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Original Article

A Serological Survey of *Neospora caninum* Infection in Urban and Rural Dogs in Ahvaz District, Southwest of Iran

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ABSTRACT

Dogs are important in the epidemiology of *Neospora caninum* because they act as definitive hosts, shedding oocysts in the environment. The aim of the present survey was to evaluate the serological prevalence of *Neospora caninum* infection in urban and rural dogs in Ahvaz district, southwest of Iran. In this study, blood samples were taken from 100 rural dogs and 50 urban dogs. The dogs were categorized into two age groups (i.e., ≤ 3 and > 3 years). Neospora agglutination test (NAT) was performed for the detection of infection. Among 150 samples, 30 (20%) showed infection in 1:50 to 1:800 dilutions by NAT (confidence interval 95%: 13.60-26.40). The antibody titers were as follows: 1:50 (n=1), 1:100 (n=14), 1:200 (n=3), 1:400 (n=10) and 1:800 (n=2). The highest serum dilution was 1:100 in 46.67% of the infected dogs and the lowest serum dilution was 1:50 in 3.33% of them. The obtained results showed a significant difference in seroprevalence between urban (10%) and rural (25%) dogs (P=0.03). Although the seroprevalence was higher in dogs above three years of age (23.33%) than below three years (17.78%), there was not a significant difference among different age groups in this regard (P>0.05). The possibility of infection in dogs above the age of three years was 1.3 more than those below three years of age (confidence interval 95%: 0.58-2.9). It can be concluded that a relatively considerable percentage of dogs in Ahvaz district are infected with *N. caninum*. These infected dogs can play an important role in the transmission of neosporosis to other animals.

Keywords: *Neospora caninum*, *Neospora* agglutination test (NAT), Seroprevalence, Ahvaz, Dog

Une Étude Sérologique sur l'Infection au *Neospora caninum* chez les Chiens Urbains et Ruraux dans le District d'Ahvaz, au Sud-ouest de l'Iran

Résumé: Les chiens jouent un rôle important dans l'épidémiologie du *Neospora caninum*, car ils agissent comme hôtes définitifs, excrétant des oocystes dans l'environnement. L'objectif de cette étude était d'évaluer la prévalence sérologique de l'infection au *Neospora caninum* chez les chiens urbains et ruraux dans le district d'Ahvaz, au sud-ouest de l'Iran. Dans cette étude, des échantillons de sang ont été prélevés chez 100 chiens en milieu rural et 50 chiens en milieu urbain. Les chiens ont été classés en deux groupes d'âge (i.e. ≤ 3 et> 3 ans). Le Test d'Agglutination du Neospora (TAN) a été réalisé pour la détection de l'infection. Parmi les 150 échantillons, 30 (20%) présentaient une infection dans des dilutions de 1:50 à 1: 800 par TAN (intervalle de confiance à 95%: 13,60-26,40). Les titres en anticorps étaient les suivants: 1:50 (n = 1), 1: 100 (n = 14), 1: 200 (n = 3), 1: 400 (n = 10) et 1: 800 (n = 2). Les dilutions de sérum les plus élevées et les plus faibles étaient de 1: 100 et 1:50 chez respectivment 46,67% et 3,33% des chiens infecéts. Les résultats obtenus ont montré une différence significative de séroprévalence entre les chiens urbains (10%) et ruraux (25%) (P = 0,03). Malgré le fait que la séroprévalence était plus élevée chez les chiens de plus de trois ans

(23,33%) comparé à ceux de moins de trois ans (17,78%), il n'y avait pas de différence significative entre les différents groupes d'âge (p> 0,05). Cependant, la possibilité d'infection chez les chiens âgés de plus de trois ans était de 1,3 fois supérieure à celle des chiens de moins de trois ans (intervalle de confiance à 95%: 0,58-2,9). Pour conclure un pourcentage relativement considérable de chiens dans le district d'Ahvaz est infecté par N. caninum. Ces chiens infectés peuvent jouer un rôle important dans la transmission de la néosporose à d'autres animaux.

