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CLINICO-PATHOLOGICAL FINDINGS IN VECTOR-BORNE PATHOGEN CO-INFECTIONS IN DOGS, FROM BUCHAREST AREA

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Abstract

Canine Vector Borne Diseases (CVBD) have a worldwide impact as some are of zoonotic concern and they lead to a variety of serious infections mostly classified by their vectors. The pathogens co-infecting the dogs are linked to their associated vector agents and with their natural habitat. Dogs with clinical signs compatible for VBDs should be tested for more than one pathogen as the signs may be often non- specific and they may vary from one individual to another. Co-infections may potentiate the disease pathogenesis, thereby changing clinical manifestations associated with singular infections. Seven cases were selected among dogs referred in the Veterinary Clinic, Faculty of Veterinary Medicine of Bucharest during of 2016, showing clinical signs compatible with VBD. They were serologically-positive for more than one pathogen. The seroreactivity revealed co-infections in dogs with four arthropod-borne pathogens: Dirofilaria immitis + Anaplasma spp. (3 dogs), D. immitis + Erlichia canis (2 dogs), E. canis + Borelia burgdorferi (1 dog) and E. canis + Anaplasma spp. (1 dog). One dog, serological positive for D. immitis and A. phagocytophilum, was also positive for Babesia canis, detected in the blood smear. The present study emphasizes the chalenge of the diagnostic, therapeutics and management of co-infected dogs and illustrates the correlation between clinical aspects that the dogs are first presented with and the full panel of paraclinical investigations like imagistical (radiography, ultrasonography) and the blood analyses (haematology, biochemistry, citology and serology).

Key words: *Co-infection, canine vector borne diseases, dogs.*

INTRODUCTION

According to WHO, there are more than 200 emergent and re-emergent zoonoses, of which almost 10 canine vector borne diseases (CVBDs), including Lyme disease, that appears to be the most common in Europe (WHO, 2014).

Climate change, together with increasing movement of dogs across Europe, have caused an increase in the geographical range of more vector borne diseases (Genchi, 2011b).

Among the vectors transmitting disease-causing pathogens, ticks play an important role as they can harbore multiple disease causing agents, sometimes completely different pathogens (Shaw et al., 2001).

The risk of exposure to ticks, mosquitoes and fleas is bigger for dogs. They can be infested with hundreds of ticks and sometimes with different tick species in the same time, therefore concurrent infections with multiple vector borne pathogens may occur (Otranto et al., 2009a).

Dogs are reservoir hosts for several arthropod-borne pathogens, some of which are of major zoonotic concern (Beugnet, 2009) and they can be infected with a large number of vector-borne pathogens such as *Hepatozoon canis, Ehrlichia canis, Anaplasma platys, A. phagocytophilum, Babesia canis, B. vogeli, Bartonella* spp, *Borrelia burgdorferi, Leishmania infantum, Dirofilaria repens* and *D. immitis (*de Caprariis et al., 2011*)*.

Some arthropods are competent vectors of more than one pathogen. Thus, dogs might be exposed to vectors infected with single pathogens at different points in time or to vectors concurrently infected with multiple pathogens, favoring the occurrence of co-infections (Otranto et al., 2009b).

Studies regarding seroprevalence, revealed that dogs from Romania are potentially at risk of major canine vector-borne diseases because of the relatively high prevalence rates of both mosquito and tick-borne pathogens in dogs (Ionita et al., 2012; Mircean et al., 2012).

The diverse tick fauna as well as the abundance of tick populations in Romania represent potential risks for both human and animal health (Ionita et al., 2016).

Anamnesis and search for specific clinical signs, along with laboratory results (biochemistry and hematology) are the key for aproaching an accurate diagnostic. These findings can be modified by the presence of a co-infection.
Therefore, in this study clinico-pathological findings from CVBDs co-infected dogs are described.

MATERIALS AND METHODS

The study describes clinical and hematological findings in seven dogs that were presented in the Veterinary Clinic, Faculty of Veterinary Medicine of Bucharest during of 2016 and were positive for more than one vector borne pathogen.
Dogs showing clinical signs compatible for VBDs were subjected for routine clinical examination, followed by blood analysis (biochemical, hematological and serological investigations), radiography and ultrasonography.
Whole blood EDTA samples were collected and tested for some selected CVBDs using blood smears and serological tests (SNAP®4Dx® Plus from Idexx Laboratories). Li-heparine tubes were used for collecting blood and biochemistry analysis was performed from plasma.
The SNAP 4Dx Plus test is an in-clinic enzyme-linked immunoabsorbent assay (ELISA) commercial kit for the detection of *Dirofilaria immitis* antigen and antibodies for *Anaplasma phagocytophilum / Anaplasma platys, Ehrlichia canis, Ehrlichia ewingii*, and *Borrelia burgdorferi*.

