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Review

TICK-BORNE INFECTIONS OF DOGS IN SERBIA: A REVIEW OF RESEARCH

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Abstract

Increased prevalence of tick-borne disease in humans and animals is an important public health issue. Dogs can be clinically ill, reservoirs of tick-borne pathogens, or sentinel populations for research. Certainly, small-scale research has been conducted in dogs and this review will focus on the recent studies of emergent and re-emergent tick-borne pathogens in dogs in Serbia. Babesiosis stands out as the most common and important tick-borne disease in dogs. In addition to this pathogen, the presence of *Hepatozoon canis* and "*Candidatus* Mycoplasma haematoparvum" in the blood of dogs was proved using molecular methods. Seroreactivity in dogs has been proven for *Borrelia burgdorferi* sensu lato (s.l.) complex, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum, Anaplasma platys, Rickettsia conorii*, and tick-borne encephalitis virus (TBEV). The presence of *B. burgdorferi* s.l. complex, *A. phagocytophilum*, *Rickettsia* spp. from the group of spotted fevers, and TBEV in ticks indicates the geographical distribution and potential for infecting humans, dogs, and other animals in Serbia. Further systematic, comprehensive, well designed, bacteriological, virological, parasitological, epidemiological, clinical (infectological), and acarological research is needed in Serbia.

Key words: dogs, review, Serbia, tick-borne pathogens

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INTRODUCTION

After the discovery of the cause of Lyme borreliosis (Burgdorfer et al., 1982; Steere et al., 1977), intensive research on tick-borne infections around the world began. Since then, the research on Lyme borreliosis and other tick-borne infections has been conducted in several directions: bacteriological, virological, parasitological, epidemiological, clinical (infectological), and acarological (Fritz and Kjemtrup, 2003). In the last two decades, researchers have warned of an increase in the incidence of tick-borne infections and the consequences they produce. For example, Lindgren and Jaenson estimated that about 85,000 new cases of Lyme borreliosis in humans occur each year in Europe, compared to between 16,000 and 20,000 per year in the United States (US) (Lindgren and Jaenson, 2006). Most tick-borne infections are naturally occurring zoonoses. In humans, vector-borne diseases account for more than 17% of all known infectious diseases (WHO, 2017). Leschnik states that in dogs, the infection risk for tick-borne pathogens has been calculated as 54% per year, leading to the assumption that vector borne diseases are even more important in companion animals than in humans (Leschnik, 2020; Leschnik et al., 2013).

Numerous factors are responsible for the newly emerging situation of tick-borne infections in public health. Summarized key factors are: 1. Tick populations are increasing and their geographic ranges are expanding, as are suitable habitats for these arthropod vectors, probably in connection with land-use, change of vegetation and current climate changes; 2. Diagnostic and treatment procedures are more developed and available, but still variable; 3. Human activity and complex socioeconomics; and 4. Different surveillance models and the varying success of control strategies (Leschnik, 2020; Madison-Antenucci et al., 2020; Franklinos et al., 2019; Semenza and Suk, 2018).

Ticks as hematophagous arthropods are a significant source of pathogen transmission to humans and animals, they can transmit bacterial, parasitic, and viral pathogens, and coinfection can often be present (Chomel, 2011; Shaw et al., 2001). The most important emergent and re-emergent tick-borne pathogens are *Borrelia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., *Babesia* spp. and tick-borne encephalitis virus (Chomel, 2011). Pathogens that can be transmitted by ticks, such as *Bartonella* spp., hemotropic hemoplasmas, *Hepatozoon* spp., and Louping ill virus, are also important for the dog population. In addition to the listed pathogens, ticks are important for the transmission of Congo-Crimean hemorrhagic fever virus (Vorou, 2009), and they can also transmit the causative agents of Q fever and tularemia, although other transmission routes are more important for these diseases (Duron et al, 2015; Angelakis and Raoult, 2010; Eliasson et al., 2006).

Chomel states that tick-borne diseases are recognized as an emerging infectious threat not only to humans but also to dogs (Chomel, 2011), and Shaw indicates that subclinically infected companion animals could provide a reservoir for human tick-transmitted infectious agents (Shaw et al., 2001). Dogs can also serve as sentinel populations of tick-borne pathogens (Thompson et al., 2020; Shaw et al., 2001).

