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Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* Infection in Stray Dogs from Sanliurfa in Turkey

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Abstract: The aim of this study was to determine the prevalence of *Dirofilaria immitis* antigen, *Ehrlichia canis* and *Borrelia burgdorferi* antibodies using an Enzyme Linked Immunosorbent Assay (ELISA) (Snap_3Dx test; IDEXX Laboratories, Westbrook, Maine, USA). This study was performed on total of 50 mixed-breed dogs (25 female and 25 male). Each dog was examined clinically, microscopic examination of stained smear. *Dirofilaria immitis* antigen infection was found in a dog (2%) and *E. canis* antibodies were present in 31 dogs (62%). None of the tested dogs (50) was positive for *B. burgdorferi* antibodies.

Key words: Dirofilariosis, ehrlichiosis, borreliosis, serology, diagnostic test

INTRODUCTION

Dirofilariasis, Ehrlichiosis and Lyme borreliosis are arthropod-borne diseases that can cause patent infections in companion and wild animals and occasionally can infect humans (Bowman *et al*., 2009; Labarthe *et al*., 2003; Pantchev *et al*., 2009).

The etiologic agent, *Dirofilaria immitis* is one of the most pathogenic nematode parasites in dogs (Bolio-Gonzalez *et al*., 2007) and in other species such as cats, foxes, bears, wolves and horses and rarely in humans (Saritas *et al*., 2005). Dirofilariosis is a parasitic disease transmitted by a mosquito (Aedes, Anopheles and Culex) and can cause cardiopulmonary disease. Environmental factors like temperature, mosquitoes plays an important role in the spread of Dirofilariosis in dogs. *D. immitis* occurs in dog, cat, fox and wolf in tropics and subtropics and in some temperature countries (Soulsby, 1986). In recent years, several epidemiological surveys of this disease have been performed in many countries. The parasite is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries (Oncel and Vural, 2005; Bolio-Gonzalez *et al*., 2007; Meriem-Hind and Mohamed, 2009; Song *et al*., 2003). Turkey is suitable country for development of this parasite due to climatic conditions and abundant intermediate hosts. Surveys of dogs from various parts of the Turkey have found from 0.5-46.2% of the animals are infected with *D. immitis* (Voyvoda *et al*., 1996; Yildirim *et al*., 2007; Yildiz *et al*., 2008; Icen *et al*., 2011).

Diagnosis of Dirofilariosis (heartworm) in companion animals is mainly performed by modified knott's technique, the microfilarial density test, rays or ultrasound and commercial serological tests such as Snap[R], Idexx, DiroCHEK[R], Synbiotics USA and Witness[R], Agen, Australia (Courtney and Zeng, 2001; Hoff *et al*., 2008; Tasic *et al*., 2008; Icen *et al*., 2011). Moreover, with recent advances in serology the potential now exist for veterinarians to use these tests in the diagnosis of heartworm disease in dogs and cats. Heartworm assays today also allow semiquantification of heartworm infection allowing the practitioner to better plan adulticide therapy (Tzipory *et al*., 2010).

Ehrlichia canis is considered to be the major agent causing canine ehrlichiosis. It is the causative agent of Canine Monocytic Ehrlichiosis (CME) in dogs which is endemic in the west region of Turkey. *E. canis* frequently often the worldwide (Breitschwerdt *et al*., 1998). Several studies have been carried out in the West part of Turkey, seroprevalence were 21% (Batmaz *et al*., 2001; Pasa and Azizoglu, 2003; Icen *et al*., 2011). This seroprevalence varied depending on the location and the dog population. Clinical diagnosis may be confirmed by demonstrating the organisms within white blood cell, seen in intracytoplasmic inclusion bodies called morulae (Rodgers *et al*., 1989; Alleman, 2005; Rodriguez-Vivas *et al*., 2005; Scorpio *et al*., 2008). More commonly, a diagnosis is made by a combination of clinical signs, positive indirect serum fluorescent antibody titer and response to treatment (Macieira *et al*., 2005;

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Rungsipipat *et al*., 2009). The serological detection of anti *E. canis* antibodies may be performed by Indirect Fluorescent Antibody Test (IFAT), Immunoblotting (IB) or Dot-ELISA. Improvements in molecular biology techniques have led to the development of DNA detection of *E. canis* (Belanger *et al*., 2002; Harrus *et al*., 2002; Nakaghi *et al*., 2008).