RESULTS

Serological evaluation revealed co-infections in seven dogs with four different arthropod-borne pathogens*: D. immitis* + *Anaplasma* spp. (3 dogs), *D. immitis* + *E. canis* (2 dogs), *E. canis* + *B. burgdorferi* (1 dog) and *E. canis* + *Anaplasma* spp. (1 dog). One dog, serological positive for *D. immitis* + *A. phagocytophilum*, *Babesia canis* was also positive, detected in the blood smear (Figure 1).
Dogs included in the study, displayed clinical signs compatible with VBDs, with the exception of one dog, presented for screening as a blood donor. The age ranged from 6 years to 14 years old. There were 5 mixed breed dogs, 1 Labrador Retriever and 1 Golden Retriever; among them, 4 were females and 3 were males.
Clinical signs in the dogs referred, included depression (2/7), fever (1/7), anorexia (2/7), weight loss (1/7), weakness (3/7), exercise intolerance (2/7), pale mucous membranes (1/7), lameness (1/7), coughing (3/7), respiratory difficulties (1/7), vomiting (2/7) and diarrhea (2/7).
The following laboratory abnormalities were registered: anemia, leucopenia, leucocytosis granulocytosis, trombocytopenia, eosinophilia, elevated liver enzymes, high blood urea nitrogen and high blood creatinine levels. (Table 1.)
Hematological parameters were determined, thus anemia and trombocytopenia were found in four dogs of seven, leucocitosis with neutrofilia in two dogs, eosinophilia in one dog and leucopenia in anoher two. Microfilaremia associated with *D.immitis* was present in three dogs of five serological positive, from the total seven.
In dogs with *Ehrlichia* spp. serological positive detected, quantitative and qualitative changes regarding leucocytes were observed as an inflammation response and antigenic stimulation (WBC >17 K/uL with Grans >13 K/uL).
Thrombocytopenia was present in four out of seven dogs, as a result of the development of anti-platelet antibodies in *E. canis* infections (three dogs) and in one dog co-infected with *Anaplasma* spp and *D. immitis* (PLT<175 K/uL).
The presence of triple infection, with *D. immitis, A. phagocytophilum, and B. canis* (fig. 1), was detected in one dog, mixed breed, male, 14 y.o with severe respiratory simptoms, anemia, leucopenia, and elevated liver enzymes.
One of the 7 dogs included in the study, was a 4 years old female, Labrador Retriever in a late stade of gestation, serological positive to *Ehrlichia* spp. and *Anaplasma* spp.
Dogs co-infected with *E. canis, B. burgdorferi and A. platys / A. phagocytophilum* were treated with doxycycline (10 mg/kg/day /PO) for more than 21 days.
In the case of the triple infection with *D. immitis, A. phagocytophilum, and B. canis,* the dog was treated with imidocarb dipropionate (4

mg/kg in a single dose) and supportive treatment was given to reduce the anemia. Doxycycline (10 mg/kg/day /PO) was also used. For co-infected dogs with *D. immitis*, a treatment with low dose ivermectine and microfilaricide combination was used.

DISCUSSIONS

Canine tick-borne pathogens have been documented in several European countries and revealed that *A. phagocytophilum* and *Borrelia* spp share the same tick vector - *Ixodes ricinus* (Straubinger et al., 2008). Another example of a shared vector is *Rhipicephalus sanguineus*, transmitting *Babesia* spp., *Ehrlichia canis, A. platys* and *Rickettsia conorii*, leading to co-infections with vector-borne pathogens in dogs.

In Romania, in a serological survey two cases of co-infection with *A. phagocytophilum* and *E. canis* were reported (Mircean et al., 2012). In a similar study, three cases of co-exposure to *D. immitis* and *A. phagocytophilum* and one case co-exposed to *E. canis* and *A. phagocytophilum* were displayed (Ionita et al., 2012).