Multidisciplinary tick-borne infection research in Serbia began intensively after the registration of the first case of a human suffering from Lyme borreliosis (Djordjević et al., 1990) and the isolation of the causative agent, *Borrelia burgdorferi* s.l., from small rodents (Stajković et al., 1993). So far, we know Serbia is an endemic area for a large number of tick-borne infections (Potkonjak et al., 2016a). Most of the research has been related to proving the presence of pathogens in ticks (Potkonjak et al., 2017; Potkonjak et al., 2016a; Potkonjak et al., 2016b; Tomanović et al., 2013; Radulović et al., 2011; Savić et al., 2010; Tomanović et al., 2010a; Tomanović et al., 2010b; Milutinović et al., 2008), using molecular biology methods or cultivation in the case of *Borrelia* species (Čakić et al., 2019). Several research studies on tick-borne pathogens in domestic and wild animals have been published in recent years (Juwaid et al., 2019; Sukara et al., 2018; Vasić et al., 2018). Small-scale research has been conducted in dogs, and this review will focus on the recent studies of emergent and re-emergent tick-borne pathogens in dogs from Serbia.

BORRELIA BURGDORFERI SENSU LATO (S.L.) COMPLEX

Lyme borreliosis is a tick-borne, zoonotic, and multisystem disease, caused by the *Borrelia burgdorferi* sensu lato (s.l.) complex. This complex is a diverse group of bacteria, distributed worldwide, and so far, the following species from ticks have been registered in Serbia: *Borrelia lusitaniae*, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia valaisiana*, *B. burgdorferi* sensu stricto (s.s.), and *Borrelia bavariensis* (Čakić et al., 2019; Potkonjak et al., 2016b; Savić et al., 2010). In Europe and largely overlapping across the continent, the most commonly reported species are *B. afzelii*, *B. garinii*, and *B. valaisiana* (Estrada-Peña et al., 2018). These spirochetes are predominantly transmitted on the European continent by ticks of the genus *Ixodes* (a principal vector is *Ixodes ricinus*), while small mammals, lizards, and birds have epidemiological significance as reservoirs of *B. burgdorferi* s.l. (Čakić et al., 2019; Steere et al., 2004). In general, the prevalence of *B. burgdorferi* s.l. in *I. ricinus* ticks from Serbia is 21.1% to 42.5% (Potkonjak et al., 2016a; Čekanac et al., 2010; Milutinović et al., 2008). According to studies on *Borrelia* distribution in Europe, their prevalence can vary widely in different regions (Potkonjak et al., 2016b). It is known that in Europe, coinfections of *B. burgdorferi* s.s. with other species (i.e., *B. garinii*) could predispose dogs to illness (Littman et al., 2018; Pantchev et al., 2015).

In the American College of Veterinary Internal Medicine (ACVIM), Littman et al. published a consensus statement arguing it is not proven that European *B. burgdorferi* s.l. causes clinical signs in dogs, although it is known that two main clinical manifestations of *B. burgdorferi* s.s. infection in dogs are Lyme arthritis and Lyme nephritis (Littman et al., 2018). So far, to our knowledge, no clinical case of Lyme borreliosis in dogs has been published from Serbia, which is in accordance with the statement of Littman et al. that most seropositive dogs show no clinical signs (Littman et al., 2018). Dogs are sensitive indicators of Lyme borreliosis prevalence because they have a high level of exposure to ticks (Stone et al., 2005). Numerous seroepidemiological studies of Lyme borreliosis in dogs have been conducted in countries across Europe in the last twenty years and showed differing seroprevalences.

