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ORIGINAL PAPER

Prevalence of antibodies to *Neospora caninum* in stray dogs of Urmia, Iran

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Abstract To investigate anti-*Neospora caninum* antibodies in stray dogs living in Urmia city, 135 blood samples were collected. Serum samples were screened for detection of anti-*N. caninum* IgG antibodies using indirect fluorescent antibody test (IFAT; ≥50). Antibodies were seen in 36 (27%) of 135 dogs. The IFAT antibody titers were as follows: 1:50 in 16 dogs, 1:100 in ten dogs, 1:200 in six dogs, 1:400 in one dog, 1:800 in two dogs, and 1:1,600 in one dog. There were no significant differences in seroprevalence of *Neospora* infection between different genders ($p>0.05$). The seropositive results were increased with age and the differences were statistically significant ($p<0.05$). The results confirm the presence and exposure of stray dogs to *N. caninum* in Urmia city and the importance of this protozoan as a cause of disease in dogs of the region.

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Introduction

Neospora caninum is an apicomplexan protozoon which was described by Dubey et al. (1988a, b). The agent can infect domestic dogs (*Canis familiaris*) (McAllister et al 1998) as well as an ample variety of hosts including ruminants (Anderson et al 1991). It has been regarded as the major cause of abortion with great economical impact in dairy cattle industry and neurologic signs in puppies and older dogs (Ruehlmann et al 1995; Dubey and Lindsay 1996; Dubey 2003).

Although the parasite can be transmitted transplacentally in several hosts and the vertical route is the major mode of its transmission in cattle, the role of the definitive host in spreading the infection through shedding of oocyst is important. Carnivores can acquire infection by ingestion of infected tissues (McAllister et al 1998; Dijkstra et al 2001). As oocysts are rarely found in dog feces (Schares et al. 2005), serological surveys provide the main clue for estimating the prevalence of canine neosporosis (Björkman et al. 1994). The agent has a cosmopolitan distribution, with reports from various countries of Europe, USA, Canada, Australia, South Africa, Japan, and Costa Rica (Dubey and Lindsay 1996). There is also serological evidence of infection in equatorial Africa and South America (Barber et al. 1997a). Seroprevalence (of infection, not disease) varies from 0.5% to 17% in Europe (Björkman et al. 1994; Rasmussen and Jensen 1996) and has been reported at 2% in the USA (Lindsay et al. 1990).

At present, only limited information is available on the occurrence and prevalence of *Neospora* infections in dogs in Iran. *N. caninum* infection was first reported by Malmasi et al. (2007) in household dogs and dogs living in dairy and

beef cattle farms. The presence of antibodies against *N. caninum* in aborted and healthy dairy cattle was detected, and neosporosis importance in cases of cattle abortion is being increasingly demonstrated in Iran (Razmi et al. 2002, 2006, 2007; Sadrebazzaz et al. 2004, 2007; Habibi et al. 2005; Haji kolaei et al. 2008). Nevertheless, no information on the prevalence of *N. caninum* infection in stray dogs is available in Iran. Because of this reason, the recent investigation was done to evaluate the seroprevalence of *Neospora* infection in stray dogs of northwest Iran.

Materials and methods

Animals

A total of 135 sera from stray dogs of mixed breeds from districts of Urmia (81 males, 54 females; <1 to >6 years of age) were randomly sampled. All of the dogs were examined after the drawing of blood samples. Information regarding access to bovines, clinical signs, gender, and age of the animals was also obtained and were registered for retrospective correlation with the anti-*N. caninum* antibody titers in the positive animals.

Preparation of the serum samples

Blood samples were drawn from cephalic vein of each dog using venopuncture. The samples were centrifuged at 700×*g* for 15 min to obtain the serum, which was aliquoted and stored in microtubes and kept frozen at −20°C until analysis.

Indirect fluorescent antibody test

The sera were screened for IgG antibodies to *N. caninum* by commercial indirect fluorescent antibody test (IFAT; MegaScreen Fluoneospora, Horbranz, Austria). All sera were screened at dilutions of 1:50 in phosphate-buffered saline (pH7.2). Serum samples showing fluorescence at the dilution of 1:50 (cutoff value) were further tittered using twofold serial dilutions (Dubey et al. 1988a, b). The entire surface fluorescence of tachyzoite was considered positive (Silva et al. 2007). On each slide, a negative and a positive control were included.

Statistical analysis

For the statistical analysis of the possible effects of the attributes of different gender and age groups range on the prevalence of anti-*N. caninum* antibodies, chi-squared test was used. Statistical significance was declared at $p \leq 0.05$. The analyses were performed by SPSS 12 statistics program for Windows.

Results

Thirty-six out of 135 (26.6%) stray dogs were found seropositive. The IFAT antibody titers were as follows: 1:50 ($n=16$), 1:100 ($n=10$), 1:200 ($n=6$), 1:400 ($n=1$), 1:800 ($n=2$), and 1:1,600 ($n=1$; Table 1). Twenty-four of 81 (30%) male and 12 of 54 (22%) female stray dogs had *Neospora* infection (Table 2). The highest infection rate was observed in male dogs (33%). There were no significant differences in seropositivity between different genders ($p>0.05$). The infection in age group 2–3 years (16%) was higher than in older dogs (2%). There was a significant difference between the infection rate of *Neospora* among different age groups ($p<0.05$).The seropositive results were being increased in the age group of 1–3 years (22%) compared to the dogs of age group 4–10 years (5%).