In Romania, tick fauna is very diverse, with up to 20 species of hard ticks identified, with the most abundant and frequent species reported *Ixodes ricinus, Dermacentor marginatus, Dermacentor reticulatus, Hyalomma marginatum, Rhipicephalus bursa* and *Rhiphicephalus sanguineus* (Mihalca et al.,2015). The tick species found more frequent parasitizing dogs in urban area of Buharest, were reported *R. sanguineus, D. reticulatus, and ocassionally I. ricinus* (Ionita et al., 2013).

Other vectors responsible for CVBDs are represented by mosquitoes (*Aedes* spp, *Anopheles* spp, and *Culex* spp), implicated in transmitting *D. immitis* and *D. repens*.

Therefore, different combinations of vector-borne pathogens and their effect on the host, should be further investigated, as the possibilty of multiple vectorial capacity can occur. Co-infections might put the clinician in difficulty, as their expression vary from sick dogs to clinical healthy ones.

Serological assays do not differentiate between current and previous infections, when it comes for the detection for antibodies; Therefore, other confirmatory test are needed (e.g. PCR). Future studies should add new insights regarding molecular charecterization of vector-borne pathogens occuring in Romania.

The results in the present study supports the geographical expansion of canine vector borne diseases in Romania and that there is a challenge for the practioners when it comes for co-infections with CVB pathogens.

In this study, the hematologic results in infections with *Anaplasma* spp and *E. canis* were similar to those in other studies, as Mylonakis et al. reported (2004). Simultaneous infection with *E. canis* and *Anaplasma* spp in dogs resulted in a more pronounced anemia (HCT 23 % with HGB 6 g/dL) and thrombocytopenia compared to the single infection with either pathogen.

Also, co-infection with *E. canis* and *Anaplasma* spp appeared to result in a more persistent infection (Mylonakis et al., 2004).

A study conducted by Latrofa et al.(2016), sustained vertical transmission of *A. platys* during the early stages of gestation, and throughout its entire course, thus increasing the importance of screening for CVBDs in dogs.

Previous studies have shown that naturally infected clinically ill dogs, suspected of having either Lyme disease, granulocytic anaplasmosis, or both diseases, were nearly twice as likely to have antibodies to both *Borrelia burgdorferi* and *A. phagocytophilum* as compared to healthy dogs from the same region, suggesting that exposure to more than one pathogen may increase the possibility of disease expression (Beall et al., 2008).

Epidemiological studies performed in Europe, evaluated the seroprevalence of *A. phagocytophilum* in dogs between 3 to 57%, but serological cross-reactivity with other *Anaplasma* spp. *(A. platys)* can potentially cause an overestimation of the true seroprevalence (Sainz et al, 2015). In Romania, values regarding seroprevalence for *Anaplasma* spp varied from 5,5% to 16% (Mircean et al, 2012; Ionita et al., 2012).

More specific diagnostic methods, such as polymerase chain reaction (PCR) are necessary, due to cross-reactivty, particulary among members of the same genus (Pantchev et al., 2010).

Table 1. Clinical signs, laboratory abnormalities and diagnostic test results in seven dogs co-infected with canine vector borne diseases causing pathogens

