

Original article

***Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Ahvaz feral cats, Iran**

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Abstract

Introduction and objective: *Toxoplasma gondii* and *Neospora caninum* are two closely related cyst forming apicomplexean protozoa that infect a wide range of warm-blooded animals. *N. caninum* has similar morphologic and biologic characteristics to *T. gondii* and until 1988 was misdiagnosed as *T. gondii*. Tachyzoites and tissue cysts of both parasites are found intracellularly in the intermediate hosts. The aim of this study was to determine the seroprevalence rate of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies in feral cats (*Felis catus*) in Ahvaz, Iran.

Materials and methods: Antibodies to *N. caninum* and *T. gondii* were determined in serum samples of 100 feral cats in Ahvaz, Khuzestan province, Iran. IgG antibodies were assayed by the modified agglutination test using whole tachyzoites of *T. gondii* and *N. caninum*, incorporating 2-mercaptoethanol, modified agglutination test and *Neospora* agglutination test, for *T. gondii* and *N. caninum*, respectively.

Results: Anti-*T. gondii* antibodies were found in 54(54%) of 100 cats but anti-*N. caninum* antibodies were detected in 19(19%) of 100 cats. There was no difference between the presence of antibodies for both parasites in male and female cats ($P>0.05$), but occurrence of antibodies was significantly increased with age for both parasites ($P<0.05$).

Conclusion: Because of high occurrence of anti- *T. gondii* antibodies in cats in this study, cats may play a serious role in human and other mammalian toxoplasmosis in Ahvaz.

Significance and impact of the study: This study was the first considering survey *T. gondii* and *N. caninum* simultaneously in cats in Iran and revealed the importance of cats in prevalence of these two parasites.

Keywords: *Neospora caninum*; *Toxoplasma gondii*; Feral cat

Introduction

Toxoplasma gondii and *Neospora caninum* are two closely related intracellular apicomplexan protozoan parasites belonging to the family *sarcocystidae* [1]. Both parasites are distributed worldwide, have a two stage life cycle including asexual in intermediate hosts and sexual stage, producing oocysts, and can induce serious fetal mortality in sheep and cattle [2-5]. *T. gondii* is also an important human pathogen [6]. Felids have an important role in the epidemiology of *T. gondii* because they are the only hosts excreting the oocysts [6]. Unlike *T. gondii*, canids are definitive hosts for *N. caninum* and oocysts of this parasite has not been demonstrated or isolated from cats [7].

Neospora caninum has similar morphologic and biologic characteristics to *T. gondii* and was first described in the 1980s [8], and now researchers showed that many warm-blooded animals including cat have antibodies against this parasite [9-13]. In animals, both parasites may be transmitted to intermediate hosts following ingestion of oocysts via contaminated food or water or the parasites may be passed vertically from mother to fetus via the placenta [14]. *T. gondii* is widely prevalent in human and animals in Iran [15-18]. There is not any study about seroprevalence of *N. caninum* in Iran and little is known about *T. gondii* in cats in Khuzestan. So, the objective of the present study was to determine the occurrence of *N. caninum* and *T. gondii* in feral cats in Ahvaz, Khuzestan, Iran.

Materials and methods

Blood samples were collected from carotid vein of 100 trapped feral Persian cats (57 male and 43 female) of the ages ranging between six months to nine years. Animals had not any clear symptom of diseases during sampling. The samples were

centrifuged at 1000g and the supernatants were frozen at -20°C until use. Sera were tested for the presence of *T. gondii* and *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. Modified agglutination test (MAT) and *Neospora* agglutination test (NAT) have been described by Dubey and Desmonts [19] and Romand *et al.*, respectively [20].

The *T. gondii* and *N. caninum* antigens were prepared in the Pasteur institute of Iran and Razi institute of Shiraz, respectively. Sera were started at 1:25 serum dilution for *T. gondii* and 1:40 for *N. caninum*. A titer of 1:25 and higher was considered as *T. gondii* infection in cats [21,22]. A titer of 1:80 and greater was indicative for *N. caninum* infection [9,10]. Sera with in border line result were re-examined. For the above two tests a complete agglutination was considered as positive result. Clear-and cut button-shaped was interpreted as a negative reaction. Logistic regression and chi-square tests were used for statistical analyses. Alpha was 0.05 for all the tests for the correlation.

Results

Antibodies to *T. gondii* in 100 examined cats were detected in 54 (54%) with titers of 1:25 in 17, 1:50 in 9, 1:100 in 7, 1:200 in 6, 1:400 in 4, 1:800 in 4, 1:1600 in 3, 1:3200 in 2, 1:6400 in 1 and 1:12800 in one cat. Antibodies to *N. caninum* also detected in 19 (19%) with titers of 1:80 in 9, 1:160 in 6, 1:320 in 2, 1:640 in one and 1:1280 in one. Only two cats had antibodies for both parasites. The seroprevalence of *T. gondii* was 30(52.63%) in males and 25(58.14%) in females. The seroprevalence of *N. caninum* was 11(19.3%) in males and 8(18.6%) in females. There was no statistical difference between the prevalence

of infection in males and females for both parasites ($P>0.05$). Logistic regression showed that the prevalence rate of seroreactivity increased significantly with age either for *T. gondii* or for *N. caninum* ($P<0.05$).

