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Original Article

Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* Infections in Stray Dogs of Hamadan Suburb, West of Iran, 2018

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Abstract

Background: Dogs, especially stray dogs, act as the major carriers of different infectious and parasitic agents in the environment; hence, their possible transmission to humans is a public health concern. The principal aim of the present investigation was to evaluate the seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infections in stray dogs of rural regions of Hamadan, West of Iran.

Methods: During this cross-sectional survey in 2018, 180 blood samples were evaluated for the presence of antibodies to *N. caninum* and *T. gondii* using enzyme-linked immunosorbent assay (ELISA).

Results: Seroprevalence of *N. caninum* and *T. gondii* infections was detected to be 5% (95% CI: 2.8-8.2%) and 35% (95% CI: 28.1-41.9%), respectively. In addition, coinfection was detected in 2.8% of animals. No significant differences were found between infection rate, sex, and age of animals regarding both parasites ($P>0.05$).

Conclusions: This study provides the first insight into the infection of dogs in a region with the prevalence of *N. caninum* and *T. gondii*.

Keywords: *Neospora caninum*, *Toxoplasma gondii*, Serology, Dogs, Coinfection

Background

Dogs exposed to zoonotic parasites (e.g., *Leishmania* spp., *Toxoplasma gondii*, *Toxocara* spp., *Echinococcus granulosus* sensu lato) represent a threat to public health (1). Moreover, some dog-associated pathogens (e.g. *Neospora caninum* and *Taenia* spp.) may frequently infect livestock and cause economic losses due to fatality, abortions, and spoilage of meat and other edible tissues (2).

Neospora caninum (Apicomplexa: Sarcocystidae) is an intracellular heterogeneous parasite with cosmopolitan distribution (3). Canines (domestic dogs, coyotes, dingoes, and gray wolves) and a wide range of herbivore animals are definitive and intermediate hosts, respectively (3). In most cases, dogs are without clinical signs of neosporosis; however, the common signs in dogs are neuropathic disorders dependent on the site parasitized ranging from paresis to paralysis, muscle flaccidity, muscle atrophy, and heart failure (4). Stray dogs play an important role in the transmission of diseases to herbivore animals. They especially cause abortions in cattle. Therefore, knowledge on the prevalence and risk factors of neosporosis in dogs is very valuable for the development and implementation of control programs in livestock of the region (5). Moreover, evaluation of the infection rate in definitive hosts and analysis of the risks in different regions is of further value (5).

Toxoplasma gondii, another important zoonotic parasite, that infects a wide range of warm- and cold-blooded animals, has a global prevalence (6). Approximately, one-third of general human populations are seropositive for toxoplasmosis (7). Dogs rarely suffer from toxoplasmosis as a primary disease; however, neurological disorders are the predominant indications in ill dogs, with signs of seizures, cranial nerve deficits, tremors, ataxia, paralysis, encephalomyelitis, choroidoretinitis, optic nerve neuritis, hepatitis, keratoconjunctivitis, and orchitis (8). *T. gondii* does not undergo the sexual reproductive cycle in dogs, and the animals are not consumed as food widely, but they can be involved in the mechanical transmission of *T. gondii* to humans (9).

Several laboratory methods including bioassay, histopathology, immuno-serology, and molecular procedures are applied for the detection of neosporosis and toxoplasmosis in animals from which, serological

techniques are adequate and suitable methods for epidemiological works. Among the serological methods, the enzyme-linked immunosorbent assay (ELISA) has the highest sensitivity and specificity for the detection of *N. caninum* and *T. gondii* infections (3,9).

Objectives

This study aimed to investigate the present status of infection of stray dogs with *N. caninum* and *T. gondii* in Hamadan region using the ELISA technique.

Methods

Sample Collection

For this cross-sectional study between June 2018 and November 2018, a total of 180 stray dogs were randomly selected from different rural regions of Hamadan, West of Iran (10). Dogs underwent a thorough clinical examination by a veterinarian and information such as sex and age of animals were recorded. Afterward, 3 mL of whole blood was taken from the saphenous vein of animals. The sera were separated by centrifugation at 1000 ×g for 10 minutes and finally stored at -20°C until laboratory evaluation.

