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Carnivores and Ixodid Ticks as Important Factors in the Emergence, Circulation and Distribution of Dangerous Infections

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Climate changes in the recent years led to a sharp rise in the tick population and an increase in the number of animals and people with tick-borne infections. The domestic and wild carnivores, especially the dogs, have a huge role for the distribution of ticks in certain areas. It is necessary to carry out a complex fight against the ticks and diseases transmitted by them, which includes systematic control of the tick population in a given area, as well as in-depth studies on their contamination with particular pathogens, especially those causing zoonoses. This work presents a short review on the recent research on the role of carnivores and ticks in the emergence, circulation and distribution of some dangerous viral, bacterial and parasitic infections.

Key words: carnivores, ixodid ticks, vector-borne diseases

Currently, multiple anthropogenic stressors - including climate change, habitat loss and fragmentation, urbanization, agricultural expansion and intensification, together with other changes in the use of water and land resources - are directly or indirectly impacting all species on earth. Such processes have also significant impacts on host-parasite interactions and infectious disease risks.

Due to the global changes (climate, economic and social) in the last decades, emergence of new serious infectious diseases of different etiology as well as spreading of already known diseases can be seen. Carnivores and ticks (Arachnida: Ixodidae) are involved in the emergence and circulation of some viral, bacterial and parasitic infections, including such with zoonotic character. In most of the cases, they appear to be the leading biotic factors in the distribution of these infections.

The aim of the present work is to perform a short overview on the studies conducted in this area in the recent years.

Prevalence data for tick-borne pathogens have been used to assess the risk for human health by Christova et al. [3]. The presence and identity of *Borrelia burgdorferi* sensu lato, *Ehrlichia*, *Anaplasma*, and *Rickettsia* species in Bulgarian *Ixodes ricinus* ticks and in non-*Ixodes* ticks from Turkey and Albania have been determined by polymerase chain reaction (PCR) and reverse line blot hybridization. In the adult Bulgarian ticks, the prevalence of *Borrelia burgdorferi* sensu lato infection has been approximately 40%, while *Borrelia afzelii* has been the predominant species, representing more than half of all *Borrelia*-positive ticks. *Ehrlichia* and *Anaplasma* species have been detected in 35% of the adult *Ixodes ricinus* ticks and in 10% of the nymphs. Sequence analysis of PCR products reacting with the *Anaplasma phagocytophila* probe has revealed a 16S rRNA gene identical to that of the *Anaplasma phagocytophila* prototype strain. *Ehrlichia* and *Anaplasma* species have been found in approximately 7% of the non-*Ixodes* ticks. Sequence analysis of some of these samples has revealed the presence of *Anaplasma ovis*, *Ehrlichia canis*, and species closely resembling *Ehrlichia chaffeensis*. About half of all adult ticks examined and approximately 20% of all nymphs have been infected with *Rickettsia* species. In *Ixodes ricinus* ticks, *Rickettsia helvetica* and *Rickettsia* species designated as IRS3 have been found in high prevalence. *Rickettsia conorii* has been found in virtually all non-*Ixodes* tick species from Albania and Turkey. The results of this study have shown that many tick-borne diseases are most probably endemic in the Balkan area. Furthermore, the results have suggested that there is a considerable chance for simultaneous transmission of tick-borne pathogens to human beings.

The occurrence of hard tick species (Acari: Ixodidae) infesting domestic dogs in Hungary has been studied by Földvári and Farkas [4]. Forty veterinary clinics from a wide geographical area have been asked to collect hard ticks from dogs and to complete a questionnaire. In total, 25 veterinary clinics submitted 900 ticks from 310 dogs. Intensity of infestation has ranged from one to 78 per dog. The most preferred sites of tick attachment in decreasing order have been head, neck and legs. The majority of ticks (91.7%) have been adults, which have been identified to species level, the others have been nymphs. Six species have been found: *Dermacentor reticulatus* (48.9%), *Ixodes ricinus* (43.2%), *Ixodes canisuga* (5.6%), *Haemaphysalis concinna* (2%) and there has been one specimen of both *Dermacentor marginatus* and *Ixodes hexagonus*. Single species infestation with *I. ricinus* or *D. reticulatus* has been found on 145 (46.8%) and 120 animals (38.7%), respectively. Mixed infestation caused by these two species has been detected on 24 dogs (7.7%). *I. canisuga* and *H. concinna* have been found on seven and five dogs, respectively. *D. reticulatus* and *I. ricinus* have been collected almost throughout the year, except for a single month. The activity peaks have been in spring and in autumn for both species. Based on clinical signs, canine babesiosis has been diagnosed by the veterinarians in 66 (21.3%) tick infested dogs. These dogs have been more frequently infested with *D. reticulatus* than the others.

