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Seroprevalence of *Ehrlichia canis* in dogs referred to Veterinary Hospital of Shahid Chamran University of Ahvaz, Iran

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ABSTRACT

Canine ehrlichiosis is a zoonotic rickettsial disease transmitted by ticks. In the present study, 198 companion dogs of different ages were examined for serum antibody detection against *Ehrlichia canis* by means of immunochromatography assay. The dogs were selected among referred cases to Veterinary Hospital of Shahid Chamran University of Ahvaz, Southwestern Iran from November 2008 to March 2010. The studied dogs were classified based on age, sex, breed, region and season. Nineteen of 198 serum samples (9.6%) had antibody against *E. canis*. Prevalence was significantly higher in adult dogs more than 3 year-old (16.18%) (P= 0.002) and 1 – 3 years (11.86%) (P= 0.016) compared with young dogs less than 1 year-old (1.41%). Prevalence was higher in male dogs (10.62%) than female dogs (8.24%), in the summer (11.32%) and west region (11.11%). There were not significant differences between the prevalence of infection and host gender, season and region (P>0.05). Typical morulae of *E. canis* were observed in monocytes of four infected dogs (2.02%). Five out of 24 (20.83%) of the thrombocytopenic dogs and 14 out of 174 (8.05%) of the non-thrombocytopenic dogs were positive for ehrlichiosis. Of 19 seropositive dogs, six (31.58%) had anemia, four (21.05%) hypoalbuminemia and five (26.32%) leukopenia. There were not statistically significant differences between hematological findings and prevalence of infection (P> 0.05). This is the first report indicating the presence of *E. canis* in companion dogs of Ahvaz district. However, the sources of infection in these dogs were not clear. Finally, the role of companion dogs in the epizootiology of *E. canis* infection needs to be further explored.

Keywords: Prevalence, *Ehrlichia canis*, Dog, Immunochromatography assay, Ahvaz

INTRODUCTION

Canine monocytic ehrlichiosis (CME) is a potentially fatal tick-borne disease, caused by the rickettsial organism *Ehrlichia* canis. Ehrlichiosis is a worldwide zoonosis, concentrated in tropical and subtropical regions due to the geographical distribution of its vector tick *Rhipicephalus sanguineus* (Perez *et al* 2006, Dantas-Torres *et al* 2008). *R. sanguineus* is widely distributed throughout the world. This tick is reported as a common tick in Iran (Rahbari *et al* 2007) but there is low epidemiological data regarding to prevalence of ehrlichiosis in different geographical parts of the

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country. Ehrlichiosis is characterized by fever, anorexia, depression, lymphadenopathy, emaciation, bleeding, epistaxis and in some cases death (Neer & Harrus 2006, Cardenas *et al* 2007). A complete blood count (CBC) is an essential constituent in the diagnosis of CME. Hematologic abnormalities can include thrombocytopenia, anemia, leukopenia, hyperproteinemia and hyperglobulinemia, but the direct relationship between laboratory findings and seropositivity is controversial (Scorpio *et al* 2008).
Specific diagnosis of ehrlichiosis can be made using a blood smear, indirect immune-fluorescence assay, ELISA, Western immunobloting, polymerase chain reaction (PCR), Immunoperoxidase test (IPT) and culturing the parasite (Hegarty *et al* 1997, Neer & Harrus 2006). Though these tests are more sensitive, specific and more reproducible, but these tests can be expensive and generally take time to be analyzed by a specialized laboratory. Immunochromatography assay (ICA) is one of the most common rapid field diagnostic methods used in clinical practice. The immunochromatography test has been reported to have a sensitivity and specificity of 79.2% and 95.3%, respectively. The aim of this study was to investigate the antibody detection against *E. canis* in the serum samples of companion dogs in Ahvaz district.

MATERIALS AND METHODS

Study area and sample size. The present study was performed in Ahvaz area that is located at an elevation of 12 meters above sea level and with warm-humid climate. In this study, a total number of 198 dogs of different age groups were examined for antibody to *E. canis* by immunochromatography assay (ICA). These dogs were referred cases to Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz from November 2008 to march 2010. Most of the dogs were apparently healthy. Information about dogs were taken from their owners and recorded. The examined dogs were grouped in three age groups (<1 year, 1– 3 years and > 3 years). Age was estimated by dental formula and owner information's. Breeds of the studied dogs were mostly German Shepherds, Doberman Pinschers and Mixed Breeds.