Lyme borreliosis is a bacterial disease caused by infection with the spirochete *Borrelia burgdorferi* in dogs, horse, cattle, sheep and humans (Gulanber *et al*., 2007; Rodgers *et al*., 1989). Hard-shelled ticks of the genus *Ixodes*, transmit *B. burgdorferi* by attaching and feeding on various mammalian, avian and reptilian hosts. Studies with dogs kept as pets in endemic areas have shown that approximately 5% of all infected dogs become ill. However, under experimental conditions, up to 75% of infected animals develop clinically apparent Lyme arthritis (Straubinger, 2000). There are no specific clinical, hematological or biochemical pathognomonic changes that would confirm the diagnosis of Lyme borreliosis. Therefore, additional tests such as antibody and organism detection, need to be considered in order to produce a specific diagnosis. ELISA, Immunoblotting or Western blotting improves, an indirect Immunofluorescence Assay (IFA) with whole celi preparations or single recombinant antigens are useful for detecting antibody responses to infection (Bowman *et al*., 2009; Liang *et al*., 2000). Lyme disease has been reported in many countries of Europe, America and Asia including in Turkey (Bhide *et al*., 2008; Guner *et al*., 2003; Gil *et al*., 2005; Labarthe *et al*., 2003; Wickle *et al*., 2006; Wright *et al*., 1997).

An ELISA test (SNAP 3Dx, IDEXX Laboratories) had been developed for simultaneous diagnosis of *D. immitis* antigen and *E. canis* and *B. burgdorferi* antibodies. This ELISA test uses a synthetic peptide (C_6) derived from Invariable Region (IR_6) as a diagnostic antigen. The test also provides for early diagnosis of *D. immitis* (specificity 100% and sensitivity 67-100%, depending on the number of adult female worms present), *E. canis* specificity 98.2% and highly specific for *B. burgdorferi* (Belanger *et al*., 2002; Labarthe *et al*., 2003; Tzipory *et al*., 2010; Choi *et al*., 2009; Bowman *et al*., 2009).

This study's aim was to investigate the seroprevalence of canine dirofilariosis, ehrlichiosis and Lyme borreliosis with ELISA test (in-office ELISA test kit SNAP 3Dx, IDEXX Laboratories) among stray dogs in Sanliurfa.

MATERIALS AND METHODS

Sample collection: This study was done 50 stray dog (25 female and 25 male) in Sanliurfa in 2012. Total 50 samples were taken from dogs living in dog shelter. Blood samples were obtained by cephalic venipuncture and the samples were collected in a tube containing EDTA as anticoagulant, stored in a cooler box at 4°C and processed within 24 h. Blood parasite examinations were performed using a light microscope for direct detection. A commercially available in-office diagnostic kit (Canine SNAP 3Dx test, IDEXX Laboratories) was used for the detection *D. immitis* antigen, *E. canis* and *B. burgdorferi* antibodies. All samples were also examined clinically and microscopical for *D. immitis*, *E. canis* and *B. burgdorferi*. Results were recorded according to age and sex on the report forms.

ELISA test procedure: The test can be conducted with canine serum, plasma or whole blood. The C_e synthetic peptide was conjugated to Bovine Serum Albumin (BSA) and to Horseradish Peroxidase (HRP) using standard methods. The HRP-C_6 peptide conjugate was contained in a conjugate diluent containing HRP-labeled anti-heartworm antibody, HRP-labeled *E. canis* peptide conjugate, nonspecific proteins and detergent. The *B. burgdorferi* or *E. canis* antibody (if present) in the sample bind to the synthetic peptide-HRP conjugate and to the synthetic peptide-BSA conjugate. Two drops of blood, serum or plasma were dispensed into a sample tube using the pipette provided with the kit. Five drops of conjugate were added to the sample and this mixture was dispensed into a sample well in the test device. The deposited blood sample and conjugate mixture flowed through the matrix of the test device which contained substrata reagents. The C_8 ELISA test was considered positive for *D. immitis*, *E. canis* and *B. burgaorferi*. If color developed in the designated reaction area of the matrix. A positive control area in the device was used to verify that the sample had been properly prepared and that the reagents were adequately reactive (Labarthe *et al*., 2003).