A broad-range 16S rRNA gene PCR assay followed by partial sequencing of the 16S rRNA gene has been used for the detection of members of the family *Anaplasmataceae* in ticks in North Africa [12]. A total of 418 questing *Ixodes ricinus* ticks collected in Tunisia and Morocco, as well as 188 *Rhipicephalus* ticks from dogs and 52 *Hyalomma* ticks from bovines in Tunisia, have been included in this study. Of 324 adult *I. ricinus* ticks, 16.3% have been positive for *Ehrlichia* spp., whereas only 3.4% and 2.8% of nymphs and larvae, respectively, have been positive. A large heterogeneity has been observed in the nucleotide sequences. Partial sequences identical to that of the agent of human granulocytic ehrlichiosis (HGE) have been detected in *I. ricinus* and *Hyalomma detritum*, whereas partial sequences identical to that of *Anaplasma platys* have been detected in *Rhipicephalus sanguineus*. However, variants of *Anaplasma*, provisionally designated *Anaplasma*-like, have been predominant in the *I. ricinus* tick population in

Maghreb. Otherwise, two variants of the genus *Ehrlichia* have been detected in *I. ricinus* and *H. detritum*. Surprisingly, a variant of *Wolbachia pipientis* has been evidenced from *I. ricinus* in Morocco. These results have emphasized the potential risk of tick bites for human and animal populations in North Africa.

From 2000 to 2004, ticks have been collected by dragging a blanket in four habitat areas in the Netherlands: dunes, heather, forest, and a city park [14]. Tick densities have been calculated, and infection with *Borrelia burgdorferi* and *Anaplasma* and *Ehrlichia* species has been investigated by reverse line blot analysis. The lowest tick density has been observed in the heather area (1 to 8/100 m^2). In the oak forest and city park, the tick densities have ranged from 26 to 45/100 m^2. The highest tick density has been found in the dune area (139 to 551/100 m^2). The infection rates varied significantly for the four study areas and years, ranging from 0.8 to 11. 5% for *Borrelia* spp. and 1 to 16% for *Ehrlichia* or *Anaplasma* (*Ehrlichia/Anaplasma*) spp. *Borrelia* infection rates have been highest in the dunes, followed by the forest, city park, and heather area. In contrast, *Ehrlichia/Anaplasma* has been found most often in the forest and less often in the city park. The following *Borrelia* species have been found: *Borrelia* sensu lato strains not identified to the species level (2.5%), *B. afzelii* (2.5%), *B. valaisiana* (0.9%), *B. burgdorferi* sensu stricto (0.13%), and *B. garinii* (0.13%). *Borrelia lusitaniae*, *Ehrlichia chaffeensis*, and the human granylocytic anaplasmosis agent have not been detected. About 1.6% of the ticks have been infected with both *Borrelia* and *Ehrlichia/Anaplasma*, which has been higher than the frequency predicted from the individual infection rates, suggesting hosts with multiple infections or a possible selective advantage of coinfection.

