Original Article

A serological survey on *Neospora caninum* infection in wild rats (*Rattus rattus*) in Ahvaz district, Iran

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Summary

Neospora caninum is an obligate intracellular protozoan parasite of the phyllum Apicomplexa which is considered as one of the major factors in abortion in dairy cows. Serologic evidence suggests that humans are exposed to the organism. The aim of the present survey was to evaluate serological prevalence of *Neospora caninum* in wild rats in Ahvaz district. Blood samples were collected from 150 adult wild rats. Antibody against *Neospora caninum* was detected in serum using *Neospora* agglutination test. Among 150 samples, nine cases (6%) showed infection in 1:20 to 1:320 dilutions. The highest titer of antibody was detected in 1:20 dilution (6%) and the lowest titer was in 1:320 (0.6%) dilution. The antibody titers were as follows: 1:20 (n=9), 1:40 (n=8), 1:80 (n=6), 1:160 (n=2) and 1:320 (n=1). Posititve samples of 6.67%, 3.33% and 10% for *N. caninum* were identified in north and west, east and center, and south of Ahvaz, respectively. This difference was not statistically ($P<0.05$) significant and odds ratio was similar in all districts. The seroprevalence was 6.02% in male and 5.97% in female rats which was not statistically ($P<0.05$). The present study showed that relatively moderate percentage (6%) of rats was infected by *Neospora caninum* in Ahvaz district. Wild rats can play an important role in the transmission of this parasite to other animals.

Keywords: *Neospora caninum*, *Neospora* agglutination test (NAT), Seroprevalence, Rat, Ahvaz, Iran.

Introduction

Neospora caninum is an obligate intracellular protozoan of the phylum Apicomplexa, first described in dogs in 1984 and recognized in a wide range of animals including wildlife species (Gondim, 2006). Canines are definitive hosts of *N. caninum*. The infection has been considered as the major cause of abortion in dairy cattle. A sylvatic life cycle involving wild canids and herbivores has maintained this infection in nature (Dubey and Lappin, 2012; King et al., 2012).

Rodents are the most abundant of living mammals in the world. Infection in the intermediate hosts such as rats may be important in maintenance of the disease in nature, and play a role in the epidemiology of the parasite (Dubey and Lappin, 2012). Wild rats have had detectable *N. caninum* DNA in their brain tissues (Huang et al., 2006). Rats are susceptible experimentally and they have shown to produce IgG

against the protozoan (Romand et al., 1998). Serological evidence in different animals indicate that many species have been exposed to *N. caninum*; however, many aspects of the life cycle of this parasite are unknown (Dubey and Lappin, 2012).

The reported species of the *Rattus genus* in Iran are included: the black rat (*R. rattus*), the brown rat (*Rattus norvegicus*), and the Himalayan rat (*R. pectoris*). Neosporosis is an important problem in many parts of the world (Akbari et al., 2009). The reported surveys have been shown that the prevalence of *N. caninum* antibodies in rodent's population are variable, depending on the used technique, number of cases, the geographic region and examined populations (Dubey and Lappin, 2012). Huang et al. (2004) showed that 16.4% of *Rattus norvegicus* were seropositive against *N. caninum* from cattle farms. Serological outbreak is also greater in farm animals than urban regions (Dubey and Schares, 2011).

In many different areas of Iran, rodents are free to interact with other animals wherever their distributions intersect and this issue increases the risk factor for other animals including dogs (Akbari et al., 2009). Nearly 30% of wild rats and 10% of wild mice have been found to contain detectable levels of the parasite (Dubey and Lappin, 2012). Some results of the studies carried out in Iran have been shown a high prevalence of *Neospora* infection in dogs. For example, a seroprevalence of *N. caninum* (31%) was detected in stray dogs in Tabriz (Garedaghi, 2012). It was also determined in dogs of three provinces of Iran as 27% in Hamadan (Gharekhani and Heidari, 2014), 11.3-28% in Tehran (Haddadzadeh et al., 2007) and 10.6% in Sarab district (Khanmohammadi and Fallah, 2011). These informations provide the basis for the seroprevalence survey of naturally infected rats.

Using immunochemical methods organisms similar in charasterisctic have been demonstrated in rodents. There are many different serological techniques such as enzyme-linked immunosorbent assay (ELISA), *Neospora* agglutination test (NAT), latex agglutination assay (LAT), indirect fluorescent antibody test (IFAT) and indirect hemagglutination assay (IHA) for diagnosis of neosporosis. The use of molecular genetics and PCR to distinguish *Neospora* from other related parasites has also been reviewed. Among the serological tests, NAT can be used as a sensitive screening test for diagnosis of neosporosis (Dubey and Lappin, 2012). To the best of our knowledge, no study has investigated the neosporosis in wild rats in Iran; so the purpose of the present survey was to investigate the serological prevalence of *N. caninum* infection by NAT in wild rats in Ahvaz district, South-West of Iran. Such informations are important in the population of wild animals for control and prevention programs.

