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Full Length Research Paper

Prevalence of *Neospora caninum* antibodies in Shepherd dogs in Sarab district, East Azerbaijan Province, Iran

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Neospora caninum **is a heteroxenous cyst-forming apicomplexan intracellular protozoan which is considered as a major cause of infectious bovine abortion worldwide. The aim of the present study was to compile the initial epidemiological data on the prevalence of *N. caninum* antibodies in Sarab area. 384 blood samples were collected. Serum samples were tested for the presence of *N. caninum* antibodies using an indirect fluorescent antibody test (IFAT; >or=50). Using cut off dilution of 1:50 for dogs. Antibodies were seen in 41 (10.6%) of the 384 dogs. The largest age group was 2-4 years, with 170 (44.3%) dogs out of which only 28 dogs (16.4%) were seropositive and the lowest age group was 4-6 years, with 87(22.6%) out of which only 5 dogs (5.7%) were seropositive. The highest rate of infection was seen in Ardaha village, with 13 (32.5%) dogs. The lowest rate of infection was detected in Khaki village, with only 3 (10%) dogs. According to dog breed, the seropositivity rate of *N. caninum* was 24.3% in pure breed dogs and 17.2% in mongrel dogs. Differences among the dog breed was found to be not significant (*P>0.05*). The antibody titres of *N. caninum* ranged from 1:50 to 1:3200. 41 Seropositive dogs with titers of 1:50 in 11, 1:100 in 18, 1:200 in 6, 1:800 in 3, 1:1600 in 2, and 1:3200 in 1 dog. In the present study, there was no correlation between antibody titers and, age or location. In this study, none of the dogs had neurological signs. The results showed the presence and exposure of Shepherd dogs to *N. caninum* in Sarab city, and this confirmed that dogs are exposed to *N. caninum* and play an important role in the epidemiology of *N. caninum* of this region.**

Key words: *Neospora caninum*, Sarab, Iran, dogs.

INTRODUCTION

Neospora caninum is a heteroxenous cyst-forming apicomplexan intracellular protozoan which is considered as a major cause of infectious bovine abortion worldwide and has been associated with endemic, epidemic and sporadic abortions (Hall et al., 2006; Dubey, 2003). This parasite was firstly identified in the brain of a dog in 1988 (Dubey et al., 1990). Dogs and wild canine are definitive hosts, while several animal species including cattle are intermediate hosts (Dubey, 2003; Gondim et al., 2004). The most prominent clinical sign in dogs is neuromuscular disorders (Basso et al., 2005). Although, a few clinical canine neosporosis have been reported, yet (4-6) there are numerous reports indicating *N.caninum* sera status of the dogs in several countries (Antony and Williamson, 2003; Sanchez et al., 2003; Ferroglio et al., 2007). As clinical diagnosis is difficult, serological tests are necessary for an exact diagnosis. Several serological tests, including the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody technique (IFAT), the direct agglutination test (DAT), and immunoblots (IB) can be used to detect anti *N. caninum*

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Abbreviations: IFAT, Indirect fluorescent antibody test; **ELISA,** enzyme-linked immunosorbent assay; **DAT,** direct agglutination test; **IB,** immunoblots.

Figure 1. Map of the northwest region of Iran.

antibodies (Bjorkman and Uggla, 1999). *N. caninum* infection has been reported in dairy cattle herds on all continents (Dubey, 2003; Yildiz et al., 2009). In the *N. caninum* life cycle, dogs (McAllister et al., 1988) and coyotes (Gondim et al., 2004) are the definitive hosts, whereas cattle and other mammals act as natural intermediate hosts (Chávez-Velásquez et al., 2004; Rodrigues et al., 2004). In cattle, *N. caninum* infection may occur by horizontal transmission due to ingestion of sporulated Oocysts shed by the definitive host (Dijkstra et al., 2001; Frössling et al., 2005). However, vertical transmission is the predo-minant route of infection (Frössling et al., 2005; Hall et al., 2006). Diagnosis of the infection in live animals can be achieved by detection of anti-*N. caninum* specific antibodies by serological tests (González-Warleta et al., 2008). The presence of antibodies to *N. caninum* in the serum of an individual indicates that, it is, or has been, infected with the parasite (Stenlund et al., 2003), although the absence of antibodies does not rule out neosporosis (Ghanem et al., 2009). The aim of the present study was to compile initial epidemiological data on the prevalence and incidence of *N. caninum* antibodies in Sarab area, Iran.

MATERIALS AND METHODS

Study area, dog population and sampling

Sarab district is located in East Azerbaijan Province in Northwestern Iran with moderate mountainous climate. It covers an area of approximately 183452 km², including 168 villages, and its population is estimated to be 148,831 of which 43% is settled in urban areas and 57% is settled in rural areas. Most of the inhabitants of Sarab district are involved in agriculture and animal husbandry, live in mud or stone houses, and maintain domestic animals, such as sheep, goats, chickens, and dogs. The city of Sarab is situated at an altitude of 1650 m above the sea level and is the closest city to Sabalan Mountain (Figure 1).

