

## WILD CANIDS AS HOSTS FOR TICKS AND TICK-BORNE ZOOBOTIC PATHOGENS IN SERBIA

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### Abstract

Wild canids are recognized as important reservoir hosts for several vector-borne pathogens and are a good sentinel species for epidemiological surveillance. Identifying the reservoir hosts for particular zoonotic pathogens in a specific area enables the implementation of adequate measures in order to prevent the occurrence of zoonotic diseases in humans and domestic animals. The present review aims to summarize the role of wild canids in the epidemiology of tick-borne zoonoses in Serbia based on previously published papers and to discuss the available data from an epidemiological point of view. The results of previously published studies indicate that wild canids are potential reservoir hosts for several zoonotic tick-borne pathogens in Serbia, and hosts for several tick species which are proven competent vectors. Based on molecular studies conducted so far, the presence of *Anaplasma phagocytophilum*, was confirmed in jackals while the presence of Candidatus *Neobabesia micurensis* (FU98), *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia lusitaniae* was proven by PCR in analyzed red foxes. There is a need for continuous epidemiological monitoring of diseases caused by pathogens previously confirmed in wild canids. Also, further research is needed to reveal the role of other wild and domestic animals in the epidemiology of tick-borne zoonoses in Serbia.

**Key words:** foxes, jackals, PCR, Serbia, vector-borne pathogens, wild canids

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## INTRODUCTION

In recent decades, vector-borne zoonoses (VBZ) have attracted considerable attention from the scientific community. Due to global climate changes, species that can serve as vectors of infectious agents with zoonotic potential (ticks, sand flies, and mosquitoes) are spreading their range year-to-year. Consequently, some VBZ occur nowadays in regions where they were not registered earlier or they occur more frequently in areas where they occurred sporadically (Dantas-Torres, 2015; Negev et al., 2015). On the other hand, shifts in population dynamics of some vertebrate species that are potential VBZ reservoirs necessitate constant epizootiological surveillance to prevent increasing risks of VBZ for human and animal health.

Wild canids are recognized as important reservoir hosts for several vector-borne pathogens and are good sentinel species for epidemiological surveillance (Tomassone et al., 2018; Aguirre, 2009), while previous faunistic studies also confirm that more tick species now parasitize wild canids in Europe (Gherman and Mihalca, 2017; Tomanović et al., 2013b).

Within the order Carnivora (*lat. carne* = flesh and *vorare* = to devour) are more than 280 species of placental mammals, mainly predators, while family Canidae is among the oldest representatives of the order Carnivora. Five wild canid species are autochthonous in Europe: golden jackal (*Canis aureus*), red fox (*Vulpes vulpes*), grey wolf (*Canis lupus*), raccoon dog (*Nyctereutes procyonoides*) and arctic fox (*Alopex lagopus*). All but the last are present in Serbia. Due to their abundance and presence over a wide area of the country, jackals and foxes have the greatest epidemiological significance among wild canids in Serbia. Despite the increase in population and the current expansion of the golden jackals' area in southeastern Europe, as well as the animals' tendency to come close to the settlements (Trouwborst et al., 2015), only a few studies so far have addressed the role of jackals in the maintenance and spread of zoonotic agents. Also, although red foxes are the most abundant medium-sized predators and range throughout Serbia (Ćirović, 2000) the role of foxes in enzootic cycles of the vector-borne zoonotic pathogens in our country is still poorly understood.

### **Role of jackals and foxes in the epidemiology of tick-borne zoonoses in Serbia**

The epidemiology of tick-borne diseases is complex since several factors are involved in their maintenance in the environment. The most important factors are: the biology of the microorganism itself, vectors, hosts, and environmental factors.