Discussion

Assessing seroprevalence, and hence the exposure of dog population of *N. caninum*, is an important part of investigating the possible transmission routes of the parasite as well as

Table 1 Seroprevalence of *Neospora* antibodies in different age groups of stray dogs

Age (years)	No. of infected dogs	Positives (IFA titers)						Total (%)
		≥50	100	200	400	800	1,600	
<1–1	8	3	2	3	0	0	0	8 (6)
2–3	22	10	7	3	0	1	1	22 (16)
4–5	4	2	1	0	0	1	0	4 (3)
6–10	2	1	0	0	1	0	0	2 (2)
Total	36	16	10	6	1	2	1	36 (27)

Table 2 Prevalence of anti-*N. caninum* based on gender of stray dogs

Antibody titer	1:50	1:100	1:200	1:400	1:800	1:1,600	Total
Infected animal (%)	16 (12)	10 (7)	6 (4)	1 (1)	2 (2)	1 (1)	36 (27)
Sex							
Male	12 (33)	7 (19)	4 (11)	1 (3)	0	0	24 (67)
Female	4 (11)	3 (8)	2 (6)	0	2 (6)	1 (3)	12 (33)

identifying populations in which neosporosis may occur. The IFAT is a well-established test for detecting anti-*N. caninum* antibodies in dogs (Dubey et al. 1988a, b).

In Iran, the presence of antibodies against *N. caninum* in dairy cattle (Sadrebazzaz et al. 2004; Razmi et al. 2006), camels (Sadrebazzaz et al. 2006) and farm and household dogs (Haddadzadeh et al. 2007; Malmasi et al. 2007) were reported. Based on the present study, this is the first report on the seroprevalence of *N caninum* in stray dogs of the northwest of Iran. This finding confirms the presence of *Neospora* infection and the important role of dogs in the region. The prevalence of 27% was lesser than the prevalence of 33% and 28% reported by Malmasi et al. (2007) and Haddadzadeh et al. (2007), respectively. This prevalence is similar to the reported prevalence of 22% in urban areas in Argentina (Basso et al. 2001). The higher seroprevalence have been reported in earlier studies on dogs conducted in several countries including USA and Canada (Cheadel et al. 1999), Belgium (Barber et al. 1997b), England (Trees et al. 1993), southern Italy (Cringol et al. 1996), the Netherlands (Wouda et al. 1999), and Turkey (Coskun et al. 2000).

Serologic investigations showed that dogs coming from dairy properties have a greater seroprevalence than those from urban areas, suggesting that farm dogs have a higher risk of exposure to the parasite (Sawada et al. 1998; Wouda et al. 1999; Félix Sánchez et al. 2003). These higher seroprevalences can be due to the risk factors (consumption of placenta, materials of aborted fetuses, uterine discharge, hunting, and close contact with potential intermediate hosts of the parasite; Dijkstra et al. 2002; Fernandes et al. 2004). The presence of anti-*N. caninum* antibodies in city dogs could be associated with subclinical transplacental transmission through several generations as well. To a lesser extent, dogs could become infected by the horizontal route if they consume sporulated oocysts of *N. caninum* (McAllister et al. 1998; Dubey 1999). For this reason, in this region, stray dogs should not be accessed to bovine placentas or uterine discharge around the farms or slaughterhouse.

There was no significant difference in seropositivity between males (n=24, 67%) and females (n=12, 33%; $p>0.05$), in agreement with the results of other surveys (Trees et al. 1993; Barber et al. 1997a; Sawada et al. 1998; Cheadel et al. 1999; Haddadzadeh et al. 2007; Malmasi et al. 2007). In this study, there is a tendency for elevated risk of pathogen contact with increasing age, suggesting postnatal exposure to *N. caninum*. Similar reports from Brazil (Fernandes et al. 2004) and Iran (Haddadzadeh et al. 2007) confirm this finding. There was an association between seroprevalence and age. The youngest seropositive animal was 2–3 years of age and the oldest was 6–10 years. However, dogs of any age (18 months to 6 years old) may be affected by neosporosis (Knowler and Wheeler 1995; Patitucci et al. 1997). The role of age in seropositivity suggests that most of the dogs acquire the infection in the postnatal period by means of horizontal transmission. The role of age has been observed in similar studies (Barber and Trees 1996; Sawada et al. 1998; Wouda et al. 1999; Haddadzadeh et al. 2007; Malmasi et al. 2007).

The anti-*N. caninum* titers encountered varied from 50 to 1600. Thirty-five (97%) of 36 seropositive dogs had the titers of 1:800 and lower. None of these cases had previous clinical symptoms of the disease, suggesting that a large portion of infection occurs in a subclinical form. In our study, one of the examined dogs showed hindlimb paraplegia and had an anti-*N. caninum* IFAT titer of 1:1,600. This may indicate an acute infection (Barber and Trees 1996). Clinical and subclinical infections with *N. caninum* in dogs have been extensively reported worldwide with seroprevalence rates ranging from 0.2% to 54% (Moore 2005).

The presence of infected stray dog could be a risk factor for the occurrence of *N. caninum*-associated abortions in cattle. Further investigations could be useful to show the correlation between stray dog neosporosis and cattle abortion in this region.

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