Statistical analysis: In statistical analysis, seropositivity to *D. immitis*, *E. canis* and *B. burgdorferi* in ELISA test was set as an outcome variable and the independent variables were sex, age (1-3, 4-5 years). The data were analyzed using SPSS Version 15 Statistical Software package. Chi-square and one-way Analysis of Variance (ANOVA) tests were used for statistical analysis and $p<0.05$ was considered significant.

RESULTS

One (2%) of the 50 samples tested with antigen detecting ELISA kits showed a positive reaction for *D. immitis* in this study. While only male (4%) dogs were affected, female dogs were not affected as shown in the Table 1. *E. canis* (62%) were positive in 31 samples. More female (72%) than male dogs (52%) dogs were positive.

Table 1: Distribution of parasites

		D. immitis		*E. canis*		*B. burgdorferri*	
Variables	n	Positive dogs	Negative dogs	Positive dogs	Negative dogs	Positive dogs	Negative dogs
Male	25	1 (4%)	24	13 (52%)	12	-	25
Female	25	-	25	18 (72%)	7	-	25
Total	50	1 (2%)	49	31 (62%)	19	-	50
1-3 years	40	1 (2.5%)	39	24 (60%)	16	-	40
4-5 years	10	-	10	7 (70%)	3	-	10
Total	50	1 (2%)	49	31	19	-	50

There was no statistically significant between female and male ($p>0.05$). There was no also a statistically significant between the ages of dogs ($p>0.05$). None of the samples had antibodies reactive for *B. burgdorferri*. Although, researchers were unable to confirm active infection on clinically and microscopic examination.

This study has shown low values for the prevalence of *D. immitis* (2%), higher values for the prevalance of *E. canis* (62%), no seropositive for *B. burgdorferri* in the dog population examined using commercially available in-office diagnostic (SNAP 3Dx, IDEXX) ELIA kit). Most dogs infected with dirofilariosis do not show any signs of disease for as long as 2 years. Unfortunately, by the time clinical signs are seen, the disease is well advanced. The signs of heartworm disease depend on the number of adult worms present, the location of the worms, how long the worms have been present and the degree of damage to the heart, lungs, liver and kidneys from the adult worms and the microfilariae.

DISCUSSION

D. immitis has been reported by many researchers in dogs in Turkey (Voyvoda *et al*., 1996; Borku *et al*., 1996). In the most of the studies was used generally modified Knott's and ELISA techniques in the diagnosis of dirofilariosis among dogs by comparing the results of the survey with those of other studies, Agaoglu for Van 46.2%, Yildiz *et al*. (2008) for Kirikkale 27.46%, Voyvoda *et al*. (1996) for Aydin 13.7%, Yildirim *et al*. (2007) for Kayseri 9.6%, Sahin for Sanliurfa 7.6%, Yildirim for Ankara 6.3%, Kozan for Afyon 3.6%, Coskun for Bursa 2.98% Kozan for Eskisehir 1.4%, Oncel and Vural (2005) for istanbul 1.52% and Icen *et al*. (2011) for Diyarbakir 2.43 were reported. Seroprevalence of present study were higher than Oncel and Vural (2005) but were lower than other studies.

Sanliurfa offers the ideal biotope for the mosquito vector of *D. immitis* (hot weather with suitable temperature; annual average temperature 19.1°C). In this study low prevalence of dirofilariosis in the dogs that live in Sanliurfa can be attributed to less opportunity for exposure to the mosquitoes, due to mosquito control programs employed by municipalities or may be the dogs have been treated for parasitism.

