

Seroprevalence of *Neospora caninum* and Coexistence with *Toxoplasma gondii* in Dogs

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SUMMARY: *Neospora caninum* is a protozoal agent causing abortion and infertility problems in dairy cattle. The objective of the present study was to detect the seroprevalence of *N.caninum* in dogs and its co-existence with *Toxoplasma gondii* in some rural and urban regions of Kirikkale province. A total of 121 blood samples were examined with indirect immunofluorescent antibody test to detect the presence of *N.caninum* IgG antibodies. The seropositivity of *N.caninum* was 28.9% in dogs. The seropositivity rate was higher in male dogs than that of females ($p<0.05$). The seropositivity rate of *N.caninum* was 36.5% and 20.7% for pure breed and mongrel dogs, respectively ($p>0.05$). There was no significant difference amongst the age groups and location of the dogs (rural or urban) ($p>0.05$). The *N.caninum* positive serum samples were also examined for the presence of antibodies for *T.gondii* by Sabin-Feldman Dye test in order to identify the possible serological co-infection, 1/16 and higher titres were evaluated as positive. According to test results, 19 out of 35 *N.caninum* seropositive dogs (54.3%) showed *T.gondii* seropositivity.

Key Words: *Neospora caninum*, seroprevalence, *Toxoplasma gondii*, dog

Köpeklerde *Neospora caninum* Seroprevalansı ve *Toxoplasma gondii* ile Beraber Görülme Oranı

ÖZET: *Neospora caninum* süt ineklerinde yavru atımı ve infertilite problemlerine sebep olan bir protozoondur. Bu çalışmada Kirikkale şehir merkezi ile köylerinde yaşayan köpeklerde *N.caninum* seroprevalansını ve seropozitif köpeklerde *T.gondii* varlığını araştırmak amaçlanmıştır. Bu amaçla 121 kan örneği *N.caninum* IgG varlığı yönünden indirekt floresan antikor testi kullanılarak incelenmiştir. Bunun sonucunda köpeklerde *N.caninum* seropozitivitesi %28,9 olarak belirlenmiştir. Seropozitifliğin erkek köpeklerde dişilere oranla daha yüksek olduğu gözlenmiştir ($p<0,05$). Saf ırklarda ve sokak köpeklerinde ise *N.caninum* seropozitifliği %36,5 ve %20,7 olarak tespit edilmiştir ($p>0,05$). Köpeklerin yaşları ve buldukları yerler (Kirikkale şehir merkezi veya köyleri) arasında ise *N.caninum* seropozitifliği bakımından önemli farklılık bulunmamıştır ($p>0,05$). *N.caninum* yönünden seropozitif bulunan köpek serumları Sabin Feldman boya testi kullanılarak *T.gondii* yönünden incelenmiş, 1/16 ve üzeri titreler pozitif kabul edilmiştir. Buna göre 35 *N.caninum* seropozitif köpeğin 19'unda (%54,3) *T.gondii* seropozitifliği belirlenmiştir.

Anahtar Sözcükler: *Neospora caninum*, seroprevalans, *Toxoplasma gondii*, köpek

INTRODUCTION

Neospora caninum, apicomplexan protozoan parasite is recognized as an important cause of abortion and infertility in dairy cattle farms all around the world (10). This parasite was firstly identified in the brain of a dog in 1988 (12). Dogs and coyotes are definitive hosts, while several animal species including cattle are intermediate hosts (10, 16). The most prominent clinical sign in dogs is neuromuscular disorders (4). Although a few clinical neosporosis have been reported as yet

(4-6) there are numerous reports indicating *N.caninum* serostatus of the dogs in several countries (1, 15, 17, 22, 24).

Toxoplasma gondii, etiologic agent of toxoplasmosis both in animals and human, is also an apicomplexan protozoan parasite resembling *N.caninum* on account of structural, antigenic and genetic features. Evenly, paraffin embedded tissue samples which had been previously misdiagnosed as *Toxoplasma gondii* infection in dogs, were re-diagnosed as neosporosis when they were re-evaluated immunohistochemically in a retrospective manner (12, 13, 20). Felids are the only known definitive host for this parasite (11). Clinical toxoplasmosis of dogs occurs mostly after such specific immunosuppressive viral infections such as canine distemper virus infection (20). Also, canine toxoplasmosis could be a sign of the risk of human toxoplasmosis epidemiologically (20).