Materials and Methods

Sample Collection

A cross-sectional survey was performed in Ahvaz region (South-West of Iran), warm and wet in climate, during July 2016 to 2017. A total of 150 wild adult rats (*R. rattus*) were trapped alive (from north, east, west, south and center regions), anaesthetized by hallotan or ether, and then blood-extracted by injection of needle into the heart. At least 3 ml of blood was collected from the heart of each rat. Blood samples were kept in test tubes without anticoagulant and allowed to clot, then centrifuged for 10 min at 5000×g. The sera were collected in plastic tubes and stored at -20°C until serologic tests. Serum samples were then examined for the detection of antibodies (chronic phase) using NAT. Sera were also examined for cross-reactivity with *Toxoplasma gondii*. The studied rats were grouped according to the area (five groups), season (four groups) and geneder (two groups). All rats seemed to be clinically healthy and no symptoms associated with any diseases were observed at the time of blood collection. Immature rats were excluded from the present survey.

Serological Test

The sera of captured rats were screened for antibodies for *N. caninum* using *Neospora* agglutination tests (NAT) based on the direct agglutination of fixed parasites with sera pre-treated with 2 mercaptoethanol to prevent non-specific IgM agglutination. Serum dilution was started from 1:20. A titer of 1:20 and higher was considered as *N. caninum* infection in wild rats. Sera with in borderline results were examined again. A complete agglutination was interpreted as positive reaction. Clear-and cut button-shaped was considered as a negative result. NAT was carried out according to the method described (Romand et al. 1998). The *N. caninum* tachyzoite antigens were prepared from the Razi institute of Shiraz. There was no cross-reaction between *N. caninum* and *T. gondii*, using positive control group.

Statistical analysis

Rats were grouped based on area, season and gender to determine whether these parameters were associated with *N. caninum* infection using chi-square test, Fisher's exact test and *Z* test. Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when $P \leq 0.05$.

Results

Among 150 samples, nine cases (6%) showed antibody against *N. caninum* in serum dilution 1:20 to 1:320 using the NAT test. The highest number of serum dilution was obtained in 1:20 dilution (6%) and the lowest number was in 1:320 (0.6%). As shown in Table 1, the NAT antibody titers were as follows: 1:20 (n=9), 1:40 (n=8), 1:80 (n=6), 1:160 (n=2) and 1:320 (n=1). The results indicated that 6.67% (two out of 30 samples) were

infected with *N. caninum* in the north and west regions. One out of 30 samples (3.33%) was positive for the parasite in eastern and central regions. Additionaly, three out of 30 samples (10%) were recognized as positive for *N. caninum* in south of Ahvaz (Table 2).

These differences were not statistically significant (P<0.05) and odds ratio was similar in all parts of Ahvaz (95% confidence interval: 0.97 to 1.15). Considering the gender, the prevalence of *N. caninum* infection was 6.02% (five out of 83) in male and 5.97% (four out of 67) in females rats (Table 1). This difference was not statistically significant (P<0.05) while odds ratio was simalr for male and female rats (95% confidence interval: 1 to 1.11). The prevalence of *N. caninum* infection in rats tended to be higher in the spring (7.89%), as the differences in the positive titers of *Neospora* infection were not significan among the seasons (winter= 7.50%, autumn= 5.13% and summer= 3.03%) (P<0.05). Results are summarized in Tables 1 and 2.

Table 1. Seroprevalence of *N. caninum* infection of wild male and female rats by NAT test in Ahvaz district, South-West of Iran.

Dilution / Gender	1:20	1:40	1:80	1:160	1:320
Male	5	4	3	2	0
Female	4	4	3	0	1
Total	9	8	6	2	1

Table 2. Seroprevalence of *N. caninum* infection (in number) of wild rats (n=150) by NAT test in different seasons and areas of Ahvaz district, South-West of Iran.

Area	Spring		Summer		Autumn		Winter	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
North	7	1	6	0	7	0	8	1
East	6	0	7	0	9	1	7	0
West	8	1	6	0	8	0	6	1
South	6	0	7	1	6	1	8	1
Center	8	1	6	0	7	0	8	0
Total (150)	35	3	32	1	37	2	37	3

Discussion

Little is known about the prevalence of neosporosis in different animals, particularly in rodents in Iran. The literature did not revealed any published research in regard to the prevalence of *Neospora* in the rat's population in Iran. Detection of antibodies against *N. caninum* is a good indicator of exposure of animals to this parasite. NAT can be used as a sensitive screening test for the diagnosis of neosporosis in many species. A titer of 1:20 cut-off in NAT is recommended and commonly used for animal sera (Romand et al., 1998). Hence, NAT was applied, in the present survey to determine the seroprevalence of *N. caninum* in rats and a titer of 1:20 dilutions was used as the positive threshold titer. The anti- *N. caninum* titers varied from 1:20 to 1:320 dilutions. Sampling of wild rats (*R. rattus*) was conducted for nearly one year, so the

obtained results can be considered as representative of the wild rat's population in the Ahvaz region, Iran. In the present study, all the studied rats were adult and the age was not included in the comparisons.

The present study showed the seroprevalence of 6% of *N. caninum* infection using NAT test in rat's population in Ahvaz district, South-West of Iran.