Azerbaijan

Serum samples

To determine the seroprevalence of anti-*Neospora caninum* antibodies in dogs in Iran and to investigate related risk factors to the infection, a study was conducted in Sarab; located in East Azerbaijan provinces of Iran. For this, 384 serum samples were collected from dogs during an 18 month period from 2009 – 2010, on thirty (30) villages of Sarab, located in the East Azerbaijan Province. The study method was descriptive cross-sectional, and the sampling method was multi stage cluster random sampling. Out

Table 1. Seropervalence of *N. caninum* infection by gender in Sarab district.

Gender	The number of dogs tested (%)	IFA*test positive	
		No.	Seroervalence (%)
Male	306 (79.7)	31	10.1
Female	78 (20.3)	10	12.8
Total	384	41	10.6

*Immunoflorescent antibody test.

Table 2. Seropervalence of *N. caninum* infection by age in dogs in Sarab district.

Age group (years)	The number of dogs tested (%)	IFA test positive	
		No.	Seropervalence (%)
0-2	126(32.8)	8	6.3
2-4	170 (44.3)	28	16.4
4-6	87(22.6)	5	5.7
≥7	1(0.3)	-	-
Total	384 (100)	41	10.6

of 168 villages in Sarab district, 30 villages (cluster) were selected randomly. Based on previous studies, and level of infection of dogs in different areas of Iran, we tested 384 dogs with a 95% confidence level and less than 2% error and studied endemic cases, approximately with ten humans and one dog per study area. The following information was obtained for each dog using a questionnaire: Owner name, age, gender, hair color, size, habitancy location, and environmental data with local information as well as other distinctive characteristics of each animal owner. Each dog was assigned a number for identification purposes. From each dog, 10 cc of peripheral blood was taken and dispensed into polypropylene tubes. To prevent lysis of blood samples after 6-10 h, blood samples were immediately taken to the Sarab Hospital laboratory, and sera were isolated by centrifugation at 2000 rpm for 10 min.

Serology for *N. caninum*

An indirect immunoflorescent antibody test (Fuller Laboratories, California, USA) consisting of *N. caninum* tachyzoites fixed on a slide was used to detect the presence of IgG antibody for *N. caninum* in the serum samples. The tests were performed according to manufacturer's recommendation procedures. The commercial test included in positive and negative control samples. A positive titre of 1:50 was considered positive. The slides were viewed using Olympus BX50 Japan, fluorescent microscope. When compared with the positive and negative control reactions, complete, sharp and regular stained tachyzoite membranes were considered as a positive result. Samples of reactivity different from that seen in positive control were considered non-specific reaction. The positive serum samples were further tested in serial at 1:3200.

Statistical analysis

Chi-squared (χ^2) was used to compare seroprevalence rates (SPR) Relative to gender and age. The differences were considered statistically significant when the probability (*P*) value is ≤0.05. The 95% confidence intervals (95%CI) of seroprevalence rates were calculated. Statistical analysis was performed using Epi Info software, version 6.

RESULTS

384 dogs from 30 random villages around Sarab were tested using immunoflo-rescent antibody test. The overall seroprevalence of *N. caninum* antibodies determined in the study was 10.6%. By municipality, a total of forty one dogs were seropositive. The seroprevalence rate (SPR) of *N. caninum* was 10.6% (95% CI; 6.4 - 12.6). 306 (79.7%) dogs were male and 78 (20.3%) were female. 31 (10.1%) male dogs and 10 (12.8%) female dogs were seropositive (Table 1). The seropositivity was more frequent among the male dogs in this study (*P<0.05*). The largest age group was 2-4 years, with 170 (44.3%) dogs out of which only 28 dogs (16.4%) were seropositive and the lowest age group was 4-6 years, with 87 (22.6%) out of which only 5 dogs (5.7%) were seropositive (Table 2).

The highest rate of infection was seen in Ardaha village, with 13 (32.5%) infected dogs. The lowest rate of infection was detected in Khaki village, with only 1 infected dog (3.3%) (Table 3). According to dog breed, the seropositivity rate of *N. caninum* was 24.3% in pure breed dogs and 17.2% in mongrel dogs. Differences among the dog breed was found to be not significant (*P>0.05*). The antibody titres of *N. caninum* ranged from 1:64 to 1:1024 (41 seropositive dogs with titers of 1:50 in 11, 1:100 in 18, 1:200 in 6, 1:800 in 3, 1:1600 in 2, and 1:3200 in 1 dog. In the present study, there was no correlation between antibody titers and age or location. In this study, none of the dogs had neurological signs. In clinical examination, nearly all seropositive dogs seemed to be healthy (92.7%), however, some dogs (7.3%) had specific symptom as dermatitis. Antibody titres ranged from 1:800 (n: 2) to 1:3200 (n: 1) in the dogs with

Table 3. Distribution of *N. caninum* infection different village of Sarab district.