Makale türü/Article type: **Araştırma / Original Research**

Geliş tarihi/Submission date: 06 Ocak/06 January 2009

Düzeltilme tarihi/Revision date: 09 Şubat/09 February 2009

Kabul tarihi/Accepted date: 02 Mart/02 March 2009

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There are some studies toward to detect *N.caninum* (5, 9) and *T.gondii* (3, 7, 14) seroprevalences of dogs in Turkey. However, none of which is related to co-incidences of these two protozoer infections. The main aim of this study was to detect seroprevalence of *N.caninum* in dogs living rural and urban regions and second aim was to detect *T.gondii* and *N.caninum* co-infection rate among examined dogs.

MATERIALS AND METHODS

Blood sampling: This study was carried out in Kırkkale Province, located in the Central Anatolia, Turkey between March and September 2007. A total of 121 blood samples were collected, 90 of which were in and around cattle farms with infertility and abortion problems in rural regions. Other samples (n: 31) were collected in urban regions randomly. The dogs were examined clinically. Then, blood samples were collected into centrifuge tubes without anticoagulant. The sera were centrifuged and stored at -18 °C until tested.

The age, breed and sex of dogs examined were recorded. The age of the dogs ranged between 1- 10 years. They were mongrel dogs (n: 58) and pure breeds such as Anatolian shepherd dogs (n: 41), German shepherd (n: 7), Pointer (n: 4), Setter (n: 4), Boxer (n: 2), Collie (n: 2), Husky (n: 1), Cocker (n: 1) and Doberman (n: 1). The majority of the dogs were males (n: 81).

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. Initial screening was conducted at 1:16 serum dilution. A positive titre of 1:16 was considered positive. The slides were viewed using Olympus BX50 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 1:512.

Serology for *T.gondii*: Sabin-Feldman Dye test (SFDT) was used to detect the presence of antibodies for *T.gondii* in the serum samples using vital antigen and methylene-blue dyeing at Laboratory of Parasitology in Ankara Refik Saydam National Institute of Hygiene. The SFDT result was regarded as positive if more than 50% of tachyzoites unstained when examined under the light microscope. An antibody titre of 1/16 and over was accepted to be positive to eliminate the cross-reactivity and false positive results.

Statistical analysis: The prevalence was estimated from the ratio of positive results to the total number of dogs. The Chi-Square goodness-of-fit test was performed. The statistical software package SPSS, version 15.0 was used for the analysis.

RESULTS

The seropositivity of *N.caninum* was 28.9% in dogs (35 of 121) in the present study. Data of sampled and seropositive animals are summarized in Table 1. The seropositivity rate was higher in male dogs than that of female dogs (34.6% v. 17.5%) (p<0.05). Although, the seropositivity was slightly higher in rural dogs (30%) than that of urban dogs (25.8%), the difference was not significant (p>0.05). The seropositivity of *N.caninum* was 30.7% and 24.2 % in dogs aged 1 to 5 years and 6 to 10 years, respectively, but the difference was not significant (p>0.05).

Table 1. The serological results of the dogs tested for *N. caninum*

Variable	Dogs (n)	Seropositive dogs (n)	(%)	P-value
Sex				
Males	81	28	34.6	< 0.05
Females	40	7	17.5	
Breed				
Pure breeds^a	63	23	36.5	> 0.05
Mongrels	58	12	20.7	
Localization				
Rural	90	27	30.0	> 0.05
Urban	31	8	25.8	
Age (years)				
1-5	88	27	30.7	> 0.05
6-10	33	8	24.2	

^a Anatolian shepherds (n: 41), German shepherd (n: 7), Pointer (n: 4), Setter (n: 4), Boxer (n: 2), Collie (n: 2), Husky (n: 1), Cocker (n: 1) and Doberman (n: 1).

According to dog breed, the seropositivity rate of *N.caninum* was 36.5 % in pure breed dogs and 20.7% in mongrel dogs. Differences among the dog breed found to be not significant (p>0.05).

The antibody titres of *N.caninum* ranged from 1:16 to 1:512 (35 seropositive dogs with titers of 1:16 in 6, 1:32 in 1, 1:64 in 15, 1:128 in 11 and 1:512 in 2) in the present study. There was no correlation between antibody titers and sex, breed, age or location.

In this study, none of the dogs had neurological signs. In clinical examination, nearly all seropositive dogs seemed to be healthy (88.6%), however, some dogs (11.4%) had nonspecific symptom as dermatitis. Antibody titres ranged from 1:128 (n: 3) to 1:512 (n: 1) in the dogs with dermatitis.

The positive serum samples were also screened for the presence of antibodies for *T.gondii* by SFDT in order to identify possible serological mixinfection. The seropositivity of *T.gondii* detected was at 1:16 titre and higher (54.3%). Table 2 shows some epidemiological data of seropositive dogs with *N.caninum* and *T.gondii*.