Faunistic studies have confirmed that several tick species important from the epidemiological standpoint parasitize on jackals and foxes in Serbia. The presence of *Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus*, *Haemaphysalis concinna* and *Rhipicephalus sanguineus* s.l. was confirmed on hunted jackals (Sukara et al., 2018; Stojanov et al., 2014), while ten tick species (*Ixodes ricinus*, *I. hexagonus*, *I. canisuga*,

*I. kaiseri*, *D. reticulatus*, *D. marginatus*, *R. sanguineus* s.l., *Haemaphysalis punctata*, *H. concinna*, and *Haemaphysalis inermis*), were confirmed as ectoparasites of red foxes in Serbia (Hornok et al., 2017a; Stojanov et al., 2014; Tomanović et al., 2013b; Pavlović et al., 2001). Several previously mentioned tick species have been confirmed to harbor pathogens important from a medical and veterinary point of view, such as *Anaplasma phagocytophilum*, *Anaplasma ovis*, *Francisella tularensis*, *Rickettsia* spp., *Borrelia burgdorferi* sensu lato complex (s.l.), *Coxiella burnetii*, and *Babesia canis* (Potkonjak et al., 2016; Mihaljica et al. 2012; Tomanović et al., 2009, 2010, 2013; Milutinović et al., 2006, 2008, 2012). Based on all available scientific papers concerning ticks in Serbia published so far, the most prevalent tick species parasitizing jackals and foxes is *I. ricinus* which is also the most common species found on vegetation and as a human parasite in Europe. This tick species is not host-specific and parasitizes a wide range of wild and domestic animals (over 300 animal species) (Gern, 2009). Also, *I. ricinus* is a proven competent vector for several zoonotic tick-borne pathogens (Medlock et al., 2013).

In addition to the epizootiological role that jackals and foxes have as potential reservoir hosts for tick-borne pathogens, these wild canids could also be responsible for the mechanical transmission and geographic expansion of specific tick species. Since jackals and foxes live near human settlements, it is likely that some of the life stages of the important tick species in Serbia parasitize domestic animals or humans, including *I. ricinus*, the most common anthrophilic species. These factors delineate the importance of jackals and foxes in enzootic cycles of tick-borne pathogens.

To reveal the exact roles of some wild canid species in enzootic cycles of vector-borne zoonotic pathogens, molecular methods are of great importance. Even though the confirmed presence of a particular pathogen by PCR assay in wild canid tissue is not valid evidence of that same species being a reservoir, this approach gives valuable information that can help elucidate the complete enzootic cycle of the tick-borne pathogen in the investigated location. Research based on molecular methods conducted in Serbia identified jackals as potential reservoirs for *A. phagocytophilum*, while the presence of Candidatus *Neorhlichia micurensis* (FU98), *Borrelia burgdorferi* sensu stricto (s.s.), *B. garinii*, and *B. lusitaniae* were confirmed in analyzed red foxes. Still, the epidemiology of VBZ caused by these agents (anaplasmosis, neorhlichiosis, Lyme borreliosis) is not completely elucidated in Serbia.

The family Anaplasmataceae (order Rickettsiales) includes the genera *Ehrlichia*, *Anaplasma*, *Neorickettsia*, and *Wolbachia*. These are small, obligatory intracellular, pleomorphic Gram-negative bacteria. More species within these genera are emerging vector-borne pathogens with zoonotic potential (Cézanne et al., 2017).

*A. phagocytophilum* is an emerging tick-borne pathogen with a global distribution that infects humans and a wide range of domestic and wild animals. The main vector of *A. phagocytophilum* in Europe is *I. ricinus*, while wild animals are considered as important reservoirs of different strains of *A. phagocytophilum*. The role of certain wildlife species

as potential reservoirs is still unknown and justifies further epidemiological studies (Stuen et al., 2013).

Data on the role of wild canids in the maintenance of *A. phagocytophilum* are scarce. The presence of DNA of this zoonotic pathogen was confirmed in foxes from Poland (2.7%), Czech Republic (4.0%), and Italy (16.6%), indicating the importance of foxes in the sylvatic cycle of *A. phagocytophilum* (Ebani et al., 2011; Karbowski et al., 2009; Hulinska et al., 2004). One case of acute granulocyte anaplasmosis in a captive wolf in Austria has also been described (Leschnik et al., 2012).