Nr. crt.	Dog's data			Clinical signs	Serology (Snap 4Dx Plus)	Biochemistry	Haematology and blood citology	Ultrasounds	Radiology
	Breed	Age (year)	Gender						
1	Golden retriever	4	F	weakness, vomiting, modified mamary glands discharge	*Ehrlichia* spp. + *Anaplasma* spp. +	no abnormalities	Mild anemia PLT ↓ Microfilaremia	Gestation	Arthtritic degenerescence of the hip joint
2	Mongrel	12	M	Coughing, vomiting	*Ehrlichia* spp. + *Borrelia burgdorferi* +	ALKP ↑ (477 U/L) GGT ↑ (25 U/L)	ESR ↑ (11,3)	Hepatomegaly Splenomegaly	Congestive thorax
3	Mixed breed	10.	F	Depression, Diarehea, anorexia, respiratory difficulties	*Ehrlichia* spp. + *Dirofilaria immitis* +	ALT ↑ (156 U/L)	Anemia Leucocitosis with neutrofilia Trombocitopenya Microfilarilaremia	Enlarged aorta	Cardiac hypertrophy Congestive pneumonia
4	Mixed breed	13	F	Coughing, depression	*Ehrlichia* spp. + *Dirofilaria immitis* +	No abnormalities	Leucopenia PLT ↓	Ventricular hypokinesia Cardiac arrhythmia	Pulmonary reactivity
5	Mixed breed	14	M	Coughing, weakness, pale mucous membranes exercise intolerance and lameness	*Dirofilaria immitis* + *Anaplasma* spp. +	ALT ↑ (163 U/L) AST ↑ (130 U/L) ALKP ↑ (680 U/L) Glu ↓ (65 mg/dL) BUN ↑ (32 mg/dL) CREA ↑(2.7 mg/dL)	MCHC↓ (28.1g/dL) Hct ↓ (32,7%) Hgb↓ (11.1 g/dL) Wbc ↓ (5.70 K/uL) PLT ↓ *Babesia* spp. + Anemia	Splenomegaly Hepatomegaly Gastritis Bile sludge	Cardiac hypertrophy Interstitial lung pattern
5	Mixed breed	6	M	Weight loss, Anorexia, Fever, Diarrhea, Exercise intolerance	*Dirofilaria immitis* + *Anaplasma* spp. +	TP↓ (5.5 g/dL) GPT↑ (173 U/L) ALKP ↑ (212 U/L)	Mild anemia Microfilaremia	Mild cardiac dilatation of the right ventricle and right atrium	Pulmonary congestion
7	Labrador	y.o.	M	Screening for blood donor Asymptomatic	*Dirofilaria immitis* + *Anaplasma* spp. +	No abnormalities	Grans↑(12.70k/uL) Neu ↑(11.14 K/uL) Eos ↑ (1.71 k/uL) Microfilaremia	No abnormalities	No abnormalities

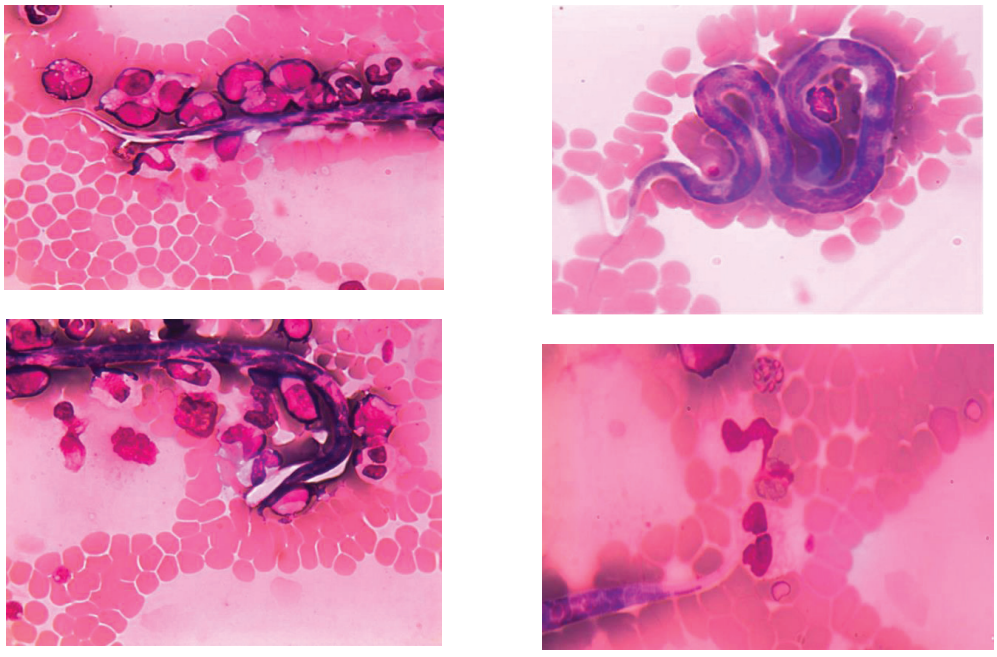

Fig. 1. Blood smears of dog showing microfilaria and merozoites of large *Babesia* spp - a case with triple infection (*B. canis, D. immitis* and *Anaplasma* spp)

CONCLUSIONS

This study emphasizes the clinical difficulties associated with assigning a specific clinical sign or haematological abnormality to a particular canine vector-borne disease.
Monitoring the response for treatment is very important in dogs with severe hematological abnormalities and multiple infections, to improve the animal clinical status before treating for a specific vector-borne pathogen.
Assigning a specific treatment, needs a complete diagnostic aproach, remainig challenging to distinguish, disease from previous exposure to one or more vector-borne pathogens.

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