Mots-clés: *Neospora caninum*, Test d'Agglutination Neospora (TAN), Séroprévalence, Ahvaz, Chien

INTRODUCTION

Neospora caninum is an intracellular protozoan of the phylum Apicomplexa. Canines (i.e., domestic dog, coyote, dingo, and grey wolf) are definitive hosts of *N. caninum* (King et al., 2012; Dubey and Lappin, 2012). Canids can be experimentally infected by ingesting parasitized tissues from the intermediate herbivore hosts (Dubey and Lappin, 2012). The infection has been regarded as the major cause of abortion with great economic impact on the dairy cattle industry. The shedding period of *N. caninum* oocysts appears to be short; nevertheless, shedding in some instances has been documented up to four months by both visual inspection and polymerase chain reaction (PCR) methods (Dubey et al., 2006; Slapeta et al., 2002). Naturally occurring infections in dogs have been found throughout the world. Previous studies have shown that the prevalence of *N. caninum* antibodies in the dog population is varied, depending on the method, number of animals, the geographical location, and whether domestic or wild populations are examined (Dubey and Lappin, 2012). Dogs being fed raw meat have a much higher prevalence of reactive antibody than dogs on commercial diets. Serologic prevalence is also greater in farm dogs than urban dogs (Dubey and Schares, 2011). In rural areas of Iran, many dogs are free to interact with wild dogs and cattle wherever their distributions intersect, and this issue increases health risks for other animals (Dubey and Lappin, 2012). Some studies carried out in Iran have shown a high prevalence of Neospora infection in dogs. For example, seroprevalence of N. caninum (31%) was detected in stray dogs in Tabriz disrtict, Iran (Garedaghi, 2012). It was also determined 29% in three provinces of Iran (Hosseininejad and Hosseini, 2011), 27% in Hamadan (Gharekhani and Heidari, 2014), 11.3-28% in Tehran (Haddadzadeh et al., 2007), and 10.6% in dogs of Sarab district (Khanmohammadi and Fallah, 2011). There are many different serological techniques for the diagnosis of neosporosis, such as enzyme-linked immunosorbent assay (ELISA), Neospora agglutination test (NAT), latex agglutination assay (LAT), indirect hemagglutination assay (IHA), and indirect fluorescent antibody test (IFAT). Among the serological tests, NAT can be used as a sensitive screening test for the diagnosis of neosporosis (Dubey and Lappin, 2012). Thus, we aimed to investigate the serological prevalence (NAT) of *N. caninum* infection in urban and rural dogs in Ahvaz district, southwest of Iran.

MATERIALS AND METHODS

Study area and sample population. A cross-sectional study was performed in Ahvaz district, situated at an elevation of 12 meters above the sea level with a warm and humid climate. In the present study, 150 dogs (i.e., 100 rural and 50 urban dogs) of different ages were examined for the detection of serum antibodies (chronic phase) from April to December 2015 by NAT. The urban dogs were selected from among large breeds referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz, southwest of Iran. Rural dogs were selected from the rural dog population of Ahvaz district. Classification was made

based on age, gender, breed, and area. The breed distribution of urban dogs was 38% for mixed breeds, 30% for German shepherd, 18% for Doberman pinscher, 10% for Great Dane, and 4% for Rottweiler. The breed distribution of rural dogs was 85% for mixed breeds, 11% for German shepherd, and 4% for Doberman pinscher. The studied dogs were divided into two groups based on age (less and more than three years), and most of them were clinically healthy and had no specific signs associated with neosporosis. Ketamine (15 mg/kg) and acepromazine (0.15 mg/kg) were injected for sedative effects. Blood samples were collected from cephalic or saphenous vein in test tubes without anticoagulant and allowed to clot, and then centrifuged for 10 min at 2400 × g. The sera were collected in plastic tubes and stored at -20 °C until serologic tests.

Serological test. All serum samples were tested for the presence of *N. caninum* antibodies using the agglutination tests based on the direct agglutination of fixed parasites with sera pre-treated with 2-mercaptoethanol to prevent non-specific IgM agglutination. Sera were started at 1:25 dilution. A titer of 1:50 and higher was considered as *N. caninum* infection in dogs. Sera with borderline results were re-examined. A complete agglutination was considered as positive result and a clear-cut button-shaped deposit of parasite suspension was interpreted as a negative reaction. NAT was carried out according to the method described by Romand (1998). The *N. caninum* antigens were prepared from Razi institute of Shiraz.

Statistical analysis. Dogs were grouped based on age, gender, breed, and area to determine whether these factors were associated with *N. caninum* infection. The data were analyzed by using Chi-square test, Fisher's exact test, and Z test. Statistical comparisons were carried out using SPSS version 16.0. P-value less than 0.05 was considered statistically significant.