Parasitological procedures. Blood samples were collected from cephalic or saphenous vein, which were divided in two tubes for hematological and biochemical evaluations. Serum samples was removed and stored at −20°C until analysis. Blood smears were prepared and allowed air drying. The smears were fixed in methanol for 5 min and stained by 10% Giemsa to detect morulae of *E. canis* in monocytes. Complete blood counts were performed for all samples. Hematological changes including anemia (Hct<37), leukopenia (WBC<6,000 cells/µl), and thrombocytopenia (platelets <200,000 /µl) were recorded (Neer & Harrus 2006). Albumin level was measured by an automated chemical analyzer (BT 3000 Plus, Biotechnica, Milan, Italy) using diagnostic kits (Pars Azmoon Co., Tehran, Iran). Antibodies against *E. canis* were detected by using BVT immunochromatography kit (SPEED EHRLI, BVT, France) in accordance with the manufacturers' recommendations. Test results were interpreted at 5-10 minutes.

Interpretation of the test. As the test began to work, purple color movement was seen across the result window in the center of the test device. A color band will appear in the left section of the result window to show that the test is working properly. This band is the control band. The presence of only one band within the result window indicates a negative result (Figure 1). If another color band appears in the right section of the result window, this band is the test band (with three forms: weak, medium and high) (Figures 2, 3 and 4). The presence of two color bands (T and C) within the result window, no matter which band appears first, indicates a positive result.

Statistical analysis. Dogs were grouped based on age, sex, breed, and season to determine whether

these factors were associated with the prevalence of *E. canis*. Chi-square test and fisher's exact test were under taken. Statistical evaluations were carried out using SPSS 16.0. Differences were considered significant when P< 0.05.

Figure 1. Negative sample of rapid *E. canis* Ag test

Figure 2. Weak positive sample of rapid *E. canis* Ag test

Figure 3**.** Medium positive sample of rapid *E. canis* Ag test

Figure 4. High positive sample of rapid *E. canis* Ag test

Table 1. Seroprevalence of *Ehrlichia canis in* companion dogs of different age groups and sexes.

Age group (year) / **Sex**	**< 1**		**1 – 3**		**> 3**	
	N	**P**	**N**	**P**	**N**	**P**
Male	41	0	33	5	27	7
female	29	1	19	2	30	4
Total	70	1	52	7	57	11

N: Negative; P: positive

Table 2. Seroprevalence of *Ehrlichia canis in* companion dogs of different age groups and regions.

Age group (year) / **Region**	**< 1**		**1 – 3**		**> 3**	
	N	**P**	**N**	**P**	**N**	**P**
North	9	0	12	2	12	1
East	12	0	14	1	8	3
West	19	1	15	0	14	5
South	12	0	8	2	6	0
Central	18	0	3	2	17	2
Total	70	1	52	7	57	11

N: Negative; P: positive

RESULTS

Nineteen of 198 serum samples (9.6%) were positive for *E. canis*. Prevalence rate was significantly higher in adult dogs >3 years-old (16.18%) (P= 0.002, df= 1, χ^2 =6.1) and 1–3 years-old (11.86%) (P= 0.016, df= 1, χ^2 =5.482) compared

with young dogs less than 1 year-old (1.41%). The difference was not differed significantly between 1-3 years-old and >3 years-old dogs (P>0.05). Prevalence rate was higher in male dogs (10.62%) than female dogs (8.24%), in summer (11.32%) and west region (11.11%). However, the difference was not observed among the prevalence of infection, genders, seasons and regions (P>0.05) (Tables 1 and 2). Typical morulae of *E. canis* were observed in monocytes of four infected dogs (2.02%). Five (20.83%) of thrombocytopenic dogs and 14 (8.05%) of non-thrombocytopenic dogs were positive for ehrlichiosis. Of 19 seropositive dogs, six (31.58%), four (21.05%) and five (26.32%) showed anemia, hypoalbuminemia and leucopenia, respectively. There were not statistically significant differences between hematological findings and prevalence of infection (P> 0.05).