A population of 731 naturally exposed pet dogs examined at a private practice in Baxter, Minnesota, an area endemic for Lyme disease and anaplasmosis, has been tested by serological and molecular methods for evidence of exposure to or infection with selected vector-borne pathogens [8]. Serum samples have been tested by enzyme-linked immunosorbent assay (ELISA) for *Aanaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Ehrlichia canis* antibodies and for *Dirofilaria immitis* antigen. Blood samples from 273 dogs have been also analyzed by polymerase chain reaction (PCR) for *Anaplasma* and *Ehrlichia* species DNA. Based on the owner history and the attending veterinarian‘s physical examination findings, dogs exhibiting illness compatible with anaplasmosis or borreliosis have been considered clinical cases, and their results have been compared to the healthy dog population. Antibodies to only *A. phagocytophilum* have been detected in 217 (29%) dogs; to only *B. burgdorferi*, in 80 (11%) dogs; and seroreactivity to both organisms, in 188 (25%) dogs. Of 89 suspected cases of canine anaplasmosis or borreliosis, *A. phagocytophilum* or *B. burgdorferi* antibodies have been detected in 22 dogs (25%) and 8 dogs (9%) respectively, whereas antibodies to both organisms have been found in 38 dogs (43%). *Ehrlichia canis* antibodies and *D. immitis* antigen have been each detected in 11 (1.5%) dogs. *Anaplasma phagocytophilum* DNA has been amplified from 7 of 222 (3%) healthy dogs and 19 of 51 (37%) clinical cases. Seroreactivity to both *A. phagocytophilum* and *B. burgdorferi* has been detected more frequently in suspected cases of anaplasmosis and/or borreliosis than seroreactivity to either organism alone. Based on PCR testing, *A. phagocytophilum* DNA has been more prevalent in suspected cases of anaplasmosis or borreliosis than in healthy dogs from the same region.

Sobrino et al. [13] have studied the ixodid tick fauna of wild carnivores in Peninsular Spain and the environmental factors driving the risk of wild carnivores to be parasitized by ixodid ticks. They have hypothesized that the adaptation of tick species to differing climatic conditions may be reflected in a similar parasitization risk of wild carnivores by ticks between bioclimatic regions in the study area. To test this, the authors have surveyed ixodid ticks in wild carnivores in oceanic, continental-Mediterranean, and

thermo-Mediterranean bioclimatic regions of Peninsular Spain. They have analyzed the influence of environmental factors on the risk of wild carnivores to be parasitized by ticks by performing logistic regression models. Models have been separately performed for exophilic and endophilic ticks under the expected differing influence of environmental conditions on their life cycle. Differences in the composition of the tick community parasitizing wild carnivores from different bioclimatic regions have been found. Modelling results partially have confirmed the null hypothesis because bioclimatic region has not been a relevant factor influencing the risk of wild carnivores to be parasitized by exophilic ticks. Bioclimatic region has been however a factor driving the risk of wild carnivores to be parasitized by endophilic ticks. The authors consider that Spanish wild carnivores are hosts to a relevant number of tick species, some of them being potential vectors of pathogens causing serious animal and human diseases.

In all, 1146 serum samples have been tested in Romania by SNAP® 4Dx® (IDEXX Laboratories, Inc., Westbrook, ME) for *Anaplasma phagocytophilum, Borrelia burgdorferi,* and *Ehrlichia canis* antibodies, and for *Dirofilaria immitis* antigen [9]. The correlation between positive cases and their geographic distribution, as well as potential risk factors (age, sex, breed, type of dog, habitat, and prophylactic treatments) have been evaluated. Overall, 129 dogs (11.3%) have been serologically-positive to one or more of the tested pathogens. The seroprevalence for the four infectious agents has been: *A. phagocytophilum* 5.5% (63/1146), *D. immitis* 3.3% (38/1146), *E. canis* 2.1% (24/1146), and *B. burgdorferi* 0.5% (6/1146). Co-infection with *E. canis* and *A. phagocytophilum* has been registered in 2 dogs (0.2%). According to the authors the geographical distribution of the seropositive cases suggests clustered foci in southern regions and in the western part of the country for *D. immitis*, and in the southeastern region (Constanţa County) for *E. canis*. *A. phagocytophilum* and *B. burgdorferi* have showed a homogenous distribution, with a tendency for Lyme-positive samples to concentrate in central Romania. Associated risk factor with infection has been the type of dog (stray dogs have been at risk being positive for *D. immitis,* shelter dogs for *E. canis*, and hunting dogs for *B. burgdorferi*). The prevalence of *D. immitis* has been significantly higher in males and in dogs older than 2 years.