Serology

Examination of samples for the detection of antibodies against *N. caninum* and *T. gondii* was performed using commercial ELISA kits (ID Screen® Neosporosis and Toxoplasmosis indirect ELISA, ID-Vet, France). According to the instructions of the manufacturer, seropositive animals were determined by calculating the S/P% (≥50% was considered positive):

S/P% = (OD of sample-OD of negative control)/ (OD of positive control-OD of negative control) ×100

Statistical Evaluation

Statistical analysis was performed using chi-square test with a confidence interval (CI) of 95% (SPSS 16.0, SPSS Inc., Chicago, IL, USA). *P* value less than 0.05 was considered significant.

Results

Based on the serological examination of 180 sera samples, the seroprevalence rates were detected 5% (95% CI: 2.8-8.2%) for *N. caninum,* and 35% (95% CI: 28.1-41.9%) for *T. gondii* infection. Co-infection with *N. caninum* and *T. gondii* was found 2.8%. In addition, the seropositivity of *N. caninum* and *T. gondii* was 7.8% and 31.3% in male animals, 3.5% and 37.1% in females, 3.2% and 33.3% in <1 year old, 1.8% and 32.1% in 1-2 years old, and 9.8% and 39.3% in >2 years old age groups, respectively. There were no statistically significant correlations between infection rates, sex, and age of animals (Table 1).

Discussion

Dogs as human companions may pose risks for human health. The possibility of an asymptomatic carrier state in dogs with some zoonotic infections is one of such risks. Dogs play an imperative role in the maintenance of different infectious and parasitic agents in the environment, and their possible transmission to humans is a public health concern (1).

Neospora caninum infection in dogs varies from 1%-100% throughout the world (11) and 0-54.6% in different regions of Iran, regarding many types of animals, samples, and diagnostic methods (12,13). This rate was also reported 28%-46% and 12.4%-27% in Center and North-West of Iran, respectively (14-16). Results similar to our seroprevalence rate (5%) was detected in previous studies from Australia, Austria, and Peru (3,11). Age plays a role in animals' seropositivity since dogs commonly acquire infection in the postnatal period through horizontal transmission pathway (5). In this work, the highest seropositivity was detected in animals over 2 years old (9.8%). In line with our findings, the effect of age in seropositivity has been reported in other researches (14-17). The high infection in older animals may be attributed to the probability of more exposure to *N. caninum* infection, as well as postnatal infection (18). In agreement with Sager et al and Sharifdini et al (19,20), no significant difference was observed in seropositivity

Table 1. Seroprevalence of *Neospora caninum*, and *Toxoplasma gondii* Infections in Stray Dogs According to Different Gender and Age Groups from Hamadan

Animals (n=180)	Gender		Age Groups (y)			Total
	Male	Female	<1	1-2	>2	
No. of samples (%)	64(35.6)	116(64.4)	63(35)	56(31.1)	61(33.9)	180(100)
Nc-infection (%)	5(7.8)[a]	4(3.5)	2(3.2)	1(1.8)	6(9.8)[b]	9(5±3.2)[ci]
Tg-infection (%)	20(31.3)	43(37.1)[c]	21(33.3)	18(32.1)	24(39.3)[d]	63(35±6.9)[ci]
Nc+Tg co-infection (%)	2(3.1)[e]	3(2.6)	1(1.6)	0(0)	4(6.5)[f]	5(2.8±2.4)[ci]

Note. Nc: *Neospora caninum*; Tg: *Toxoplasma gondii*; ci: 95% confidence interval

[a] χ^2=1.653, *P*=0.198; [b] χ^2=0.197, *P*=0.905; [c] χ^2=0.613, *P*=0.433; [d] χ^2=0.598, *P*=0.741; [e] χ^2=0.044, *P*=0.833; [f] χ^2=0.164, *P*=0.920.

between males and females in this study. However, such result was not in line with that of Goździk et al (21). Some studies reported the highest infection rate in male dogs (15,16,20). *N. caninum* antibodies increased markedly during gestation in a pregnant bitch, suggesting that there is parasitic recrudescence during pregnancy (3).

