Seroprevalance of Canine Brucellosis and Toxoplasmosis in Female and Male Dogs and Relationship to Various Factors as Parity, Abortion and Pyometra

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ABSTRACT
The objective of this study was to determine the seroprevalance of canine brucellosis and toxoplasmosis in female and male dogs and also determine the relationship to various factors as parity, abortion and pyometra. Brucella canis is a disease of the reproductive tract that may cause late abortion, infertility and fail of conception with optimum insemination time in females and infection of the sexual organs in males. Toxoplasma gondii is an important obligate intracellular protozoan parasite which can affect all warm-blooded mammals and humans which may cause fatal diseases with severe problems, such as abortion. As a result, in this study B. canis was determined in low seroprevalence in some cases on the island (North Cyprus), T. gondii was determined as an important contagious parasite. Also reproductive parameters like parity, spaying, cyclicity could be important too and it was presented that extended evaluation of these factors is needed with further studies.

Key words: Brucella, Dogs, Seroprevalance, Toxoplasma.

INTRODUCTION
Canine Brucellosis caused by Brucella canis is a disease of the reproductive tract that may cause late abortus, infertility and fail of conception with optimum insemination time in females and infection of the sexual organs in males (Wanke, 2004; Castrillon-Salazar et al., 2013; Yuksekayak, 2013). The period between infection and reproductive signs are variable; abortions occur mostly at approximately 7-9 weeks of gestation. Early embryonic deaths have also been reported two to three weeks after venereal transmission. Congenitally infected pups can be born normal and later develop brucellosis. A year after infection dogs with brucellosis may recover spontaneously, but recovery is more common after two to three years, and some dogs remain chronically infected for years. Chronically infected dogs can shed this organism in normal vaginal secretions, urine, aborted fetus and placenta and semen despite being seronegative and blood culture negative. Low concentration of bacteria may be excreted in saline, milk and nasal secretions. Uninfected dogs living with infected animals of the same sex were found to acquire the infection within 6 months (Wanke, 2004; Makloski, 2011; Kaden et al., 2014; Castillo et al., 2014; Kang et al., 2014). The Microplate Agglutination Test (MAT), Rapid Slide Agglutination Test (RSAT) and the Tube Agglutination Test (TAT) are often used to detect antibodies to B. canis in dogs (Thakur et al., 2003; Tuemmers et al., 2013). In MAT, titers above 1:160 are accepted as seropositive and titers below 1:160 are considered as cross reaction or previously infected.

Toxoplasma gondii is an important obligate intracellular protozoan parasite which can affect all warm-blooded mammals including humans which may cause fatal diseases with severe problems, such as abortion. The clinical symptoms of toxoplasmosis in dogs are generally characterized by respiratory disorders, diarrhea and ataxia. Abortion from Toxoplasma gondii has been reported following maternal signs of emaciation, lymphadenopathy, dyspnea, lethargy, diarrhea and central nervous system disturbance. Alimentary and congenitaly infections were reported (Elshiekhka, 2008; Paula de Dreer et al., 2013). Clinical signs cannot be observed in all Toxoplasma gondii infections, so laboratory methods are required to confirm the diagnosis (Pretzer, 2008). There are a number of serologic tests used for Toxoplasma diagnosis such as the Sabin-Feldman dye test (DT), Complement Fixation (CF), Indirect Hemagglutination Assay (IHA), Enzyme Linked Immunosorbert Assay (ELISA), Indirect Fluorescence Antibody Test (IFAT) and the Latex Agglutination Test (LAT). Among these DT is a specific and sensitive for detection of T. gondii and titers of 1:16 and above were considered as positive for T. gondii (Balkaya et al., 2010; Gicik et al., 2010; Paula de Dreer et al., 2013, Souvik et al., 2013).

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The aim of this study was to investigate the seroprevalence of *Brucella canis* and *Toxoplasma gondii* in dogs in North Cyprus, and to evaluate the rate of canine brucellosis and toxoplasmosis in females and males. In this study, also the relations between clinical manifestations such as abortions, pyometra, number of parity and the presence of the causative agents *Brucella canis* and *Toxoplasma gondii* were evaluated.

**MATERIALS AND METHODS**

This study was conducted in three big regions of North Cyprus (TRNC) in 2014 and 2015 (Nicosia, Famagusta, and Kyrenia). A total of 101 dogs of different breeds including 12 stray dogs, aged 2-10 years and fed with commercial dog food or leftovers were included in the study. There were 68 females and 33 males. To determine the seroprevalence of *Toxoplasma gondii* and *Brucella canis*, blood samples were collected and separated sera were stored at -20 °C until use for serological tests. All tests were carried out at Public Health Institution of Turkey in Ankara.