Since the life cycle of *N. caninum* has not been completely elucidated, based on the present results, it can be postulated that the wild rats can be served as an intermediate reservoir host.

Althoght all seropositive rats were clinically healthy, they might spontaneously be infected with *N. caninum* and thus served as an intermediate host becoming an indicator of this protozoan infection. It seems that the climatic conditions (warm and wet) in Ahvaz district are suitable for the survival of the parasite's oocysts.

Antibody titers were much lower in the present study than those reported in France (1:800 to 1:6400) (Romand et al., 1998). In experimental surveys, the number of the infesting protozoan is higher than that of natural infection. Serologically positive animals without clinical signs are considered as an important source of infection for others (Almeria, 2013). Wildlife needs to be taken into account in the prevention measures to reduce the economic losses.

The seroprevalence of *N. caninum* is variable depending on age, number of animals, their living type, the used diagnostic techniques, and geographic region in different animals (Coskun et al., 2000; Romanelli et al., 2007). In the present study, the prevalence tended to higher in male rats than females, but did not show any statistically significant difference. These results were in accordance with those described by Huang et al. (2004) that did not show any correlation between gender and seroprevalence for neosporosis.

Serological investigations have been shown that the animals coming from rural areas, have a higher prevalence than those from urban areas that it can be due to the consumption of the placenta, aborted fetuses, uterine discharge, and close contact with potential hosts of this parasite (Fernandes et al., 2004). Haddadzadeh et al. (2007) reported a high infection rate of *Neospora* infection in farm dogs as compared to urban and household dogs. The high prevalence of infection in some area of Iran indicates the necessity of preventive strategies to be performed in dogs and cattle populations.

The role of subspecies in rats is not well established in the epidemiology of neosporosis and requires further research. The role of rodents was investigated by assaying brain tissue of feral mice and rats for *Neospora caninum*. Of the 105 feral mice and 242 rats tested, 10% and 30% were positive in PCR assays, respectively (Jenkins et al., 2007). Lindsay et al. (1995)

used mouse as model for central nervous system *N. caninum* infections.

The prevalence of *N. caninum* infection has been studied in some other regions of Iran. A seroprevalence study of *N. caninum* in urban and rural dog population of Tehran was detected 11.3 and 28% infection, respectively (Haddadzadeh et al., 2007). 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 serum of the feral cats in Ahvaz district. Garedaghi (2012) reported that 31% of stray dogs in Tabriz region had antibody against *N. caninum*. 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. Different serological tests, climatic variations and species are the major causes of variation in the results (Almeria, 2013).

DNA of *N. caninum* was not detected in any of the samples of *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus* captured in Sao Paulo in Brazil (Muradian et al., 2012). Rodents around farms had been shown to be a plausible of *N. caninum*, with demonstration of *N. caninum* DNA in feral rats and mice. The species of rodents in which *N. caninum* DNA had been detected includes the field or wood mouse (*Apodemus sylvaticus*), rat (*Rattus norvegicus*), house mouse (*Mus musculus*), capybara (*Hydrochaeris hydrochaeris*), common vole (*Microtus arvalis*), and water vole (*Arvicola terrestris*) (Dubey and Schares, 2011). Very recently, *N. caninum* had been reported in rock squirrel (*Spermophilus variegates*) in the Netherlands. The DNA of the parasite was reported in harvest mouse (*Micromys minitus*) (15.4%) and in two species of insectivores: the common shrew (33.3%), and white-toothed shrews (10.8%) (Almeria, 2013). In an area relatively free of farm animals, it was observed low *N. caninum* prevalence in field mice (3.4%) and house mice (3.1%) (Dubey and Lappin, 2012). Dogs may be infected by eating infected house mice and this nessecitates new stragedies for *N. caninum* control. In addition, ingestion of dead rodents by farm animals, either by accident or on purpose can lead to the transmission of infection (Almeria, 2013). The fact that small mammal's particularly rodents can easily be harbouring the parasite makes them a candidate for a good indicator species for parasitic infections in farms.

Many wild rats live in different areas of Iran. When they are infected with *N. caninum* they become a transmission vector for other animals. Infection of *N. caninum* in dogs is through the ingestion of aborted fetal materials, as well as, infected rodents. Prevention methods should focus on educating farm owners about the importance of collecting wild rodents and reducing their numbers. At

present, there is no effective treatment or vaccine for neosporosis. No drugs are known to prevent transplacental transmission. The exact role of rodents and other wildlife in the life cycle and transmission of *N. caninum* needs to be confirmed and better understood.

Conclusions

From the results of this research, it is concluded that a relatively moderate percentage of wild rats in Ahvaz district, South-West of Iran are infected with *N. caninum*. These seropositive rats might play a role in the transmission of neosporosis to other animals. It is nessecary to conduct broader surveys in extended areas to detect the overall epidemiological status of neosporosis in rodent population's particularly in wild rats.

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Ethics Statement

The present protocol was reviewed and approved by the guideline of Animal Welfare Committee of Shahid Chamran University of Ahvaz. I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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