *N. caninum* infection.

Key words: *Neospora caninum*, Seroprevalence, Dog

Introduction

Neospora caninum is an intracellular protozoan parasite belonging to the phylum Apicomplexa. Neosporosis was first reported in some Boxer puppies with myositis and congenital encephalitis in Norway in 1984 when Bjerkas witnessed parasites similar to Toxoplasma (Bjerkås et al, 1984; Barker et al, 2021). Today, the disease has a global distribution. There are many studies about the prevalence of this parasite around the world (Anvari et al, 2020; Dubey, 2003). The life cycle of this parasite involves an asexual division stage in intermediate hosts and a sexual division stage in definitive hosts. The definitive hosts are canids, including dogs, coyotes, wolves, and dingoes. Additionally, dogs can serve as intermediate hosts as well. Vertical transmission of the parasite is possible during several gestations, but not all the puppies are necessarily affected. A wide range of herbivores including domestic and wild ruminants, equids, camelids, rodents and birds can act as intermediate hosts.

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Experimental infections have been conducted in other species, such as mice, rats, foxes, cats, pigs, rabbits, monkeys, and gerbils (Dubey, 2003; Gharekhani et al, 2020; Reichel et al, 2020; Nematollahi et al, 2022). The definitive hosts become infected by consuming tissues contaminated with *Neospora* cysts, which then develop into oocysts in their intestines and are shed in the environment through feces. Intermediate hosts become infected either by ingesting sporulated oocyst-contaminated food or water or by vertical transmission during gestation especially in cattle which seems to be an important reason for the remaining *Neospora* for several generations. The sporozoites transform into motile tachyzoites which replicate rapidly and later form bradyzoites in various tissues such as muscles and brain of intermediate host, leading to cyst formation (Dubey, 2003; Gharekhani et al, 2020).

Neosporosis in cattle is of great importance. The damages caused by the disease in this industry include fetal abortion, weak calf births, increased calving intervals, reduction in milk production and increased culling and veterinary costs (Ayati et al, 2023; Nematollahi and Foroozanfar, 2023; Hazrati Kalbi Beki et al, 2023). Neosporosis in dogs has various clinical forms affecting all ages, mainly showing nervous disorders. Some cases exhibit a septicemic blood profile. Also, there is a cutaneous form of the disease that seems to be found solely in adults. The most severe signs occur in congenitally infected puppies. Some puppies are clinically unaffected and some are born asymptomatic followed by developing ascending hind limb paralysis 21 days postpartum. Jaw paralysis, dysphagia, and heart failure may also develop over time. In one experimentally infected dog, a stillbirth occurred along with some apparently normal puppies (Beugnet et al, 2018; Salama et al, 2022, Kennedy et al, 2024; Morganti et al, 2024).

Numerous studies have been conducted worldwide on the epidemiology of this parasite in dairy cows and dogs. An important aspect in this regard is the role of free-ranging dogs in spreading *Neospora* oocysts between farms (Mosallanejad et al, 2018). Detection of anti-*Neospora* antibodies is the method of choice for mass screening in epidemiological studies. Several diagnostic techniques such as enzyme-linked immunosorbent assay, modified Agglutination Test, latex agglutination test, indirect hemagglutination assay and indirect fluorescent antibody test can be put to work to diagnose this parasite (Dubey, 2003). The present study was aimed to investigate seroprevalence and risk factors of *N. caninum* infection in dogs from the Kermanshah and Khorramabad areas.

Material and Method

Sampling procedure and risk factors

The sampling was done in Khorramabad and Kermanshah areas, west of Iran. Between January and February 2023, 120 blood samples were collected from domestic dogs (60 from Khorramabad and 60 from Kermanshah) based on the Cochran sample size formula:

$$n = \frac{Z^2 p(1-p)}{e^2}$$

where n is the sample size; Z is the confidence interval (1.96); e is the precision (0.05); and p is the prevalence in target population. A prevalence of 8.6% was adopted based on the findings of Gharekhani and Yakhchali (2019).

$$n = \frac{(1.96)^2 \times 0.086 \times (1-0.086)}{(0.05)} \approx 120$$

Of these, 58 (48.3%) and 62 (51.6%) were respectively male and female. The dogs were grouped into three age sub-groups according to dental abrasion and tartar (Smith, 1999): group I, age < 6 months (n=37), group II, 6 months – 6 years (n=46) and group III, age > 6 years (n=37). Furthermore, animals were grouped as household dogs (n=11), homeless dogs (n=79), and farm dogs (n=30).

Blood samples were taken from cephalic vein and collected in clot activator tubes. Sera were removed by centrifugation at 1500 rpm for 10 min. All sera were subjected for heat treatment at 56 °C for 30 min to inactivate the endogenous complement components (Hariri et al, 2021; Turlewicz-Podbielska et al, 2024).

***Neospora*-modified agglutination test (N-MAT)**

The sera were initially diluted at 1:25, followed by serial dilution up to 1:400 in 96 round-bottom-well microplates as described by Romand et al, (1997) and Mosallanejad et al, (2018). A negative agglutination was indicated by a central discrete opaque dot in the well, while positive agglutination appeared as diffuse opacity throughout the entire well. The positive sera with 1:25 dilution and above were considered positive for neosporosis. All samples with inconclusive results were retested (Dubey et al, 2007).

Statistical analysis

The seroprevalence of *Neospora* infection in dogs was estimated by the ratio of positive results of the N-MAT test to the total number of examined dogs. Assessment of the associations between the seroprevalence with age, sex, lifestyle and location of the dogs was made with Chi-square and Fisher's exact tests. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA). P values of ≤ 0.05 were considered statistically significant.

