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EVIDENCE OF CANINE VECTOR-BORNE DISEASES IN TWO COUNTIES FROM EASTERN ROMANIA

CONFIRMAREA PREZENŢEI BOLILOR CU TRANSMITERE VECTORIALĂ LA CARNIVORE ÎN DOUĂ JUDEŢE DIN ESTUL ROMÂNIEI

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ABSTRACT

The canine vector-borne diseases (CVBDs) are an emerging problem in veterinary medicine. The present work investigated the seroprevalence of three pathogens (*Anaplasma phagocytophilum, Borrelia burgdoferi* and *Ehrlichia canis)* causing vector-borne diseases (VBDs) in dogs from two counties in Eastern Romania. Between March and May 2019, blood samples were collected from 92 dogs originating in Iași (n=72) and Brăila (n=20) counties. All the serum samples were tested using three commercial enzyme-linked immunosorbent assays (ELISA) with the purpose to detect the specific IgG antibodies against *Borrelia spp.*, *Anaplasma phagocytophilum* and *Ehrlichia canis*, respectively. Following the ELISA testing, 14 out of 92 tested dogs were identified as seropositive for specific IgG antibodies, of which 6 for *Borrelia spp*. (6.52%), 5 for *Anaplasma phagocytophilum* (5.43%) and 3 for *Ehrlichia canis* (3.26%). Simultaneous detection of IgG antibodies against two pathogens was noticed in four dogs. Our results highlight that veterinarians should be aware of the existence of canine VBD pathogens and should inform pet owners to take measures to protect their pets from vector-borne diseases using prophylactic treatments. Also,veterinarians should advise wherever appropriate undertaking diagnostic tests to establish the health status in order to provide the best possible treatment.

Keywords: dog, IgG antibodies, tick-borne diseases

REZUMAT

Bolile vectoriale canine reprezintă o problemă emergentă în medicina veterinară. Prezentul studiu analizează seroprevalența a trei agenți patogeni (*Anaplasma phagocytophilum, Borrelia burgdorferi și Ehrlichia canis*) care cauzează afecțiuni la câini în două județe din Estul României. În perioada martie-mai 2019 s-au recoltat probe de sânge de la 92 de câini din județele Iași (n=72) și Brăila (n=20). Toate probele de ser au fost testate utilizând trei kituri comerciale de imunoabsorbție enzimatică (ELISA) cu scopul de a detecta anticorpii IgG specifici împotriva *Borrelia spp*., *Anaplasma phagocytophilum* și respectiv *Ehrlichia canis.* În urma testării ELISA, 14 din cei 92 de câini testați au fost identificați ca fiind seropozitivi pentru anticorpii specifici IgG, dintre care 6 pentru *Borrelia spp*. (6.52 %), 5 pentru *Anaplasma phagocytophilum* (5.43%) și 3 pentru *Ehrlichia canis* (3.26%). Detecția simultană a anticorpilor IgG împotriva a doi agenți patogeni a fost identificată la patru câini. Rezultatele noastre evidențiază faptul că medicii veterinari ar trebui să fie conștienți de existența patogenilor care se transmit la câini, informând proprietarii despre tratamentele profilactice existente împotriva vectorilor. De asemenea, medicii veterinari trebuie să facă, acolo unde este cazul, teste corespunzătoare de diagnostic pentru a stabili starea de sănătate a animalelor, oferind cel mai bun tratament posibil.

Cuvinte cheie: câine, anticorpi IgG, boli transmise de căpușe

Canine vector-borne diseases (CVBDs) have become a focus of interest in recent years (20). In dogs, bacteria, such as *Anaplasma phagocytophilum, Borrelia burgdoferi* and *Ehrlichia canis* are among the major CVBDs (7). These important pathogens are transmitted by tick bites. *Borrelia burgdorferi*, the agent of the Lyme disease, is transmitted to humans and animals by *Ixodes spp.* ticks, during the blood meal.

Lyme disease is a potential health threat to dogs in Europe. Dogs seem to be the most susceptible domestic animal, and by some authors, serve as sentinel as well as an appropriate animal model to investigate human Lyme disease (11). The first specific symptom in dogs is lameness, due to myositis or arthritis, which generally occurs weeks or months after infection, but most dogs show no clinical signs, since active infection does not necessarily cause the illness (13). In 2011, Coipan and Vladimirescu, detected in the South-East of Romania 3 *B. burgdorferi s.l*. genospecies in *Ixodes ricinus* ticks: *B. afzelii, B. garinii,* and *B. valaisiana* (6).

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Anaplasma phagocytophilum is transmitted in Europe by *Ixodes ricinus* ticks and produces granulocythic anaplasmosis. The early cases in dogs were reported in the United States in 1982 and in Europe in 1988 (14). Clinical findings are represented by anorexia, lethargy, fever, lameness, thrombocytopenia, hypoalbuminemia, lymphopenia (12) and increased liver enzyme levels (5). In Romania, Matei et al. (2015) reported a prevalence of *Anaplasma phagocytophilum* of 3.4% after testing over 10.000 *Ixodes ricinus* questing ticks, using molecular biology techniques (15).