Table 2. The data of the dogs seropositive with *N. caninum* and *T. gondii*

Variables	Dogs (n)	Seropositive dogs (n)	(%)	P-value
Sex				
Male	28	14	50	> 0.05
Female	7	5	71.4	
Breed				
Pure breeds ^a	25	14	56	> 0.05
Mongrels	10	5	50	
Localization				> 0.05
Rural	27	14	51.8	
Urban	8	5	62.5	
Age				
1-5 years	28	14	50	> 0.05
6-10 years	7	5	71.4	

^a Anatolian shepherds (n: 19), German shepherd (n: 3), Pointer (n: 1), Boxer (n: 1) and Cocker (n: 1).

DISCUSSION

Infertility and abortions are the main reproductive problems in animal breeding. One of the parasitic agents responsible for abortions in cattle farms is *N. caninum*. Neosporosis caused important economical losses in the world. Seropositivity of neosporosis in dogs was reported in different countries (8, 22, 25). In Turkey, only one clinic neosporosis has been detected in Doberman pinscher pup with paraplegia and progressive pelvic limb hyper-extension as yet (5). Also, seroprevalence of neosporosis in dogs was reported as 10 % in Turkey (9). In present study, the prevalence of *N. caninum* was observed as 28.9% in dogs serologically.

The most important factor of epidemiology of dog neosporosis is to eat or contact with infected tissue from cattle and other intermediate hosts (12). Naturally, this situation is more frequent in rural regions than those in urban areas. Some researchers (1, 15, 24, 25) reported that dogs from rural areas were significantly more seropositive for *N. caninum* than those from the urban area. Hornok *et al.*, (17) reported that seroconversion were common in rural dogs than that of urban dogs. This situation indicated that dogs in the rural area might have been contacted with infected tissue from intermediate hosts. In the present study, the differences between rural and urban dogs were found to be not significant. In urban region, seropositivity was especially detected dogs living in garden. It was thought that dogs living in urban could have been eaten some uncooked meat pieces given by the owners.

Some researcher suggested that the male dogs were predisposed to neosporosis (18). However, some suggested that the same is true for female dogs (26). The others reported no significant difference in seropositivity between the males and the females (15, 24, 25). 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.*, (25) reported that the significant differences in dog breed seropositivity with *N. caninum*. However, significant differences were not observed among the breed of dogs in the present study ($p > 0.05$). Anatolian shepherd dogs and mongrel dogs are more common in livestock farms in Turkey. 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 could be playing an important role for epidemiology of disease among the hunting dogs.

In present study, the seroprevalences of neosporosis and coinfection with toxoplasmosis in rural dog living in and around cattle farms with infertility and abortion problems were 30% and 51.8%, respectively. We also screened the sera samples of cattle living these farms with seropositivity dogs in Turkey serologically with respect to neosporosis and toxoplasmosis in another study (unpublished). Also, a clinic neosporosis in a calf was seen in one of the sampled cattle farms (19).

The seropositivity of neosporosis increased slightly with age in some study (25). In the present study, significant difference was not detected amongst the age groups ($p > 0.05$).

The seroprevalence of *T. gondii* in dog population is quite variable depending on the methods and the geographic areas. The seroprevalence of toxoplasmosis in dog reported as from 11.7 to 85% in different region of Turkey (3, 7, 14). The Sabin–Feldman dye test is highly sensitive and specific for serology of *T. gondii* (11). However, the test is made only in Reference Laboratories due to obligation for live tachyzoites (23). Some researchers determined the seroprevalence of *N. caninum* and *T. gondii* in same dogs as 4.9 - 10.6% (2, 21). The seropositivity of *T. gondii* was detected as 54.3% in positive serum samples due to *N. caninum* in this study with SFDT.

Toxoplasma gondii seropositivity was related to the age of dogs and living with cats in the home (2). In rural areas of Turkey, dogs and cats live together. Naturally, dogs can easily contact with cat faeces possibly including *T. gondii* oocysts in the environment. In urban areas, stray cats are play an important role in the epidemiology of toxoplasmosis beside some uncooked meat pieces might also been eaten by dogs living in home. In our study, toxoplasmosis seropositivity was nearly equal in rural and urban dogs (51.8% v 62.5%).

In conclusion, the seropositivity rate of *N. caninum* was as 28.9% in dogs in Turkey. Nearly all seropositive dogs were seemed healthy. These clinically healthy but serologically positive dogs are important risk in cattle farm. Especially high antibody titres of *N. caninum* were detected in dogs with dermatitis in our study; there was attention to dogs with dermatitis symptoms in cattle farms with infertility and abort problems.

ACKNOWLEDGEMENTS

This work was partly supported by a grant from the Kırıkkale University Research Fund (KU BAP, No: 2006/24).

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