According to the report of Dubey et al, the high risk of infection was seen in farm dogs with a close contact with seropositive animals and secreted materials (3). These factors are: the possibility of consumption of cattle fetuses or placentas infected with tachyzoites or cysts of *N. caninum*, materials of aborted fetuses, or uterine discharge of dogs living in the neighborhood of farms (3). Dogs' commuting between farms and rural areas play an important role in spreading *Neospora* infection owing to a direct contact with wild and other domestic animals (4).

The seroprevalence rate of *T. gondii* in dogs was reported 19.6%-91% in the global scale (6). In addition, this rate was detected 10.1% in Sarab county, East-Azerbaijan province, Iran (22), 10.7% in West region (23), 20% in Ahvaz (24), 22.4% in Tehran (25), and 26.8% in Chaharmahal and Bakhtiari, Isfahan, and Khoozestan provinces (18) of Iran. In the present study, 35% of animals were seropositive for *T. gondii*; this rate was greater than that reported by other Iranian studies (18,22-25), and that of United Kingdom (6.7%) and China (10.8%) (26,27). However, the prevalence rates less than 43.1% and 68% were also detected in Brazil and Senegal, respectively (28,29).

Moreover, in this study, higher seroprevalence was seen in >2 year-old animals, like the result that was observed in the study of Hosseininejad et al (25). High infection is due to a greater chance of exposure to disease agent (18). Furthermore, in the study of Wu et al, akin to our study, no significant correlation was seen between seropositivity and age ($P < 0.05$) (27). High seroprevalence in lower ages may be due to congenital infection; the fetus may have initially acquired *Toxoplasma* infection during pregnancy of female canines (27). In the present research, no significant association was found between *T. gondii* infection and gender, suggesting that gender is not a crucial factor contributing to infection (25,27). In a report from Brazil, there was no age and gender predisposition, despite the prevalence being higher in adult dogs. This observation relates to the longer exposure to the agent (28).

In the study of Hosseininejad and Hosseini, the simultaneous presence of *N. caninum* and *T. gondii* infections was detected in 8.9% of the dogs selected from three Iranian provinces (18). This co-infection rate was 36% ± 9.4% in Senegal (29). Additionally, *N. caninum* and *Leishmania infantum* co-infection was 9.4% in North-West of Iran (20). In the previous studies from Hamadan, *N. caninum* and *T. gondii* infections in stray dogs were reported 52.8% and 24.3%, respectively (23,30). Difference in diagnostic techniques, study design, and experimental strategies, as well as climatic variations are the main causes of varied findings.

The role of *N. caninum* as a zoonotic agent is ambiguous (3,1); however, different profiles of antibodies to *N. caninum* have been found in immunosuppressive humans (3). These findings are of uncertain significance, because neither the parasitic DNA nor the parasite has been confirmed in human tissues. Nevertheless, *N. caninum* has been successfully cultured in human cell lines. A concern for *N. caninum* infection in farm dogs may be a risk factor for the farmers' infection (31). The role of dogs in the transmission of *T. gondii* to humans has been postulated based on serological surveys and observations in that dogs ingest cat feces and often roll in cat feces, as well as other foul-smelling substances (32). The oocysts of *T. gondii* were determined in feces of dogs in Germany (33). Moreover in an experimental study, viable sporulated oocysts were detected in dog feces for up to 2 days after ingestion (34). The close biological association of *N. caninum* with *T. gondii,* as well as accidental ingestion of oocysts are considered as the main evidence (31).

Conclusions

This study at first reported the *N. caninum* and *T. gondii* co-infection in dogs from Hamadan. The role of stray dogs in the maintenance and transmission of infectious diseases cannot be emphasized, since these dogs not only wander the streets in scavenging garbage, drinking pools of water on the streets and possibly hunting natural reservoir of diseases, such as rodents to feed themselves, but they may be exposed to an environment contaminated with infective pathogens.

Ethical Issues

All ethical standards were respected. Samples were taken from the dogs with official permission from and under supervision of Institutional Animal Ethics and Research Committee of Iranian Veterinary Organization (IVO, Iran), Hamadan Office (Certificate No. 32/1397.4.1).

Conflict of Interests

Authors declare no conflict of interests associated with this study.

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