Ehrlichia canis is the etiological agent behind canine monocytic ehrlichiosis. *Rhipicephalus sanguineus* is the primary vector of *E. canis*, which is the cause of monocytic ehrlichiosis (9). The disease was first described in Algeria, in 1935 (8). In dogs, the most important clinical findings that may raise suspicion for ehrlichiosis include pyrexia, depression, pale mucosa, epistaxis and melena. Amongst these, epistaxis may be considered as the single most frequent attributing suspicion of ehrlichiosis (20).

In Romania there is still not sufficient data concerning canine vector-borne diseases. Serological surveys conducted in dogs for the detection of specific antibodies against *Borrelia spp*., *A. phagocytophilum* and *E. canis* offer a promising perspective in identifying the potential risk areas for humans. The aim of this study was to assess the presence of IgG antibodies against *B. burgdorferi*, *A.phagocytophilum* and *E.canis* in dogs in Eastern Romania in 2019.

MATERIALS AND METHODS

A total of 92 dog serum samples originating from two counties located in Eastern Romania (Iași and Brăila) were collected, between March and May 2019. Of these, 53 were sampled from males and 39 from females. The samples were collected from dogs kept in public shelters from both counties, Iași - Tomești shelter (n=37) and Brăila shelter (n=20). Another number of 35 samples were collected from dogs examined in veterinary private clinics in Iași (Table 1). All the samples collected from the veterinary clinics came from dogs diagnosed with babesiosis. In all cases informed con-sent was obtained from the owners and from the shelter veterinarian.

Blood samples were collected aseptically from the cephalic vein in tubes with clot activator for serum separation. The serum samples were centrifuged for 10 minutes at 3000 rotations per minute (RPM) and stored at – 20°C until use.

Table 1

Location and number of dogs sampled

County	No of samples		
	Males	Females	Total
Iași	41	31	72
Brăila	12	8	20
Total	53	39	92

Dog serums were analyzed using commercial enzyme-linked immunosorbent assays with the purpose to detect specific antibodies of the immunoglobulin G class (IgG) against *Borrelia* VIsE antigen (Anti-VIsE ELISA dog IgG, Euroimmun 23560 Lübeck, Germany), *Anaplasma phagocytophilum* (Anti-*Anaplasma phagocytophilum* ELISA Dog IgG, Euroimmun 23560 Lübeck, Germany), *Ehrlichia canis* (Anti - *Ehrlichia canis* ELISA Dog IgG, Euroimmun 23560 Lübeck, Germany), respectively. Serum samples were diluted 1:101 according to the manufacturer's instructions. Absorbance values were measured using a Microplate Reader (Tecan Sunrise, Tecan Trading AG, Switzerland) at 450 nm with 620 nm as a reference wave length.

RESULTS AND DISCUSSIONS

Details regarding gender, age, origin and living conditions were collected for each dog. In the study were included dogs aged between 4 months and 16 years. No formal randomized selection of the dogs was applied and none of the dogs were vaccinated against the Lyme disease.

Following the ELISA testing, 14 out of the 92 tested samples were positive for specific IgG antibodies against *Borrelia spp*. (n=6), *Anaplasma phagocytophilum* (n=5) and *Ehrlichia canis* (n=3).

The prevalence of specific IgG antibodies against *Borrelia spp*. was 6.52%. Differences in seroprevalence between locations were observed, dogs from Iași county showing the highest seroprevalence (8.69%) in comparison to Brăila county (2.17%). The highest seroprevalence in Iași suggests a higher exposure of dogs to ticks. Correlating the obtained results with gender, 3 (5.66%) out of 53 males and 3 (7.69%) out of 39 females were found positive.

The overall seroprevalence of anti-*Anaplasma phagocytophilum* IgG antibodies was 5.43% (n=5). Seropositive dogs were identified only in Iași county, of which 4 (10.25%) were females and 1 male (1.88%).

Specific IgG antibodies against *Ehrlichia canis* were detected only in Iași in 3 dogs (3.26%), of which 2 fe-

males (5.12%) and one male (1.88%).

Simultaneous detection of IgG antibodies against *B. burgdorferi* and *A. phagocytophilum* was registered in 3 dogs (3.26%). One of the three dogs was also identified with a borderline result for IgG *E. canis* antibodies. Simultaneous detection of IgG antibodies against *A. phagocytophilum* and *E. canis* was registered in one dog (1.08%). Details related to age, gender and origin of the seropositive dogs are presented in Table 2.