DISCUSSION

This as the first report on prevalence of canine ehrlichiosis in companion dogs in Ahvaz district. These findings showed that 9.6% of dogs had antibody against *E. canis*. These results indicate that the parasite survives in companion dogs of Ahvaz region. Prevalence of ehrlichiosis was reported from other regions of Iran. Akhtardanesh *et al* (2009) noted that, the seroprevalence of canine monocytic ehrlichiosis using IFA and ICA was 13.8% and 10.6%, respectively. In West Azerbaijan, 67% of wild dogs and 38% of domestic dogs were serologically positive for *E. canis*. These rate of infection reported 58% and 39%, respectively, in east Azerbaijan (Asri & Mahmoudian 2001). Large variation in seroprevalence was found due to certain epidemiological factors, especially geographical distribution of biological vectors, the average age, lifestyle and clinical status of the examined population (Neer & Harrus 2006). Studies indicate that the incidence of Ehrilichiosis can vary greatly between countries and regions. The high seroprevalences of *E. canis* infection were detected as 54.2% in Tunisia (Mighirbi *et al* 2009), 44.4% in Saudi Arabia (Sacchini *et al* 2007) and 21% in Turkey (Batmaz *et al* 2001), whereas low prevalence (2.9- 9.7%) were obtained in Italy (Solano-Gallego *et al* 2006). In recent investigation, prevalence was significantly differed among age groups. This is inagreement with other researches (Watanabe *et al* 2004, Rodriguez-Vivas *et al* 2005, Costa *et al* 2007). Possible explanations can be attributed to the increased probability of a dog being exposed to *E. canis*. In some other studies, age was not associated with level of antibody to *E. canis* (Matthewman *et al* 1993, Inokuma *et al* 1999, Waner *et al* 2000). Seroprevalence of infection in male dogs (10.62%) was higher than females (8.24%), however, it was not significantly differed. It can be explained by the territorial habits associated with males, as they have a wider area of operation than females and this was in agreement with other researchers (Waner *et al* 2000, Watanabe *et al* 2004, Rodriguez-Vivas *et al* 2005, Solano-Gallego *et al* 2006). However, findings were not in agreement with Batmaz *et al* (2001). Sainz *et al* (1996) reported no differences between breeds, although, they found a correlation between the utility of dogs and seropositivity to *E. canis*. Thrombocytopenia is the most common and consistent hematological finding in the affected dogs with acute CME as well as experimentally infected dogs, so it could be a sensitive, but not very specific screening test (Troy *et al* 1980, Waner *et al* 1995, Macieira *et al* 2005). This is a common finding in the subclinical stage of the disease; however severe thrombocytopenia is most frequently evident during the chronic stage (Troy *et al* 1980, Waner *et al* 1995). There was a high prevalence (26.8%) of *E. canis* infection in thrombocytopenic in contrast to low prevalence of infection (3.5%) in non-thrombocytopenic dogs in Rio de Janeiro, Brazil (Macieira *et al* 2005). In the

present study, the prevalence of ehrlichiosis was higher in dogs with thrombocytopenic (20.83%), though the difference was not significant (P>0.05). Therefore thrombocytopenia should not be used alone to establish a diagnosis of *canine ehrlichiosis*. Correlation was not observed between seronegative and seropositive dogs for anemia, hypoalbuminemia and leukopenia. No specific hematologic differences were apparent by Scorpio *et al* (2008). These results can be due to differences in strain pathogenicity (Neer & Harrus 2006). So far, no vaccine is available to prevent ehrlichiosis infection. Veterinarians should be aware that ehrlichiosis seems to be endemic in Iran. Because ehrlichiosis is a zoonotic disease that is mostly transmitted by ticks *Rhipicephalus sanguineus*, a complex study has to be done, where both dogs and humans are included with an appropriate sample size and the evaluation of risk factors. Also, preventive and control measures to control ticks should be established in order to minimize the risk of infection. It is concluded that further investigation and additional molecular studies are necessary to identify the strains of the organism.

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