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Seroprevalence of *Neospora caninum* in Stray Dogs of Tabriz, Iran

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Abstract: To investigate anti-*Neospora caninum* antibodies in stray dogs living in Tabriz city, 100 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 31 (31%) of 100 dogs. The IFAT antibody titers were as follows: 1:50 in 11 dogs, 1:100 in 10 dogs, 1:200 in 6 dogs, 1:400 in 1 dog, 1:800 in 2 dogs and 1:1,600 in 1 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 Tabriz city and the importance of this protozoan as a cause of disease in dogs of the region.

Key words: Seroprevalence, *Neospora caninum*, dogs, infection, Tabriz, Iran

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; Kamga-Waladjo *et al.*, 2009a, b) 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 (Bjorkman *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 0.5-17% in Europe (Bjorkman *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; Hajikolaei *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 100 sera from stray dogs of mixed breeds from districts of Tabriz (63 males, 37 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 (pH 7.2). Serum samples showing fluorescence at the dilution of

1:50 (cutoff value) were further tittered using two fold 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-square (χ^2) test was used. Statistical significance was declared at $p \leq 0.05$. The analyses were performed by SPSS 12 statistics program for Windows.

RESULTS AND DISCUSSION

A total 31 out of 100 (31%) stray dogs were found seropositive. The IFAT antibody titers were as follows: 1:50 (n = 11), 1:100 (n = 10), 1:200 (n = 6), 1:400 (n = 1), 1:800 (n = 2) and 1:1,600 (n = 1; Table 1). About 21 of 63 (33%) male and 10 of 37 (27%) female stray dogs had Neospora infection (Table 2). The highest infection rate was observed in male dogs (29%). There were no significant differences in seropositivity between different genders ($p>0.05$). The infection in age group 2-3 years (20%) was higher than in older dogs (1%). 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 (27%) compared to the dogs of age group 4-10 years (4%).

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 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 Tabriz Northwest of Iran. This finding confirms the presence of Neospora infection and the important role of dogs in the region. The prevalence of 31% 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 (Cringoli *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; Sanchez *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 = 21, 68%) and females (n = 10, 32%; $p>0.05$) 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 6 years old) may be affected by neosporosis

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

Age (years)	No. of infected dogs	Positives (IFA titers) ≥50	100	200	400	800	1600	Total (%)
<1-1	7	2	2	3	0	0	0	7 (7)
2-3	20	7	7	3	0	1	1	20 (20)
4-5	3	2	1	0	0	1	0	3 (3)
6-10	1	0	0	0	1	0	0	1 (1)
Total	31	11	10	6	1	2	1	31 (31)

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 (%)	11 (11)	10 (10)	6 (6)	1 (1)	2 (2)	1 (1)	31 (31)
Male	9 (29)	7 (22)	4 (13)	1 (3)	0	0	21 (68)
Female	1 (3)	3 (10)	2 (6)	0	2 (6)	1 (3)	10 (32)

(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 50-1600. The 30 (97%) of 31 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.

CONCLUSION

In this 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 0.2-54% (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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