PRELIMINARY DATA ON SEROLOGICAL SURVEY OF EXPOSURE TO ARTHROPOD-BORNE PATHOGENS IN STRAY DOGS FROM BUCHAREST, ROMANIA

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Abstract

Canine vector-borne diseases (CVBDs) represent an important group of illnesses affecting dogs around the world. In addition to their veterinary importance, some CVBD-causing pathogens are of major zoonotic concern, with dogs potentially serving as reservoirs and sentinels for human infections. The present study aimed at assessing the seroprevalence of some selected arthropod-borne pathogens (*Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi sensu lato*, and *Anaplasma phagocytophilum*) in stray dogs from Bucharest's areas, using point-of-care assays: SNAP® Heartworm test (n=16) and SNAP 4DX (n=75), IDEXX Laboratories, Westbrook, ME. The SNAP heartworm detects only *D. immitis* antigen, whereas the SNAP 4DX detects *D. immitis* antigen and antibodies against *E. canis*, *A. phagocytophylum*, and *B. burgdorferi*. All animals displayed no clinical signs at the physical examination, therefore they were assumed as clinical healthy. Overall, 30.77% (28/91) of the dogs were serologically-positive to one or more of the tested pathogens. The prevalence of positive test results was as follows: *D. immitis*, 18.68% (17/91), *E. canis*, 4.00% (3/75), *A. phagocytophilum*, 16.00% (12/75). Three dogs (4.00%) were co-exposed to *D. immitis* and *A. phagocytophilum* and one (1.33%) was co-exposed to *E. canis* and *A. phagocytophilum*. There was no evidence for *Borrellia* infection. This study provides on insight of exposure to certain pathogens infecting stray dogs in some areas of Bucharest, emphasizing high risks for vector-borne diseases.

Key words: *arthropods, stray dogs, pathogens, vector.*

INTRODUCTION

Dogs are competent reservoir hosts of several zoonotic agents and can serve as a readily available source of nutrition for many blood feeding arthropods. The explosion of the canine population, and their increasingly close relationship with humans in both urban and rural areas pose new concerns for human public health (Otranto et al., 2009a; Genchi et al., 2011a).
Canine vector-borne diseases (CVBDs) represent an important group of illnesses affecting dogs around the world. These diseases are caused by a

diverse range of pathogens, which are transmitted to dogs by different arthropod vectors, including ticks and insects (fleas, mosquitoes, phlebotomine sandflies) (Otranto et al., 2009b). In addition to their veterinary importance, some CVBD-causing pathogens are of major zoonotic concern, with dogs potentially serving as reservoirs and sentinels for human infections.

The growing medical interest in canine vector-borne diseases CVBDs) is directly related to both animal welfare and public health (Beugnet and Marié, 2009).

Stray dogs (free-roaming) are often present in urban areas representing an increasing public health concern (Slater et al., 2008). Despite of the great concern worldwide on vector-borne diseases generally (Knols and Takken, 2007), and on CVBDs particularly, little is known about the occurrence and prevalence of vector-borne pathogens in dogs in different areas of Romania. There is only one recent epidemiological study on the prevalence of vector-borne pathogens in dogs in Romania (Mircean et al., 2012), in which Bucharest' area was not included. Therefore, the present study aimed at assessing the seroprevalence of some selected arthropod-borne pathogens (*Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi sensu lato*, and *Anaplasma phagocytophilum*) in stray dogs from Bucharest's areas.

MATERIALS AND METHODS

We evaluated the prevalence of arthropod-borne pathogens in stray dogs from Bucharest's areas using point-of-care assays: SNAP® Heartworm test (n=16) and SNAP 4DX (n=75), IDEXX Laboratories, Westbrook, ME. The SNAP heartworm detects only *D. immitis* antigen, whereas the SNAP 4DX detects *D. immitis* antigen and antibodies against *E. canis*, *A. phagocytophylum*, and *B. burgdorferi*. Stray dogs (n=91), originated from two different areas of Bucharest (in southeastern Romania), which were subjected to the sterilization procedure, were included in the study. All animals displayed no clinical signs at the physical examination, therefore they were assumed as clinical healthy.

RESULTS AND DISCUSSIONS

The seroprevalence of infection or exposure and co-exposure to several arthropod-borne pathogens in stray dogs in Bucharest's area are displayed in Table 1. Overall, 30.77% (28/91) of the dogs were serologically-positive to one or more of the tested pathogens.

In decreasing order, the seropositivity was as follows: to *D. immitis*, 18.68% (17/91), *A. phagocytophilum*, 16.00% (12/75), *E. canis*, 4.00% (3/75). Three dogs (4.00%) were co-exposed to *D. immitis* and *A. phagocytophilum* and one (1.33%) was co-exposed to *E. canis* and *A. phagocytophilum*. There was no evidence for *Borrellia* infection in this study.

These findings strongly indicate that dogs from the studied area are potentially at risk of major canine vector-borne diseases some of them of zoonotic concern.