Village	The number of dogs tested (%)	IFA Test positive	
		No.	Seropervalence (%)
Ardeha	40(10.4)	13	32.5
Jahizdan	15(3.9)	4	26.6
Bahraman	16(4.1)	4	25
Arzang	31(8.1)	5	16.1
Baraghosh	22(5.7)	4	18.1
Asbfroshan	70(18.2)	2	2.8
Jaldabakhan	27(7.03)	5	18.5
Khaki	30(7.2)	1	3.3
Asfestan	28(7.2)	3	10.7
Total	279(72.6)	41	14.7

dermatitis.

DISCUSSION

N. caninum is an obligate intracellular protozoan that can infect domestic and wild canines (Schares et al., 2001; Wapenaar et al., 2007), as well as ruminants and equines, and is described as causing neuromuscular alteration and death in dogs. The role of domestic dogs in the epidemiology of *N. caninum* as well as the relationship between *N. caninum* infection of farm dogs and cattle were demonstrated, however, evidence is scarce regarding the role of wild canines in domestic animal neosporosis (Steinman et al., 2006). *N. caninum* infection provokes neurological disorders, recurrent abortion and death in dogs and cattle. Dogs are both intermediate and definitive host of *N. caninum*. Thus, the development of sensitive and specific immunoassays to diagnose canine neosporosis is essential to control this disease (Jesus et al., 2007). The most important factor of epidemiology of dog neosporosis is to eat or contact with infected tissue from cattle and other intermediate hosts (Dubey et al., 1990). Seropositivity of neosporosis in dogs was reported in different countries (Capelli et al., 2004; Ploneczka and Mazurukiewicz, 2008; Wanha et al., 2005; Coskun et al., 2000; Basso et al., 2005; Ferroglio et al., 2007). The study of Razmi (2009) on seroprevalence of neosporosis in dogs was reported as 4 (2.2%) fecal samples was positive in Mashhad area, Khorasan province, Iran. This was the first report of finding *N. caninum* DNA in feces of farm dogs in Mashhad area and fecal samples were examined by *N. caninum* specific PCR, two of the samples were positive for *N. caninum*. Other studies in west and central parts of Iran using two indirect ELISA tests from a total of 548 dogs, showed that 159 (29%) were positive for *N. caninum* (Hosseininejad et al., 2011). Some serological assays have been done in Iran to clarify the extent of *N. caninum* infection. These studies were performed in a limited population of dogs in Tehran and Urmia (Malmasi et al., 2007; Yakhchali et al., 2010) as well as in cattle (Razmi et al., 2006) and camel (*Camelus dromedarius*) populations (Sadrebazzaz et al., 2006). In present study, the prevalence of *N. caninum* was observed as 10.6% in dogs serologically. Naturally, this situation is more frequent in rural regions than those in urban areas. Some researchers (Antony and Williamson, 2003; Sanchez et al., 2007; Ferroglio et al., 2003; Wanha et al., 2005; Haddadzadeh et al., 2007; Malmasi et al., 2007; Hosseininejad and Hosseini, 2011) reported that dogs from rural areas were significantly more seropositive for *N. caninum* than those from the urban area. Some researcher suggested that the male dogs were predisposed to neosporosis (Klein and Muller, 2001). However, some suggested that the same is true for female dogs (Wouda et al., 1999). The others reported no significant difference in seropositivity between the males and the females (Sanchez et al., 2007; Ferroglio et al., 2003; Wanha et al., 2005; Haddadzadeh et al., 2007; Malmasi et al., 2007). The seropositivity was more frequent among the male dogs in this study ($P<0.05$). Obviously, the male dogs are more frequently preferred in animal farms. Therefore, the male dogs might have been infections more than the female dogs. Wanha et al. (2005) reported significant differences in dog breed seropositivity with *N. caninum*. However, no significant differences were observed among the breed of dogs in the present study ($P>0.05$). Anatolian Karabash shepherd dogs and mongrel dogs are more common in livestock farms in Iran. Pure breed dogs are preferred for family pets or hunting purposes. Wild intermediate hosts of *N. caninum* can be accepting in dogs during the hunting. The wild intermediate hosts such as foxes (*Vulpes vulpes*), wolfs (*Canis lupus*), jackals (*Canis aureus*) and coyotes (*Canis latrans*) could be playing an important role for epidemiology of disease among the dogs. The seropositivity of neosporosis increased slightly with age in some study (Wanha et al., 2005). In the present study, significant difference was not detected amongst the age groups ($P>0.05$). The largest age group

was 2-4 years, with 170 (44.3%) dogs out of which only 28 dogs (16.4%) were seropositive and the lowest age group was 4-6 years, with 87 (22.6%) out of which only 5 dogs (5.7%) were seropositive. In conclusion, the seropositivity rate of *N. caninum* was as 10.6% in dogs in Sarab (East Azerbaijan Province, Iran). Nearly all seropositive dogs were seemed healthy. These clinically healthy but serologically positive dogs are of important risk to other animals. Especially high antibody titres of *N. caninum* were detected in dogs with dermatitis and local alopecia in our study; there was attention to dogs with dermatitis symptoms in animal's farm with infertility and abort problems.

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