The eco-epidemiology of *A. phagocytophilum* in Serbia is largely unknown and insufficiently researched. The presence of *A. phagocytophilum* was confirmed by molecular methods in *I. ricinus* ticks collected from vegetation at several localities in central Serbia, Vojvodina, and the Belgrade area, with average prevalence of 13.9% (Milutinović et al., 2008). No cases of human granulocytic anaplasmosis (HGA), a disease caused in humans by *A. phagocytophilum*, have been officially confirmed in Serbia (Dumler, 2005). So far, only two cases of human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis* belonging to the Anaplasmataceae family have been described (Arsić et al., 2014; Đokić et al., 2006). There is a possibility that HGA occurs as a febrile illness of unclear etiology, but remains undiagnosed due to its nonspecific symptoms.

Serological studies conducted in dogs in Serbia confirmed they are exposed to *A. phagocytophilum*. Using the immunofluorescence antibody test (IFAT), a seroprevalence of 15.5% was recorded in analyzed dogs from Vojvodina (Potkonjak et al., 2015), while dogs from Belgrade had a seroprevalence of 26.1% (Kovačević Filipović et al., 2018). Although previous studies indicate that dogs are exposed to the causative agent of canine anaplasmosis, there have been no confirmed cases of this disease in Serbia to date. A recent study conducted in dairy cows confirmed the presence of antibodies to *A. phagocytophilum* in 2.45% of the tested animals from the areas of Gruža and Požarevac (Vasić et al., 2018).

For the first time in Europe, a recently conducted study in Serbia has indicated to jackals as potential reservoirs of *A. phagocytophilum*. Two animals (0.9%) originating from the locality Smederevo and Velika Plana were positive for *A. phagocytophilum* DNA (Sukara et al., 2018). This finding and the fact that jackals inhabit around 70% of the country (Ćirović et al., 2018) together with the confirmed presence of *I. ricinus* as the main vector of *A. phagocytophilum* on jackals (Sukara et al., 2018), give us a basis to conclude that jackals have a potential role in the maintenance of the causative agent of human and animal anaplasmosis in Serbia. Further research is needed on jackals and other wild canids (foxes and wolves) to elucidate the role of these animals in the epidemiology of *A. phagocytophilum*.

A recent study on the role of foxes in the epidemiology of VBZ in Serbia, has confirmed the presence of a newly discovered member of the family Anaplasmataceae, *Candidatus* Neoehrlichia sp., sequence type FU98, and indicated foxes could be potential reservoir

hosts for the causative agents of Lyme borreliosis, the spirochetes *Borrelia burgdorferi* s.l. (Sukara et al., 2019).

*Neoehrlichia mikurensis*, an emerging tick-borne zoonotic pathogen, and *Candidatus* *Neoehrlichia lotoris* (non-zoonotic) are currently proposed to be members of the family Anaplasmataceae. *Candidatus* *Neoehrlichia* sp. FU98, which is distinct from *N. mikurensis*, but more closely related to *Candidatus* *Neoehrlichia lotoris*, was confirmed for the first time in foxes from Austria (Hodžić et al., 2015), then in Czech Republic (Hodžić et al., 2017), and recently in Serbia, where an overall prevalence of 4.7% was recorded (Sukara et al., 2019). Positive foxes originated from three localities: Svilajnac, Veliko Gradište, and Surčin.

The potential reservoir role of other wild animals in Europe has been proposed after the confirmed presence of *Candidatus* *Neoehrlichia* sp. FU98 in raccoon dogs and badgers (Hildebrand et al., 2018; Hornok et al., 2017b). More recently, a strain similar to *Candidatus* *Neoehrlichia* sp. FU98 was reported in domestic dogs from Hungary (Hornok et al., 2018). Further research is needed to investigate the pathogenic and zoonotic potential of this zoonotic pathogen and the exact role of foxes and other animals in the epizootiology of *Candidatus* *Neoehrlichia* sp. FU98.

Maintenance of the causative agents of Lyme borreliosis in the environment occurs in enzootic cycles that include vertebrates and ticks. In Europe, *I. ricinus* is the main vector, but the roles of many vertebrates as potential reservoir hosts are still unknown (Franke et al., 2013). Previous seroepidemiological studies in Serbia confirmed that domestic dogs are exposed to *B. burgdorferi* s.l. (Obrenović et al., 2015; Potkonjak et al., 2013), while a high diversity of *Borrelia* species (*B. burgdorferi* s.s., *Borrelia afzelii*, *B. lusitaniae*, *B. garinii*, *Borrelia bavariensis*, and *Borrelia valaisiana*) were confirmed in *I. ricinus* ticks collected from vegetation (Čakić et al., 2019; Tomanović et al., 2008).