Results

Out of 120, 18 samples (15%) were positive (Table 1). The seroprevalence of infection in Kermanshah (16.66%) was higher than in Khorramabad (13.33%; $P > 0.05$). Furthermore, there was no significant difference in the seroprevalence between male (18.96%; 11/58) and female (11.29%; 7/62; P>0.05). The seroprevalence of infection in dogs younger than 6 months old (21.62%; 8/37) was higher than other age groups (X^2=1.69, df = 1, $P = 0.19$; Table 1). There was also no statistically significant difference ($X^2 = 0.14$, df = 1, $P = 0.69$) in the seroprevalence among household dogs (27.27%), farm dogs (16.66%) and homeless dogs (12.65%; Table 1).

Table 1: Seroprevalence of *Neospora caninum* infection in different sex, age and lifestyle groups of examined dogs from Kermanshah and Khorramabad, western Iran

Risk factors	No. of examined animals	No. of seropositive animals	Seroprevalence (%)	95% CI
Gender				
Male	58	11	18.97	8.88-29.05
Female	62	7	11.29	3.41-19.17
Age				
<6 months	37	8	21.62	8.36-34.89
6 months – 6 years	46	6	13.04	3.31-22.78
>6 years	37	4	10.81	0.81-20.82
Lifestyle				
Household	11	3	27.27	0.95-53.59
Farm	30	5	16.67	3.33-30.00
Homeless	79	10	12.66	5.33-19.99
Total	120	18	15	8.61-21.39

Discussion

Canids play a significant role in the life cycle of *N. caninum* as they serve as both definitive and intermediate hosts of the parasite. The modified direct agglutination test (N-MAT) is rapid, highly sensitive, and easy to use, making it ideal for screening large numbers of animal samples for *N. caninum* antibodies. The pooled prevalence of *N. caninum* infection in canine populations, as determined by the employed diagnostic technique, varied according to the data: ELISA (22.27%), NAT (16.05%), and IFAT (15.45%; Anvari et al, 2020).

A meta-analysis study on available data up to 2018 shows a global prevalence of 17.14% with regional prevalence of 26.59% in Africa, 21.35% in Australia, 19.01% in Asia, 17.72% in Europe, and 15.03% in America (Anvari et al, 2020). In the present study, the examined dogs (household, homeless and farm dogs) from Kermanshah and Khorramabad were seropositive for *N. caninum*. In other regions of Iran, prevalence rates have been reported as follows: 19.4% in Tehran (urban dogs; IFAT), 20% in Ahvaz (rural and urban dogs; N-MAT), 22% in Isfahan (household and kennel dogs; PCR), 32% in Chaharmahal-va-Bakhtiari (household and homeless dogs; IFAT), 45% in Tabriz (household, homeless and kennel dogs; microscopy), and 44.44%-54.62% in Shiraz (household, homeless and farm dogs; N-MAT and dot-ELISA) (Haddadzadeh et al, 2007; Mosallanejad et al, 2018; Motamedi et al, 2020; Hosseininejad et al, 2010; Nematollahi et al, 2022; Khordadmehr et al, 2012).

According to the serological results, the difference between the two genders and between the different age groups was not significant, being consistent with findings from other studies (Mosallanejad et al, 2018; Khordadmehr et al, 2012). On the other hand, some investigations reported a significant difference between these variables and the infection rates (Haddadzadeh et al, 2007; Hosseininejad et al, 2010; Altaee et al, 2022).

Our findings highlight the need for concentration on preventative efforts in dogs of these areas. Infected dogs, as the only hosts that can shed *N. caninum* oocysts, play a crucial role in transmitting the infection to livestock, especially dairy cows, potentially leading to abortion. It is essential to implement the necessary measures to control the parasite and the population of infected dogs. Moreover, preventative efforts such as educating dog owners about the importance of collecting feces, reducing the number of homeless dogs and improving the sanitary conditions should be considered.

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Conflict of interest

The authors declared no conflict of interest.

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شیوع سرمی *نئوسپورا کانینوم* در سگ‌های خرم آباد و کرمانشاه، غرب ایران

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چکیده

نئوسپروزیس با توزیع جهانی تأثیر زیادی بر صنعت پرورش گاوهای شیری دارد. این مطالعه با هدف تعیین شیوع سرمی و عوامل خطر آلودگی به *نئوسپورا کانینوم* در سگ‌های صاحب‌دار و بدون صاحب در نواحی غرب ایران (خرم‌آباد و کرمانشاه)، انجام شده است. در مجموع تعداد ۱۲۰ قلاده سگ (هر استان ۶۰ قلاده) به طور تصادفی انتخاب شدند. نمونه خون‌ها از ورید سفالیک گرفته شد و سرم‌ها به منظور تشخیص آنتی‌بادی‌های ضد *نئوسپورا کانینوم* با استفاده از آزمون آگلوتیناسیون اصلاح شده *نئوسپورا* (N-MAT) آنالیز شدند. در مجموع، ۱۸ قلاده (۱۵ درصد) از سگ‌های مورد بررسی به *نئوسپورا کانینوم* آلوده بودند. شیوع سرمی آلودگی در کرمانشاه (۱۶/۶۶ درصد) از خرم آباد (۱۳/۳۳ درصد) بیش‌تر بود. شیوع سرمی در بین سگ‌های خانگی (۲۷/۲۷ درصد)، سگ‌های مزرعه (۱۶/۶۶ درصد) و سگ‌های بدون صاحب (۱۲/۶۵ درصد) تفاوت معنی‌داری نداشت. انجام اقدامات لازم به منظور کنترل انگل و مدیریت جمعیت سگ‌های آلوده به *نئوسپورا کانینوم* در این نواحی ضرورت دارد.

کلمات کلیدی: *نئوسپوروا کانینوم*، شیوع سرمی، سگ

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