The first more extensive seroprevalence study of Lyme borreliosis in dogs from Serbia was conducted by Savić et al. (2010). They tested blood sera from 486 dogs from four regions of Vojvodina province (Ada-Mol region, Novi Sad region, Fruška Gora region and Novi Bečej region) by ELISA (recomWell Borrelia canis IgG, Mikrogen, Germany) and Western blot assay (recomBlot Borrelia Canis IgG, Mikrogen, Germany). This study showed the seroprevalence of IgG antibodies to *B. burgdorferi* s.l. in dogs was 25.5% by ELISA and 26.1% by Western blot assay (Savić et al., 2010). In another study from Vojvodina, Potkonjak et al. reported 8.1% of dogs were positive for anti-*B. burgdorferi* s.l. IgG antibodies by ELISA (recomWell Borrelia canis IgG, Mikrogen, Germany) and Western blot assay (recomBlot Borrelia Canis IgG, Mikrogen, Germany). In that study, most of the positive findings were in South Bačka district area (Potkonjak et al., 2013b). In accordance with Potkonjak et al. (2013b), Kovačević Berić et al. reported lower average seroprevalence (0.07%) in dogs from North Bačka district (Berić Kovačević et al., 2017). The first report on the seroprevalence of IgG antibodies to *B. burgdorferi* in dogs in the Belgrade area was published by Obrenović et al. (2015). They tested sera of 215 dogs from different subpopulation groups and reported overall *B. burgdorferi* seroprevalence of 25.1% by ELISA, 24.7% by Western blot assay and 22.3% by IFA test. The same authors reported the highest seroprevalence of 31.2% for the group of shelter dogs and slightly lower seroprevalences for groups of hunting (19.5%) and companion dogs (17.6%) (Obrenović et al., 2015). Contrary to these results, Kovačević Filipović et al. found 1.8% of dogs (from shelters) were seroreactive to *B. burgdorferi* by qualitative ELISA test (SNAP® M-A, IDEXX Laboratories, Inc., Westbrook, Maine, USA). They tested 111 dogs, also from different subpopulation groups from suburban and rural Belgrade municipalities (Kovačević Filipović et al., 2018). Other authors (Gajić et al., 2016) also reported a low seroprevalence (2.33%) of Lyme borreliosis in dogs from the Belgrade area. These authors tested 86 blood samples from apparently healthy stray dogs by ELISA (SNAP® 4Dx Plus®Test, IDEXX Laboratories, Inc., Westbrook, ME, USA) (Gajić et al., 2016). One explanation for the differing prevalences of *B. burgdorferi* in dogs in the Belgrade area could be the use of different diagnostic tests that have different sensitivities and specificities. Robertson et al. indicated that differences in the regional distributions of borrelial species could further influence the preferential reactivity of sera (Robertson et al., 2000). This can also be a consequence of antigenic heterogeneity among European isolates (Jovičić et al., 2003). This suggests differences with US borrelial isolates is important and that tests designed for the US market might not be suitable to detect antibodies to European isolates.

EHRLICHIA SPP. AND *ANAPLASMA* SPP.

It is known that, for now, three of these species are important for dogs in Europe: *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Anaplasma platys*. According to the guideline by Sainz et al., other *Ehrlichia* species (*Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia muris*, and *Ehrlichia ruminantium*) have not been detected in dogs from Europe. *Ehrlichia canis* causes canine monocytic ehrlichiosis (CME), which manifests clinically differently in different phases of the disease and can potentially be fatal (Sainz et al., 2015). Dogs in the chronic phase exhibit bone marrow hypoplasia and severe pancytopenia (Mylonakis et al., 2004). The zoonotic potential of *E. canis* is still an open question because a bacterium closely related to *E. canis* was found in Venezuela in humans (Perez et al., 1996).

To our knowledge, no clinical case of CME in dogs has been registered and nor has *E. canis* DNA been amplified from dog blood in Serbia so far (Kovačević Filipović et al., 2018). It is known that CME is endemic in the Mediterranean region and southeastern Europe (Schafer et al., 2019), and the presence of *E. canis* DNA in dogs in neighboring countries has been proven in Romania (Morar et al., 2015), North Macedonia (Petrov et al., 2018), and Greece (Mylonakis and Theodorou, 2017; Mylonakis et al., 2004). According to our first seroepidemiological research of CME in dogs from Vojvodina, using indirect immunofluorescence antibody assay (IFAT), the seroprevalence of IgG antibodies to *E. canis* antigens was 16% (Potkonjak et al., 2013a). Similar to the previous research, Bogićević et al. found the overall seroprevalence of IgG to *E. canis* antigens was 11.06% in dogs from Serbia (Bogićević et al., 2017). Epidemiological cross-sectional studies indicate the seroprevalence of *E. canis* in European countries ranges from 0.16% in Hungary (Farkas et al., 2014) to 50% in Albania (Hamel et al., 2009). Although findings of *E. canis* morula in monocytes (Pavlović et al., 2017) and *Ehrlichia* spp. morula in neutrophils (Potkonjak et al., 2010) from dog blood in Serbia have been reported, without evidence of *Ehrlichia*-DNA and sequential genome analysis, these findings should be received with caution, due to the low sensitivity and specificity of microscopic examination of stained blood smears in regard to the possible presence of other inclusions or artifacts. It should be noted that Kovačević Filipović et al. did not detect *Ehrlichia* spp. morulae in blood smears from dogs, but they were not expected to be found as the dogs were apparently healthy (Kovačević Filipović et al., 2018). The main vector for *E. canis* in Europe is *Rhipicephalus sanguineus* s.l. According to our research, this tick species is most often present in dogs in Serbia but, so far, has been negative for the presence of *E. canis* DNA (Potkonjak et al., 2016a). However, Hornok et al. published molecular evidence of *E. canis* in ixodid ticks from carnivores in South Hungary (Hornok et al., 2013). In addition, Ionita et al. found *E. canis* DNA in ticks from dogs in a greater urban area in Southeastern Romania (Ionita et al., 2016).