RESULTS

In the present study, 150 dogs were tested for the presence of antibodies against *N. caninum* using the NAT. The overall recorded seroprevalence of *N. caninum* in dogs was 20% (30 out of 150; confidence interval 95%: 13.60-26.40). As shown in tables 1 and 2, the NAT antibody titers were as follows: 1:50 (n=1), 1:100 (n=14), 1:200 (n=3), 1:400 (n=10), and 1:800 (n=2). The obtained results showed a significant difference in seroprevalence between urban dogs (10%) and rural (25%) dogs (P=0.03). The possibility of infection in rural dogs was detected three times more than that in urban dogs (confidence interval 95%: 1.07-8.4). The seroprevalence was 20% (17 out of 85) in male dogs and 20% (13 out of 65) in females based on NAT method. Although the seroprevalence was more in dogs above the age of three years (23.33%: 14 out of 60) than below three years (17.78%: 16 out of 90), there was not any significant difference between age groups (P>0.05). The possibility of infection in dogs above three years of age was detected to be 1.3 more than dogs below three years old (confidence interval 95%: 0.58-2.9). The seroprevalence of *N. caninum* infection was 17.39% (8 out of 46) in purebred dogs and 21.15% (22 out of 104) in mixed dogs, showing no significant difference by breed (P>0.05). The results are summarized in tables 1 and 2.

Table 1. Seroprevalence of N. caninum infection by Neospora agglutination test based on age in urban and rural dogs in Ahvaz district, southwest of Iran

	Positives (antibodies titers)					
Age (years)	1:50	1:100	1:200	1:400	1:800	Total
≤ 3	1	8	2	4	1	16
> 3	0	6	1	6	1	14
Total	1	14	3	10	2	30

Table 2. Seroprevalence of *N. caninum* infection by *Neospora* agglutination test based on gender in urban and rural dogs in Ahvaz district, southwest of Iran

	Positives (antibodies titers)					
Gender	1:50	1:100	1:200	1:400	1:800	Total
Male	1	9	2	4	1	17
Female	0	5	1	6	1	13
Total	1	14	3	10	2	30

DISCUSSION

The present study exhibited that the seroprevalence of *N. caninum* infection was 10-25% by NAT method in urban and rural dog population in Ahvaz district, southwest of Iran. NAT can be used as a sensitive screening test for the diagnosis of neosporosis in dogs. A 1:50 cut-off in NAT has been recommended and commonly used for canine sera. Therefore, in the present survey, we applied NAT to determine the seroprevalence of *N. caninum* in dogs and used a titer of 1:50 as the positive threshold titer. The anti-*N. caninum* titers encountered varied from 1:50 to 1:800. All seropositive dogs were clinically healthy. Serologically positive asymptomatic dogs are considered an important source of infection for other animals. The seroprevalence of *N. caninum* is variable depending on age, number of animals, their living type (stray or companion), the used diagnostic methods, and geographic area in dogs (Dubey and Lappin, 2012). In our survey, the prevalence was non-significantly higher in dogs aged above three years than those below three years. These results were agreement with those described by Coskun et al. (2000) and Romanelli et al. (2007) that did not show any correlation between age and seroprevalence for canine neosporosis. On the other hand, seroprevalence rate increased in dogs with age, which suggests postnatal exposure to *N. caninum* through horizontal transmission. High infection was attributed to greater chance for exposure to *Neospora* over time (Wanha et al., 2005; Malmasi et al., 2007; Haddadzadeh et al., 2007; Basso et al., 2009; Hosseininejad and Hosseini, 2011). In the present study, there was no significant difference in gender, which is in agreement with some other reports (Haddadzadeh et al., 2007; Hosseininejad and Hosseini, 2011; Sharifdini et al., 2011; Gozdzik et al., 2011; Nguyen et al., 2012). Farmers in Iran mostly rear male dogs in their farms. Therefore, male dogs might become infected with *Neospora* more than females (Hosseininejad et al., 2010; Khanmohammadi and Fallah, 2011). However, different serological tests, climatic variations, and frequency of canids are the main causes of discrepant results on the farms (Dubey and Schares, 2011). Serologic investigations have been shown that dogs coming from rural areas have a greater seroprevalence than those from urban areas, suggesting that rural dogs are at higher risk for exposure to the parasite, which can be due to consumption of placenta, materials of aborted fetuses, uterine discharge, hunting, and close contact with potential intermediate hosts of the parasite (Fernandes et al., 2004). Wouda et al. (1999) found a positive correlation between the seropositivity of farm dogs and increased seroprevalence in cattle, indicating a relationship between infections in dogs and in cattle. Based on our finding, *Neospora* infection rate in rural dogs (25%) was higher than that in urban dogs (10%). Haddadzadeh et al. (2007) and Malmasi et al. (2007) reported high rate of *Neospora* infection in farm dogs as compared to urban and household dogs. The results were in support of findings of Nguyen et al. (2012) who conducted studies in South Korea. Most reports have not found specific breed susceptibility or a higher seroprevalence of *N. caninum* in mixed breed dogs (Dubey and Lappin, 2012). In the present study, although the seroprevalence was higher in mixed breeds (21.15%) than purebreds (17.39%), the difference was not significant. In this regard, Jesus et al., (2006) in Brazil did not find any statistical difference among breeds in the frequency of anti-*N. caninum* antibodies. However, Robbe et al. (2016) suggested higher seropositivity in purebred dogs. The role of breed in the epidemiology of canine neosporosis is not well established and requires further research. The prevalence of *N. caninum* infection has been studied in some cities of Iran, for example, the seroprevalence of *N. caninum* was detected to be 11.3% and 28% in dogs from urban and rural environments in Tehran, respectively (Haddadzadeh et al., 2007). Malmasi et al. (2007) reported the rate of infection in 20% of household dogs and in 46% of farm dogs in Tehran. Razmi et al. (2006) reported the seroprevalence of neosporosis to be 2.2% in dogs in Mashhad, Iran. Another seroprevalence study of *Neospora* in three