A South African strain of *Ehrlichia canis* has been isolated and used to infect a laboratory-bred Beagle dog [5]. *Rhipicephalus sanguineus* nymphs, fed on this dog, moulted to adult ticks with infection rates of *E. canis* between 12% and 19% have been used in a series of *in vivo* and *in vitro* experiments. Five groups of 6 dogs have been challenged with the infected *R. sanguineus* ticks, which have been removed 24 h, 12 h, 6 h or 3 h after the ticks had been released onto the dogs. The animals have been monitored for fever and thrombocytopenia and have been considered infected if they became serologically positive for *E. canis* antibodies as well as PCR positive for *E. canis* DNA. Seven dogs have been infected with *E. canis* in the following groups: Group 1 (24 h tick challenge) 1 out of 6; Group 2 (12 h) 1 of 6; Group 3 (6 h) 2 of 6; Group 4 (6 h) 2 of 6 and Group 5 (3 h) 1 out of 6. Six of those 7 infected dogs have developed fever and a significant thrombocytopenia. One dog has not shown any symptoms, but has been found PCR positive on several occasions. Five additional dogs have been PCR positive on one test sample only but have not been considered infected because they have not developed any specific *E. canis* antibodies. *In vitro*, *R. sanguineus* ticks have attached and fed on bovine blood through silicone membranes with attachment rates up to 72.5% after 24 h increasing to 84.2% at 72 h. The ticks have transmitted *E. canis* as soon as 8 h post application as demonstrated by *E. canis* DNA found in the nutritive blood medium. The authors have concluded that transmission of *E. canis* by *R. sanguineus* ticks starts within a few hours after attachment, which is earlier than previously thought. These findings underpin the need for acaricides to

provide either a repellent, an anti-attachment and/or a rapid killing effect against ticks in order to decrease the risk of transmission of *E. canis*.

To monitor the emergence of thermophilic, Mediterranean ixodid tick species and tick-borne pathogens in Southern Hungary, 348 ticks have been collected from shepherd dogs, red foxes and golden jackals during the summer of 2011 [6]. Golden jackals have shared tick species with both the dog and the red fox in the region. *Dermacentor* nymphs have been collected exclusively from dogs, and the sequence identification of these ticks has indicated that dogs are preferred hosts of both *D. reticulatus* and *D. marginatus* nymphs, unlike previously reported. Subadults of three ixodid species have been selected for reverse line blot hybridisation (RLB) analysis to screen their vector potential for 40 pathogens/groups. Results have been negative for *Anaplasma*, *Babesia* and *Theileria* spp. Investigation of *D. marginatus* nymphs has revealed the presence of *Ehrlichia canis*, *Rickettsia massiliae* and *Borrelia afzelii* for the first time in this tick species. *Ehrlichia canis* has been also newly detected in *Ixodes canisuga* larvae from red foxes. The authors have concluded: in absence of transovarial transmission in ticks this implies that Eurasian red foxes may play a reservoir role in the epidemiology of canine ehrlichiosis.

Blood samples and ticks have been collected from 100 shepherd dogs, 12 hunting dogs and 14 stray dogs in southern Hungary, in order to screen them for the presence of *Hepatozoon* spp. by PCR [7]. Out of 126 blood samples, 33 have been positive (26%). Significantly more shepherd dogs (31%) have been infected, than hunting (8%) and stray dogs (7%). Three genotypes of *Hepatozoon canis* have been identified by sequencing, differing from each other in up to six nucleotides in the amplified portion of their 18S rRNA gene. In *Dermacentor marginatus* larvae/nymphs and *Dermacentor reticulatus* nymphs, *H. canis* has been present only if they had been collected from PCR-positive dogs, and the genotypes have been identical in the ticks and their hosts. However, two *Haemaphysalis concinna* nymphs removed from a PCR-negative dog have been found positive for *H. canis*, and the genotype detected in specimens of this tick species differed from that in the blood of their respective hosts. These results have indicated that canine hepatozoonosis may be highly prevalent in regions where *Rhipicephalus sanguineus* is considered to be non-endemic. Canine hepatozoonosis has been significantly more prevalent west of the Danube river (where higher densities of red fox and golden jackal populations occur), suggesting a role of wild carnivores in its epidemiology.