Table 1. **Seropositivity (number positive and percentage) of stray dogs from southeastern Romania to some selected arthropod-borne pathogens**

Location	Samples positive/samples tested (percentage)					
	***D.im.* (Ag)[a]**	***E.c.* (Ab)[b]**	***A.ph.* (Ab)[c]**	***B.b.* sl (Ab)[d]**	***D.im.* + *A.ph.***	***A.ph.* + *E.c.***
Area A	10/59	1/44	10/44	-	3	-
Area B	7/35	2/31	2/31	-	-	1
Total	**17/91** (18.68%)	**3/75** (4.00%)	**12/75** (16.00%)	**-**	**3/75** (4.00%)	**1/75** (1.33%)

[a]Antigen of *Dirofilaria immiitis*; [b]Antibody to *Ehrlichia canis*; [c]Antibody to *Anaplasma phagocytophilum*; [d]Antibody to *Borrelia burgdorferi* sensu lato.

In a similar study, Mircean et al. (2012) have been reported lower values of seroprevalence of *A. phagocytophilum* (5.5%), *D. immitis* (3.3%), and *E. canis* (2.1%). However, focal regions were found in the southeast of Romania for all these pathogens, with the highest prevalence, up to 31.00% for *D. immmitis*, 17.00% for *E. canis*, and 10.3% for *A, phagocytophilum*, respectively (Mircean et al., 2012).

These findings can be explained by the particular ecological conditions (climate, biotopes) associated with the distribution and abundance of vector competent arthropods, ticks (for *A. phagocytophilum, E. canis*) and mosquitoes (for *D. immitis*) in the studied areas (southeastern Romania, Bucharest's area included). Moreover, stray dogs are at high risk of acquiring vector-borne pathogens, mainly because they are often untreated against ectoparasites, thus, representing an easy feeding source for them. In addition, the general conditions of these animals (e.g., poor nutrition) may contribute to susceptibility to some VBDs. Likewise, when infected, stray dogs are often neither monitored nor treated against vector-borne pathogens (Otranto and Dantas-Torres, 2010).

A serological study of selected vector-borne diseases in shelter dogs in central Spain using also point-of-care assays reported similar data for *A. phagocytophilum* (19.0%), but lower for *E. canis* (5%) (Couto et al., 2010).

In Portugal, Cardoso et al. (2012) had reported high risks of healthy dogs, serological tested, for CVB-pathogens, like *D. immitis* (3.6%), *E. canis* (4.1%), *B. burgdorferi* (0.2%), *Anaplasma* spp. (4.5%),

Similarly, in a study in Germany, 41.9% (26/62) of healthy dogs were found to be seropositive for *A, phagocytophilum* (Jensen et al., 2007).

The prevalence of *E. canis* infection in dogs in Italy, estimated by serological surveys varied from 14.9% in southern Italy (Otranto et al., 2008) to 46.7% in Sardinia (Cocco et al., 2003), emphasizing some varieties among foci according to local factors (e.g., vector population density and activity patterns).

Prevalence rates/ranges (%) reported for *D. immitis* in some European countries, were very different, like: from 0.6 to 80% in Italy, 0.6 to 6.8% in France, 1.6% in Switzerland, 6.2% in Serbia, from 10 to 34% in Greece (as reviewed by Traversa et al., 2010). Moreover, canine dirofilariosis by *D. repens* has been considered for a long time to be mainly diffused in southern regions of Italy, while *D. immitis* is considered endemic in northern regions with prevalence rates ranging from 22 to 80% in dogs untreated with prophylactic drugs (Rossi et al., 1996; Genchi et al., 2001; Genchi et al, 2011b).

Although positive serological results may suggest prior exposure and not necessarily disease, they can alert veterinarians to take into consideration further clinical and diagnostic evaluation of individual dogs (Carrade et al., 2011). Many dogs infected with vector-borne agents remain asymptomatic for months or even years, but diagnosis of subclinical infection is important (Ionita et al., 2012), as those animals might still serve as reservoirs of pathogens to other hosts including humans. Therefore, especially in areas of endemicity, an annual serological screening would be recommended to promote early detection and treatment (Otranto et al., 2009).

Travelling of dogs from arthropod-borne diseases endemic areas into non endemic areas and *vice versa* poses a risk for the introduction and dissemination of exotic pathogens if competent vectors are present (Otranto and Dantas-Torres, 2010).

In Germany, some of CVB-pathogens, like *Babesia* spp., *Leishmania* spp., *D. immitis* or *E. canis* have repeatedly been recorded in travelling and imported dogs (including from Romania) (Hamel et al., 2012).

The introduction of non-endemic pathogens, and sometimes their vectors, by dogs is documented also in Austrian dogs (Leschnik et al., 2008).

The above phenomenon highlights the importance of establishing effective surveillance systems to avoid the importation of infected animals into and from different regions. A future risk may arise from an increasing number of imported dogs, carrying vectors that may be host to various pathogens, to areas still free of those pathogens. A further problem is the probability, that these vectors may become native when climate conditions are going to be favorable to them (Daugschies, 2001; Deplazes et al., 2006).

CONCLUSIONS

This study provides on insight of exposure to certain pathogens infecting stray dogs in some areas of Bucharest (southeastern Romania), emphasizing high risks for vector-borne diseases, some of them of zoonotic concern. Therefore, the findings are expected to serve as a reference for future investigations and control actions in order to protect dogs and limit the risk of transmission of vector-borne agents to humans.

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