Turk. J. Vet. Anim. Sci.
2007; 31(1): 73-74

Short Communication

Seroepidemiology of Leishmania among Healthy Dogs in Bulgaria

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Received: 24.11.2005

Abstract: A seroepidemiological study of canine leishmaniasis was carried out in 220 healthy pet dogs from 11 different regions in Bulgaria allocated in 5 regions in southern Bulgaria: Plovdiv (n = 20), Stara Zagora (n = 20), Yambol (n = 20), Burgas (n = 20), and Blagoevgrad (n = 20), and 6 regions in northern Bulgaria: Varna (n = 20), Silistra (n = 20), Rousse (n = 20), Veliko Tarnovo (n = 20), Pleven (n = 20), and Montana (n = 20). The indirect fluorescent antibody test was used.

All blood sera examined yielded negative results for leishmaniasis, thus suggesting that healthy household dogs in Bulgaria are not infected by *Leishmania*.

Key Words: Dog, *Leishmania*, Bulgaria, IFA test

Canine leishmaniasis (CL) is an infectious disease caused by protozoa belonging to the genus *Leishmania* and transmitted by bites of female sand flies (*Phlebotomus* in the old World). Dogs usually present with cutaneous lesions along with the visceral form of the disease (1,2). The prevalence rate of canine leishmaniasis in Bulgaria remains to be clarified. The country is situated between 41° and 44° north latitudes, thus being a part of the natural zones of leishmaniasis, but a low number of human visceral leishmaniasis cases have been recorded during the past years and stray animals have been found to be occasionally infected (3).

The purpose of the present study was to investigate the seroprevalence of *Leishmania* among healthy pet dogs in Bulgaria. Eleven regions of Bulgaria were included in the study: area 1: 5 regions in southern Bulgaria: central - Plovdiv, Stara Zagora and Yambol; eastern – Burgas; western - Blagoevgrad; area 2: 6 regions in northern Bulgaria: central - Silistra, Rousse, Veliko Tarnovo, Pleven; eastern – Varna; western - Montana.

One hundred canine blood sera were collected from 5 regions in southern Bulgaria, namely Plovdiv (n = 20), Stara Zagora (n = 20), Yambol (n = 20), Burgas (n = 20), and Blagoevgrad (n = 20). Forty-nine of these samples were obtained from female dogs and 51 were from male dogs, all of various breeds and ages (mean 3.8, SD 0.39 years). The areas selected included all regions where human visceral leishmaniasis had been detected, while some others were added in order for a representative sample of the whole country to be investigated.

Additionally, 120 blood sera were obtained from 6 regions in northern Bulgaria, namely Varna (n = 20), Silistra (n = 20), Rousse (n = 20), Veliko Tarnovo (n = 20), Pleven (n = 20), and Montana (n = 20). Fifty-one of these samples were obtained from female dogs and 69 were from male dogs, all of various breeds and ages (mean 4.26, SD 0.43 years).

All blood samples were collected from healthy pet dogs during routine health care visits in veterinary clinics situated in the aforementioned towns. Blood samples were obtained during all seasons between 2002 and 2004.

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All sera were assayed by the indirect fluorescence antibody (IFA) test.

The test was performed according to the manufacturer's instructions (BIO VET test –France). Briefly, plates of 10 ready-to-use wells with Fluoleish were used along with FITC-labeled anti-dog IgG conjugate (Sigma F-4012, Germany) diluted 1:25 in PBS. All blood sera were diluted in working titers of 1:100 and 1:200. Each series included both positive and negative controls. The plates were examined under fluorescent microscope, through slide cover glasses. Titers of 1:100 and higher were considered positive.

All serum samples scored negative for anti-leishmania antibodies. Given the lengthy incubation period of CL (months to years), blood samples were not collected during a restricted time period or season.

Although the role of leishmania-infected dogs in the epidemiology of human visceral leishmaniasis is still controversial, the dog is considered one of the main reservoirs of the parasite in nature (4). In the neighboring country Greece, the canine population of pet animals has been found to be infected with *Leishmania infantum* with an incidence ranging between 6.8% and 48.4% (5).

It was surprising to find that all dogs examined in the present study had no antibodies against *Leishmania*. Thus, a lack of contact with the vector (*Phlebotomus*) could be the only explanation for our results. Since all animals examined were pet dogs, it is possible that sand fly repellents regularly implemented by the owners are quite effective and prevent sand fly biting. Another explanation could be that *P. papatasi* and *P. sergenti*, which are the main sand fly species in Bulgaria (6), have a higher tropism towards humans than towards household dogs, as opposed to *P. neglectus*, which is the main sand fly species encountered in Greece (7). Furthermore, seroprevalence rates may change over time due to human intervention along with the natural dynamics of *Leishmania* transmission, and there is evidence that serology alone is a non-reliable tool in the investigation of canine leishmaniasis epidemiology (8). On the other hand, IFA test has been found to be very sensitive in detecting leishmania antibodies in the canine (9). Therefore, the possibility of false negative results seems to be very low and does not significantly interfere with the results. In order for this important issue to be clarified, further research has to be done, mainly focusing on the vector behavior in Bulgaria, its tropism and infectivity in particular in areas of the country in which human visceral leishmaniasis has already been diagnosed. Since not all infected dogs develop positive antibody titers (10), it is possible that some of the examined animals in this study had a latent infection along with adequate activation of the cellular immunity and undetectable levels of antibody titers against leishmania (11). Finally, dogs living in kennels and wild animals should also be examined since a certain species could be an additional reservoir of leishmania and more accessible to sand flies than household dogs.

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