The CME is an infectious disease with a high incidence. *E. canis* can be detected for a short period of time in monocytes but they cannot be found during subclinical and chronic stages of infection. Even so the search for morulae in circulating monocytes is stili the routine diagnostic method for ehrlichiosis (Moreira *et al*., 2005) but in most cases unrewarding. The diagnostic is in some cases, a combination of clinical and hematological signs (Cohn, 2003) but this signs may be confusing and variable (Waner *et al*., 2001). The CME has a worldwide distribution and a significant seroprevalence in dogs accordin to Asia, Africa, Europe, North America and South America (Zhang *et al*., 2008) was reported. The seroprevalence of *E. canis* antibodies is reported considerable in some Middle East country such as Egypt (33%), Israel (30%) and Iran (14.63%), (Botros *et al*., 1995; Baneth *et al*., 1996; Harrus *et al*., 1997; Akhtardanesh *et al*., 2010). The presence of *E. canis* is confirmed in Turkey (Pasa and Azizoglu, 2003; Ulutas *et al*., 2007). Batmaz *et al*. (2001) reported that seroprevalance of *E. canis* 21% were found West of Turkey. In the present study, seroprevalence of CME has been determined 62% in Sanliurfa. The finding was higher than reported seropositivity in Egypt (33%), Israel (30%) and Iran (14.63%), 21% in Western of Turkey. The lower percentage of prevalence of *E. canis* in West of Turkey due to exposure to tick-less dogs living in the city center may be. The higher percentage of prevalence of *E. canis* in this study were obtained. Dog shelters may be a favorable environment for ticks and pesticides parasitological in dog shelters may not be enough for ticks. Thus, veterinarians should be aware that CME seems to be in Southeast of Turkey.

Active infection of dogs seropositive for *E. canis* could not be confirmed in the samples as clinical signs and hematological evaluation. Researchers conclude that they were infected or earlier exposed to *E. canis*. Many researchers already described that serology is the most appropriate test for the diagnosis of *E. canis* natural infection in dogs, especially in the chronic stage when *E. canis* is rare in circulating blood.

Lyme Borreliosis is a commonly diagnosed, vector-borne disease in humans, dogs, cats, horse, cattle and sheep most often caused by infection with *B. burgdorferi* (Bhide *et al*., 2008; Gulanber *et al*., 2007). Not all animals infected with *B. burgdorferi* develop clinical disease. Evidence of clinical disease is low, suspected to be only 5-10% of infected dogs (Alleman, 2005). Seropositivity may be as high as 75% in

endemic areas but this may be the result of cross reactivity of IFA or ELISA with nonpathogenic borrelia or antibodies resulting from earlier vaccination (Straubinger, 2000).

Lyme disease in dogs has been reported in several countries with seropositivity ranging from very low to 53.7% (Bhide *et al*., 2004). Bhide reported that Anti-Borrelia antibodies were found in 93 (23.2%) of the 400 dogs Western Turkey. Gulanber *et al*. (2007) reported clinical case of lyme disease in a Saint Bernard dog. But in this study has not been determined *B. burgdorferi* infection.

Weather is a critical factor in the prevalence of the disease. Transmission depends on the intermediate host which have certain climate requirements. Hot weather and suitable temperatures are necessary for development of mosquitoes and ticks. In this study, the lower prevalence of *D. immitis* in dogs that live in such areas can be attributed to less opportunity for exposure to the mosquitoes, due to mosquito control programs employed by municipalities. However, higher percentage of prevalence of *E. canis* determined because of application of parasitological pesticides for ticks may be inadequate.

CONCLUSION

As a result, this was the first serological survey of Sanliurfa dogs for antigen to *D. immitis*, *E. canis* and *B. burgdorferi*. These results indicate that the positive serology results indicate that those agents are present while Lyme disease is not an important disease in the region but further epidemiologic studies should be performed to determine the distribution of lyme disease. The data will provide veterinarians with an increased awareness of the vector-borne disease agents in their practice areas and elevate their consideration of these infections that veterinarians should pay attention to this disease in their clinical practice and include it within the differential diagnosis and choosing appropriate diagnostic or prophylactic procedures. *D. immitis*, *E. canis* and *B. burgdorferi* can also cause zoonotic disease in humans. For this reason, results of this study are important to public health. In addition, this study was revealed the presence of *D. immitis* and *E. canis* in the stray dogs of Sanliurfa and this study will shed light on future studies in Sanliurfa.

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