In order to detect *Ehrlichia* spp. in cats from the central-western region of Brazil, blood and serum samples have been collected from a regional population of 212 individuals originated from the cities of Cuiabá and Várzea Grande [1]. These animals have been tested by the Immunofluorescence Assay (IFA) and the Polymerase Chain Reaction (PCR) designed to amplify a 409 bp fragment of the *dsb* gene. The results obtained have shown that 88 (41.5%) of the cats have been seropositive by IFA and 20 (9.4%) of the cats have been positive by PCR.

The seroprevalence of important canine vector-borne diseases (CVBDs) in 167 dogs from Central-Southern Bulgaria (Stara Zagora), with special emphasis on hitherto uninvestigated babesiosis and angiostrongylosis, on poorly investigated Lyme borreliosis and canine granulocytic anaplasmosis, and on the potentially zoonotic dirofilariosis and leishmaniosis has been determined by Pantchev et al. [11]. Relatively high prevalence rates have been documented for anti-*Babesia canis* antibodies, *Dirofilaria immitis* antigen (16.2 %; 27/167 each), anti-*Ehrlichia canis* (21 %; 35/167) and anti-*Anaplasma phagocytophilum* antibodies (30.5 – 46.1 %; 51 – 77/167), while *Borrelia burgdorferi* seroprevalence has been low (2.4 %; 4/167). All samples have been negative for *Leishmania infantum* antibodies and *Angiostrongylus*

vasorum antigen and antibodies. In total, 64.7 % (108/167) of the samples indicated infection or exposure to at least one agent and a high proportion of dual infections (39.8 %; 43/108) has been demonstrated. Multiple infections with up to four different organisms have been also detected. The authors underline the importance of CVBDs and especially of co-infections which could influence the clinical outcome in dogs.

Çetinkaya et al. [2] have studied the presence of *Anaplasma* spp., and *Ehrlichia* spp. in dogs and ticks in the Thrace region of Turkey. A total of 400 blood samples and 912 ticks have been collected from dogs living in shelters that are located in four cities (Istanbul, Edirne, Tekirdag and Kirklareli) of the Thrace Region. Blood and buffy coat smears have been prepared for microscopic examination. Hematologic and serologic analyses have been performed using cell counter and commercial Snap3Dx test kit, respectively. Eight hundred fifty of collected ticks have been classified as *Rhipicephalus* sanguineus, 33 as *Rhipicephalus turanicus* and 29 as *Ixodes ricinus*. After DNA extraction from blood samples and pooled ticks (127 tick pools, in total), nested PCR has been performed to detect the DNA of *Anaplasma* spp., and *Ehrlichia* spp. The seroprevalence of Ehrlichia *canis* has been 27.25% (109) by Snap3Dx test and the total molecular positivity has been 11.75% (47) in dog blood samples and 21.25% (27) in tick pools by nested PCR. The frequencies of the infected blood samples with *E. canis*, *Anaplasma phagocytophilum* and *Anaplasma platys* have been detected as 6%, 4% and 6%, respectively. *E. canis* and *A. platys* have been detected in *R. sanguineus* pools with a ratio of 15.75% and 0.7%, respectively. In addition, *A. platys* has been also detected in *R. turanicus* pools (0.7%). *A. phagocytophilum* has been found only in *I. ricinus* pools (3.93%). Morulae of three species have been detected in buffy coat and blood smears. While anemia has been observed in dogs infected with *E. canis* and co-infected (with one or more species), thrombocytopenia has been observed only in co-infected dogs. Based on the results of the tests used in this study, the authors have recommended the combined use of serologic, molecular, cytologic, hematologic analyses and physical examination of tick exposure for an accurate diagnosis of ehrlichiosis and anaplasmosis.