All sera were examined for *Toxoplasma gondii* antibodies using the Sabin Feldman dye test (DT) as described (Sabin and Feldman, 1948). Healthy 3-4 weeks aged white Swiss albino mice were used for the preparation of test antigen. *T. gondii* RH antigen was derived from the peritoneal fluid of these mice 48 hours after injecting them with a virulent strain. As an activator serum, human serum (known to be seronegative for *T. gondii*) was used. *T. gondii* stained tachyzoites were examined under light microscope with 40X objective. If more than 50 % of tachyzoites on one microscopic field were not stained, this dilution step was accepted as positive. Titters of 1:16 and above were considered as positive.

Microplate agglutination test (MAT) was performed by using the antigen prepared as described by Kimura et al. (2008) for detection of *Brucella canis*. The titers were expressed as a reciprocal of the highest dilution of sera showing agglutination. Positive and negative controls were used in each series of test runs. The plates were read by a microtiter mirror. Titters of 1:160 and above were considered as positive. A titer of 1:40 were considered as cross reaction or previously infected and evaluated as negative.

Information of the dogs like age, spaying, regular cycle, parity, history of pyometra and abortion were recorded for the study.

All statistical analyses were performed using SPSS® (Version 14.0 for Windows, SPSS Inc., Chicago, IL, USA). Chi-square test was used to determine the difference between the groups, when sample size was small Fisher’s exact-test was used. Bivariate correlation was used to measure the strength of the relationship between the groups. Pearson’s Correlation Coefficients was calculated to measure the strength and the direction of the relationship.

**RESULTS AND DISCUSSION**

In the present study, a total of 101 dogs (68 females and 33 males) from TRNC were examined by SF dye test and 25 dogs (24.75%) were found seropositive for *T. Gondii*. Out of these seropositive dogs, 14 were female and 11 were male. Seroprevalence varied in different age groups and also 3 of 12 (25%) stray dogs were found *T. gondii* positive. The investigation also showed that the prevalence was higher in male animals (11/33 = 33.33%) than in female (14/68 = 20.58%) animals (P<0.05) (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Seroprevalence of <em>Toxoplasma gondii</em> and <em>Brucella canis</em> infections in dogs in relation to age, sex, reproductive problems and living status of the dogs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxoplasma (n=101)</strong></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Castration</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>&lt;5</td>
</tr>
<tr>
<td>Regular Cycle</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>Reproductive Problem</td>
</tr>
<tr>
<td>Pyometra</td>
</tr>
<tr>
<td>Living Status</td>
</tr>
<tr>
<td>Household</td>
</tr>
</tbody>
</table>

aP<0.05
In MAT, the investigation showed that only 1 of the male animals (3.03%, 1/33) was infected (titer=1:160), and 0 (0.00%, 0/68) of female animals. Cross reaction (titer 1:40) was found in 4 (12.12%, 4/12) of male animals and 12 (17.64%, 12/68) of females.

Eight of the seropositive 14 dogs for *T. gondii* (57.14%) did not gave birth before and 6 dogs (42.85%) were found with 1 and more parity. In the clinical history, abortion was found in 3 females and 2 of them were found to be seropositive for *T. gondii* and 1 of them was seronegative (Table 1-2). A strong negative correlation was determined \( r = -0.934; \) \( P < 0.06 \) between parity and seropositivity in *T. gondii* when all titers was evaluated together (Table 2).

### Table 2: Relation between parity and titer in *T. gondii* and *B. canis* seropositive dogs.

<table>
<thead>
<tr>
<th>Parity</th>
<th><em>Toxoplasma gondii</em> (+)</th>
<th><em>Brucella canis</em> (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

Correlation between parity and *T. gondii* (All titers evaluated together) \( R = -0.934; P = 0.06 \)

By the anamnesis pyometra has been determined in 1 *T. gondii* seropositive and 1 *T. gondii* seronegative dogs. Also it has been found in 3 of the *B. canis* seronegative dogs were aborted and pyometra was developed in 2 of them. No reproductive disorders were developed in the dogs which were *T. gondii* and *B. canis* seropositive at the same time. When we compared the *T. gondii* seropositive dogs according to the castration status, 21 dogs (24.42%) were found to be non-castrated and 4 dogs (26.67%) were found to be castrated \( (P > 0.05) \). Also *T. gondii* seropositive dogs had regular sexual cycle with the ration of 20.84% and 20.00% of the dogs had irregular cycle \( (P < 0.05) \) (Table 1).

Most important clinical case due to *Brucella canis* infections is abortus (Makloski, 2011; Hofer et al., 2012). Infected animals shed infection by vaginal secretions, urine, aborted fetus, placenta, semen and rarely by saliva, milk and nasal secretions (Johnson and Walker, 1992; Wanke, 2004; Hofer et al., 2012). Toxoplasmosis is very important for the public health due to the contamination risk during the pregnancy. The infection can be acquired by alimentary intake of tissue cysts in infected meat or food and water contaminated with oocysts (Paula de Dreer et al., 2013). Also dogs play a role in the transmission of oocysts to humans (Lindsay et al., 1997). The oocysts can contaminate the environment mechanically or by oocyte transporting dogs (Jittapalapong et al., 2007). Spontaneous abortion and fetal death have been observed in dogs infected with toxoplasma oocysts or tachyzoites (Bresciai et al., 2007; Paula de Dreer et al., 2013).