provinces of Iran revealed the overall prevalence of infection in 29% of dogs with regional variations (Hosseininejad and Hosseini, 2011). The overall infection rate for *N. caninum* was 10.6% in shepherd dogs in Sarab district, East Azerbaijan (Khanmohammadi and Fallah, 2011). Hamidinejat et al. (2011) showed that the prevalence of infection was 19% in the serum of feral cats in Ahvaz, Iran. Garedaghi (2012) reported that 31% of stray dogs in Tabriz had antibodies against *N. caninum*. In a study, nine positive samples of *N. caninum* were detected by PCR from 428 fecal specimens collected from dogs living in dairy farms. In another survey in Hamadan, 27% of dogs were positive for *Neospora* antibody (Gharekhani and Heidari, 2014). Abdoli et al. (2015) stated that the seroprevalence of *N. caninum* was 3.68% in house sparrows by nested PCR targeting the *Nc-5* gene in Iran. Seroprevalence of infection varies from 0.5% to 17% in Europe, 27% in Australia and 2% in the USA (Dubey and Lappin, 2012; King et al., 2012). The prevalence of 20% for *N. caninum* was found in our study, which was higher than that reported for dogs in Turkey (10%) (Coskun et al., 2000), Brazil (6.7-10%) (Mineo et al., 2001; Gennari et al., 2002), Italy (6.4%) (Cringoli et al., 2002), and China (15%) (Wang et al., 2016). In intermediate hosts, the prevalence of *N. caninum* infection in *Rattusrattus*, *Rattusnorvegicus*, and *Mus musculus* captured in urban areas of Sao Paulo revealed a strikingly low frequency of occurrence of these infections (Muradian et al., 2012). The high prevalence of infection in Iran indicates the necessity of control strategies to be adopted for dogs and cattle. It seems that climatic conditions in this area (warm and humid) are relatively suitable for the spread and survival of the oocysts. After the confirmation of dog as a final host, the presence of dogs in farms has been assumed to provide a higher chance of horizontal transmission through ingestion of oocysts shed by the infected dogs (Dubey and Lappin, 2012). Sanitary conditions and animal health must be improved to reduce the transmission risk of *N. caninum* by dogs. Preventive efforts should focus on educating dog owners about the importance of collecting dog feces and reducing the number of stray dogs. Our results will provide the basis for further studies that will deepen our understanding of the epidemiology of *N. caninum*. Further studies will be necessary in various areas to survey the overall epidemiological status of neosporosis in the dog population. In conclusion, a relatively considerable percentage of dogs in Ahvaz district, southwest of Iran, are infected with *N. caninum*. These infected dogs can play an important role in the transmission of neosporosis to other animals.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article. Most animals were clinically healthy and had not specific signs associated with neosporosis. All procedures that might be associated with discomfort including venipuncture were performed by an experienced veterinarian.

Conflict of Interest

The authors declare that they have no conflict of interest.

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