Nader et al. [10] have performed a study intended to detect the prevalence of tick-borne bacteria and parasites occurring at the Black Sea in Bulgaria and evaluate the zoonotic potential of the tick-borne pathogens transmitted by ticks in this area. In total, cDNA from 1541 ticks (*Dermacentor* spp., *Haemaphysalis* spp., *Hyalomma* spp., *Ixodes* spp. and *Rhipicephalus* spp.) collected in Bulgaria by flagging method or from hosts has been tested in pools of ten individuals each for *Anaplasma phagocytophilum*, *Babesia* spp., *Borrelia burgdorferi* (s.l.), *Rickettsia* spp. and "Candidatus Neoehrlichia mikurensis" via conventional and quantitative real-time PCR. Subsequently, samples from positive pools have been tested individually and a randomized selection of positive PCR samples has been purified, sequenced, and analyzed. Altogether, 23.2% of ticks have been infected with at least one of the tested pathogens. The highest infection levels have been noted in nymphs (32.3%) and females (27.5%). Very high prevalence has been detected for *Rickettsia* spp. (48.3%), followed by *A. phagocytophilum* (6.2%), *Borrelia burgdorferi* (s.l.) (1.7%), *Babesia* spp. (0.4%) and "Ca. Neoehrlichia mikurensis" (0.1%). Co-infections have been found in 2.5% of the tested ticks (mainly *Ixodes* spp.). Sequencing has revealed the presence of *Rickettsia monacensis*, *R. helvetica*, and *R. aeschlimannii*, *Babesia microti* and *B. caballi*, and *Theileria buffeli* and *Borrelia afzelli*. This study has shown very high prevalence of zoonotic *Rickettsia* spp. in ticks from Bulgaria and moderate to low prevalence for all other pathogens tested. The authors underline the risk that the tick bites from this area could lead to *Rickettsia* infection in humans and mammals.

As a conclusion to the current literature review, the following can be said:

In the recent years carnivores and ixodid ticks most often have been studied as factors in the development of the ehrlichiosis, anaplasmosis and borreliosis. Fewer studies in this connection have been conducted to babesiosis, dirofilariosis, and ricketsiosis. Single ones have about leishmaniasis, *Hepatozoon* sp. and "Candidatus Neoehrlichia mikurensis".

The studies carried out have mainly aimed at determining the species composition of ticks that have been spread and affected carnivores in different geographic areas, as well as the pathogens identified in them. For this purpose both standard immunological methods of investigation and newer molecular-biological methods are used.

Conclusions

Here are some conclusions from different research:

● The combined use of serologic, molecular, cytologic, hematologic analyses and physical examination of tick exposure for an accurate diagnosis of vector-borne diseases is recommended.

● The percentige of the ticks, infected with both *Borrelia* and *Ehrlichia/Anaplasma*, which has been higher than the frequency predicted from the individual infection rates, suggesting hosts with multiple infections or a possible selective advantage of coinfection.

● Multiple infections with up to four different organisms have been also detected in other study. Its authors underline the importance of Canine vector-borne diseases and especially of co-infections which could influence the clinical outcome in dogs.

● Wild carnivores are hosts to a relevant number of tick species, some of them being potential vectors of pathogens causing serious animal and human diseases.

● Eurasian red foxes may play a reservoir role in the epidemiology of canine ehrlichiosis.

● Red fox and golden jackal may have an important role in epidemiology of Canine hepatozoonosis.

● Associated risk factor with the vector-borne diseases is the type of dog (stray dogs have been at risk being positive for *Dirofilaria immitis,* shelter dogs for *Ehrlichia canis*, and hunting dogs for *Borrelia burgdorferi*).

● Cats have been positive for *Ehrlichia* spp.

● Many tick-borne diseases are most probably endemic in the Balkan area.

● The prevalence of zoonotic *Rickettsia* spp. in ticks from Bulgaria established in one of the studies has been very high. The risk that the tick bites from this area could lead to *Rickettsia* infection in humans and mammals has been underlined.

● Based on clinical signs, canine babesiosis has been diagnosed by the veterinarians in dogs, more frequently infested with *Dermacentor reticulatus* than other tick species.

● Human granulocytic ehrlichiosis have been detected in *Ixodes ricinus* and *Hyalomma detritum*.

● Transmission of *E. canis* by *Rhipicephalus sanguineus* ticks starts within a few hours after attachment, which is earlier than previously thought.

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