Discussion

This study aimed to estimate the seroprevalence of *N. caninum* and *T. gondii* in feral cats distributed all over Ahvaz, Iran. In the current study, we used MAT as well as NAT for detecting antibodies against *T. gondii* and *N. caninum*, respectively. Two of serum samples had antibodies for both parasites. Several studies have used the MAT for detecting the antibodies against *T. gondii* as an accurate and easy procedure [6,8]. Moreover, NAT revealed a good sensitivity and specificity in comparison with Indirect Fluorescent Antibody Test (IFAT) [23,20]. Furthermore, as reported by Dubey *et al.* [9], a good correlation was found between NAT and Western blotting at dilutions of 1:80 or higher for screening *N. caninum* positive cats.

The results of this study show a high prevalence of anti-*T. gondii* antibodies (54%) in feral cats in Ahvaz. The overall prevalence of *T. gondii* IgG antibodies (LAT titre $\geq 1:1$) were found in 40 of 100 (40%) of stray cats, with regional variations. Overall 16 of 100 (16%) of stray cats had diagnostically significant antibody titres (LAT $\geq 1:64$). Serological surveys of cats from Spain by Gauss *et al.* [24], found *T. gondii* antibodies in 45% of 220 cats from Barcelona, Spain. In other studies, seroprevalence of *T. gondii* was reported to be 84.7% in Majorca, Spain [13], 32.3% in Mainland, Spain [25] and 47.6% in Hungray [13]. Also Bresciani *et al.* [11] detected 25% seropositivity in domestic cats in Brazil.

Toxoplasmosis is a globally distributed zoonosis that affect fetus in human and

some animals such as sheep and in the immunosuppressed individual [26]. Because of the great importance of *T. gondii* as a causative zoonosis agent, public health organizations, such as the World Health Organization, have repeatedly advised the collection of accurate epidemiological data on this parasite [8]. Toxoplasmosis is also one of the important diseases caused fetus abnormalities in animals, especially in sheep [8]. Cat could excrete the oocysts of *T. gondii* via feces, so this animal has a key role in infecting humans and other animals.

To our knowledge, this is the first study to investigate the occurrence of *N. caninum* antibodies among cats in Iran. Supporting the studies of Dubey *et al.* [9] (11.9%), Ferroglio *et al.* [10] (24.8%) and Millan *et al.* [13] (6.8%), our results confirmed the serological presence of specific antibodies to *N. caninum* in feral cats as an intermediate host (19%) and indicate that populations of feral cats are exposed to *N. caninum* infection. Different prevalence in such studies may be based on the different methods, geographical distribution of the parasites, sampling criteria and cut-off values.

Current data suggest that occurrence of anti-*N. caninum* antibodies in feral cats of Ahvaz is relatively considerable, but similar to most of the studies, lower than that to *T. gondii*. Sorbino *et al.* [27] in 2008 have also suggested that the prevalence of anti-*N. caninum* antibodies in cats are lower than that to *T. gondii*. This suggests that *N. caninum* is less widespread in the environment. Dogs excrete few oocysts when, infected with *N. caninum* in comparison with cats that shed large amounts of oocysts in the environment. On the other hand, most warm-blooded animals are susceptible to *T. gondii* as an intermediate host [14].

In the present study, there was no significant difference between genders

neither for *T. gondii* nor for *N. caninum*. These findings have been also reported by other authors [10,28-30]. However, the age of cats had influence in seroprevalence of infection by both parasites, and these results have been supported by other investigators [25,29]. Clinical neosporosis has not yet been reported in naturally infected cats, but experimental infection in immunocompetent cats and cats given corticosteroids showed lesions similar to those observed in dogs [31].

Cats usually acquire toxoplasmosis by eating tissues of infected animals such as small mammals, rodents or birds [8]. Studies show that toxoplasmosis may have a great role in abortion of ewes in Ahvaz [32], so aborted lambs and afterbirths may also spread *T. gondii* infection to cats. The existence of tissue cysts in the brain and meat of animals is potentially important in infection in human and cats. So, cats have to forbidden reaching these parts of carcasses [33].

Conclusion

The strains of *T. gondii* in the present study are left unknown and the most infections in cats are asymptomatic, therefore, *T. gondii* infection in cats is important clinically. It is important for public because of shedding oocysts in the environment. Based on the relatively high occurrence of this parasite in cats in Ahvaz, we have to consider it as a major risk factor for acquired toxoplasmosis in humans and animals of this area. On the other hands, this study revealed that cats in Ahvaz are the suitable intermediate hosts for *N. caninum* and may have a role in epidemiology of this parasite in the other hosts.

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Conflict of interest statement: All authors declare that they have no conflict of interest.

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