The represented study is aimed to determine the incidence of these two infectious agents which are important both for fetal death, infertility and public health North Cyprus as well as interaction between parity, pyometra, cyclicity etc. in this study.

There are no seropositive female dogs but only 1 male dog (3.03%) determined as seropositive which were evaluated at e” 1:160 titer for *B. canis*. MAT was used for the serological analysis of *B. canis* in this study. d” 1:50 titer is considered as noninfected negative and e” 1:200 titer is considered as active infection for *B. canis* in serological controls with Rapid Slide Agglutinasyon Test (RSAT) (Fredrickson and Barton, 1974; Flores-Castro and Carmichael, 1978, Higgins et al., 1979; Castillo et al., 2014). Titors 1:50 are not considered as positive and some authors have mentioned that cross-reactions between *B. canis* and other microorganisms (Bordetella bronchiseptica, Pasteurella multocida and Moraxella spp.) could be responsible for such low titers (Carmichael and Kenney, 1970; Hoff et al., 1974). Abort in 3 dogs and pyometra in 2 dogs were determined by investigation of the records in *B. canis* seronegative dogs. On the other hand there was not an important relation between castrated and non castrated dogs, cyclicity in females and *B. canis* titer. When considering all titers it was presented there was no relation between age and infection. Also this was shown in different studies (Gicik et al., 2010; Bigdeli et al., 2011).

Pyometra is influenced by different factors like age, administration of exogenous progesterone, mating etc. (Smith, 2006; Pretzer, 2008). Although it is known *B. canis* can cause abortion, also noninfectious agents, bacterial agents like Campylobacter spp., Salmonella spp., *E. coli*, *L. monocytogenes*, Mycoplasma spp. and protozoal agents as Toxoplasma gondii, Neospora caninum can cause abortions in dogs (Pretzer, 2008).

Dahlbom et al. (2009) reported seroprevalence of *B. canis* was 0.0% in Finnish kennel dogs. On the other hand Ucan et al. (2010) determined the seroprevalence of *B. canis* between 21.5% and 25.2% in a study comparing different tests. Seroprevalence of *B. canis* varies depending on the geographical conditions and housing of the dogs together with others (Bigdeli et al., 2011). No positive seroprevalence of *B. canis* infection in female dogs (0.00%) is thought to be a reason of being an island of North Cyprus and hence limited dog entrance.

In this study toxoplasma seropositive dogs were found 24.75% of all dogs, 20.58% of female dogs and 33.33% of male dogs were determined *T. gondii* seropositive by serological tests. It was reported ratio of toxoplasma seropositive dogs to all dogs was found 76.4% in Brasil (Canon-Franco et al., 2004), 46.3% in South Korea (Lee et al., 2008), 13.30% in China (Li et al., 2016) and 46-97.5%
in different cities of Turkey in stray dogs (Babür et al., 1997; Aktaş et al., 1998; Sevinc et al., 2000; Eren et al., 2002; Babür et al., 2007). This ratio was similar to the findings of studies reporting 10.81-17.50% seropositivity in pet dogs (Zhang et al., 2010; Wu et al., 2011). T. gondii seroprevalence is affected by ecological and geographical factors, as well as feeding and animal welfare conditions for dogs (Wu et al., 2011).

T. gondii seroprevalence percentage was found higher in male dogs (33.3%) than females (20.58%) in this study (P>0.05). Wu et al. (2011) reported higher prevalence in male dogs (12.5%) than females (8.9%) but not statistically important. Similarly Li et al. (2016) reported 13.00% in females and 13.40 in males.

When parity relation between parity and T. gondii was evaluated an interesting result was found. When parity decreased a decrease was observed in the number of dogs infected by T. gondii. The correlation -0.934 (P=0.06) supports this finding. This situation shows a resistance against T. gondii can develop by the increased parity.

Studies showed that amniotic concentrations of IgG is significantly (P < 0.05) higher in samples collected from multiparous compared with primiparous bitches (Dall’Ara et al., 2014) and humans (Gelber et al., 2007). According to these results infection risk by these types of agents could decrease due to the development of defense system in multiparous bitches.

There were no differences between the seroprevalence positive dogs with and without pyometra/abortion, and castrated (26.67%) and non castrated in dogs (24.42%) with seropositivity for toxoplasma gondii infections (P>0.05). While Dubey et al. (2008) determined a significant difference between castrated and non castrated dogs (P<0.01), they could not present a significant difference between the dogs infected with another agent and non-infected dogs.

In this study while B. canis was determined in low seroprevalence in some cases on the island (North Cyprus), T. gondii was determined as an important contagious parasite. Also reproductive parameters like parity, spaying, cyclicity could be important too and it was presented that extended evaluation of these factors is needed with further studies.

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REFERENCES