Table 2

Identification of seropositive dogs from Iași and Brăila counties

Gender and age	County	*Borrelia burgdorferi* IgG antibodies	*Anaplasma phagocytophilum* IgG antibodies	*Ehrlichia canis* IgG antibodies
Female, 12 years	Iași	+	-	-
Male, 1 year	Brăila	+	-	-
Male, 6 years	Iași	+	-	-
Male, 4 years	Iași	+	+	±*
Female, 1 year	Iași	+	+	-
Female, 3 years	Iași	+	+	-
Female, 2 years	Iași	-	+	-
Female, 2 years	Iași	-	+	+
Female, 5 years	Iași	-	-	+
Male, 4 years	Brăila	-	-	+

*Legend: +: Positive ; -: Negative ; ±: Borderline

In the recent years, worldwide distribution of vector-borne diseases in dogs (CVBDs) has been constantly changing. In the context of the current research, vector-borne diseases and in particular tick-borne diseases can significantly impact canine health status. Many factors are involved in the increased risk of developing vector-borne diseases, for example the increase of density of arthropod vectors can be explained by climate changes (3). Also, the impact and the importance of CVBDs are increased by the fact that most of them are zoonotic, transmissible from animals to humans.

The present study demonstrated the presence of canine tick-borne diseases in dogs from Eastern Romania, in 2019. Our study revealed similar results with a previous publish research on *Borrelia burgdoferi* antibodies prevalence in dogs and horses (10). However, a more recent study conducted by Raileanu et al. in 2015, reported a lower prevalence for *B. burgdoferi* in the Eastern Romania compared to our results (21). This facts highlights that in the recent years the prevalence of *B. burgdorferi* in dogs has increased in the region assessed in our study.

The highest seroprevalence in our study was obtained for *B. burgdoferi* antibodies. These findings could be explained by the significant number of samples coming from the public shelter dogs, which did not receive any prophylactic repellents or acaricides for protection. In accordance with the previously published studies, presence of seroreactivity to *B. burgdorferi* was not related to gender or age in the dogs' population (10), considering the fact that antibodies were detected in both males and females, with a wide age range (1 to 12 years).

In recent studies performed in Romania, Mircean et al. (2012) tested 1146 samples from 16 counties, collected from randomly selected dogs, using an ELISA SNAP 4Dx (IDEXX Laboratories, Inc., Westbrook, ME) (16). This study revealed a lower seroprevalence for *Borrelia burgdorferi sensu lato* (3.3%), approximately the same seroprevalence for *Anaplasma phagocytophilum* (5.5%) and a lower seroprevalence for *Ehrlichia canis* (2.1%), compared to our findings.

Several studies have reported the presence of CVB Ds using various diagnostic methods in Europe (1, 4, 18, 20). A study carried out in a neighbouring country, Bulgaria, in 2015 (20), reported a similar prevalence for *B. burgdorferi* (2.4%) and a much higher prevalence for *E. canis* (21%) and *A.phagocytophilum* (46.1 %). Furthermore, the seroprevalence values recorded in dogs in Italy, from 2013 to 2017, was lower for *A. phagocytophilum* (3.31%) and higher for *E. canis* (16.8%). These results could be related to the climatic conditions, which are known to strongly influence the presence of ticks, the increasing vector exposure depending on animals' age and/or immunological status (4). In Greece, Angelou et al. (2019) tested 1000 dogs, reporting a higher prevalence (7.9%) for *Ehrlichia spp*. and lower (2.7%) for *Anaplasma spp* (2). Other studies from the Balkan Peninsula have indicated an overall prevalence of CVBDs ranging from 25.7% in Croatia to 25.1% in Albania (18) while the highest prevalence rates have been observed in Spain (37.1%) (17) and Portugal (66%) (1).

CONCLUSIONS

Canine vector-borne diseases (CVBD) represent a growing challenge that practicing veterinarians are facing in Romania. Our results highlight that in Eastern Romania, seroprevalence of *B. burgdoferi spp* and *E. canis* increased compared to 2015 (21). Compared to the seroprevalence obtained in 2012 (16), the seroprevalence for *A. phagocytophilum* was similar.

Limited reports of CVBD prevalence, low index of suspicion for CVBD and poor availability of sensitive PCR-based diagnostic assays locally may lead to incorrect diagnosis and treatment of the animal. Additionally, more investigations need to be performed in order to establish the potential risk of canine vector-borne diseases in Eastern Romania. The emergence of parasitic and zoonotic diseases due to global warming, an increase in pet travel and expansion of disease vectors and in particular ticks are likely to have considerably influenced the distribution of CVBD agents in our country. Even though dog owners are informed by veterinarians about the risk of pet infections by tick-borne pathogens and even though prophylaxis against ticks is applied, *B. burgdorferi*, *E. canis* and *A. phagocytophilum* infections are present in Eastern Romania. The findings of this work indicate that veterinarians should be aware of the existence of CVBD pathogens and consider them in the differential diagnosis of dogs showing CVBD clinical signs and/or laboratory abnormalities. Most importantly, infection prevention should be carried out in a routine, on a regular basis, using prophylactic treatments in pets.

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