Although it has been previously confirmed that foxes are competent reservoirs for *B. burgdorferi* s.l. (Liebisch et al., 1995), only a few studies to date have addressed the role of red foxes in the epizootiology of Lyme borreliosis in Europe (Dumitrache et al., 2015; Heidrich et al., 1999). Recently, for the first time, the potential role of red foxes as reservoir hosts for *B. burgdorferi* s.s., *Borrelia g* (proven causative agents of Lyme borreliosis), and *B. lusitaniae* was proposed in Serbia. The total prevalence of these zoonotic pathogens in red foxes was 5.4%, recorded by molecular methods (Sukara et al., 2019). So far there is no available data on the role of other wild animals in the epidemiology of Lyme disease in our country. Future research should be directed towards identifying the exact role of foxes and other vertebrate hosts in the maintenance of Lyme borreliosis agents in Serbia.

## CONCLUSION

Due to increased contact between wildlife, domestic animals, and humans worldwide, and the fact that these interactions in the future will be more frequent, there is a need

for constant epidemiological surveillance of VBZ in wildlife populations. Identification of the reservoir hosts in a specific area is a prerequisite for the implementation of adequate measures to prevent the occurrence of zoonotic diseases in domestic animals and humans. The results of previous studies conducted in Serbia confirmed that jackals and red foxes are good hosts for several tick species and are potential reservoir hosts for several tick-borne zoonotic pathogens. Further research is needed to discover the exact role of wild canids and other wild and domestic animals in the epidemiology of VBZ in Serbia.

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### Authors' contributions

SR and TS designed the content of manuscript; SR wrote the manuscript; TS, MD, and GV participated in manuscript writing and revised it critically.

### Competing interests

The authors declare they have no competing interests

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## DIVLJE KANIDE KAO DOMAĆINI ZA VEKTORIMA PRENOSIVE ZOONOZNE PATOGENE U SRBIJI

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### Kratak sadržaj

Divlje kanide su prepoznate kao važni domaćini rezervoari za nekoliko vektorima prenosivih zoonotskih patogena i predstavljaju dobre sentinel vrste za epidemiološki nadzor. Identifikacija domaćina rezervoara za pojedine zoonotske patogene na određenom području omogućava primenu odgovarajućih mera kako bi se sprečila pojava zoonoza kod ljudi i domaćih životinja. Cilj ove studije bio je da se predstavi uloga divljih kanida u epidemiologiji zoonoza koje se prenose vektorima u Srbiji na temelju prethodno objavljenih radova i da se dostupni podaci prodiskutuju sa epidemiološkog stanovišta. Na osnovu dosadašnjih molekularnih istraživanja u Srbiji, divlje kanide su prepoznate kao potencijalni rezervoari za nekoliko krpeljima prenosivih zoonotskih patogena. Kod šakala je potvrđeno prisustvo *Anaplasma phagocytophilum*, dok je prisustvo Candidatus *Neobabesia micurensis* (FU98), *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* i *Borrelia lusitaniae* molekularnim metodama potvrđeno kod lisica. Rezultati pokazuju da su divlje kanide potencijalni domaćini rezervoari i izvor zoonotskih patogena u Srbiji i da predstavljaju dobre domaćine za više vrsta krpelja koji su potvrđeni kompetentni vektori pojedinih patogena.

Potrebno je kontinuirano epidemiološko praćenje bolesti uzrokovanih patogenima čije je prisustvo prethodno potvrđeno kod divljih kanida. Takođe, potrebna su dodatna istraživanja kako bi se otkrila uloga drugih divljih i domaćih životinja u epidemiologiji zoonoza koje se prenose vektorima u našoj zemlji.

**Ključne reči:** lisice, šakali, PCR, Srbija, vektorima prenosivi patogeni, divlje kanide