E. ewingii causes canine and human granulocytic ehrlichiosis, while *E. chaffeensis* causes canine and human monocytic ehrlichiosis (Sainz et al., 2015). So far, there have been sporadic serologically-positive findings for these pathogens in Europe, mainly in humans (Sainz et al., 2015; Topolovec et al., 2003). In accordance with that, two clinical cases of *E. chaffeensis* infection have been registered in humans from Serbia (Arsić et al., 2014; Dokić et al., 2006). To our knowledge, there are no other significant findings for these pathogens in Europe. It should be mentioned that Kovačević Filipović et al. found one case of an *E. ewingii* seropositive dog and concluded it could be a cross reaction with other rickettsia species (Kovačević Filipović et al., 2018). In accordance with this conclusion and the known cross-reactivities between *E. canis-E. ewingii* (Cardenas et al., 2007), and *E. canis-A. phagocytophilum* (Harrus and Waner, 2010), positive serological findings should be interpreted with caution. *E. ewingii* and *E. chaffeensis* are transmitted by the *Amblyomma americanum* tick, which is widespread primarily in the southcentral and eastern US (Raghavan et al., 2019). Kramer et al. reported detection of *E. chaffeensis* in *Dermacentor variabilis* and *Ixodes pacificus* (Kramer et al., 1999). To our knowledge, these tick species are not present in Europe or in Serbia. Regardless, *E. ewingii* and *E. chaffeensis* infections should be considered in cases of imported dogs or blood transfusions, as in the case of *E. canis* infection (Sainz et al., 2015).

Two bacteria from the genus *Anaplasma* are important for the clinical pathology of dogs – *A. phagocytophilum* and *A. platys*. *A. phagocytophilum* causes granulocytic anaplasmosis, which is mainly characterized by general infectious syndrome. In addition to dogs, this pathogen can infect other animal species (domestic ruminants, horses, cats, and several wild animals (Silaghi et al., 2017)) and humans (Sainz et al., 2015). According to our information, no clinical case of the disease or *A. phagocytophilum* DNA has been registered in the blood of dogs in Serbia so far (Kovačević Filipović et al., 2018). Seroepidemiological studies showed the seroprevalence of *A. phagocytophilum* in dogs was 15.5% by IFAT in Vojvodina (Potkonjak et al., 2015), and 26.1% by qualitative ELISA test in Belgrade (SNAP® M-A, IDEXX Laboratories, Inc., Westbrook, Maine, USA) (Kovačević Filipović et al., 2018). The geographical spread of *A. phagocytophilum* in Europe is mainly limited to northern and central Europe, and seroepidemiological studies showed that 3 to 57% of dogs have antibodies to A. *phagocytophilum* (Petrov et al., 2018). In our geographical area, the vector for *A. phagocytophilum* is the *I. ricinus* tick, which is very abundant in the environment. According to previous research, *A. phagocytophilum* DNA has been detected in *I. ricinus* ticks in Serbia using molecular methods, and with a prevalence of infection in the ticks from 1.4% to 13.9% (Potkonjak et al., 2016a; Tomanović et al., 2013; Milutinović et al., 2008). Although the presence of *A. phagocytophilum* DNA in *I. ricinus* ticks has been proven, and Tomanović et al. assumed that strains with potential infectivity for humans and domestic animals were present in Serbia (Tomanović et al., 2010b), the question as to why the disease is rarely clinically manifested in dogs remains open. One of the answers could be the high genetic diversity of the European *A. phagocytophilum* strains, which could be a consequence of adaptation of *A. phagocytophilum* to different host species. Circulation of four *A. phagocytophilum* ecotypes has been established, and it is known that ecotype/cluster (I) is isolated significantly more often from dogs than the other ecotypes (Matei et al., 2019; Jahfari et al., 2014).

A. platys causes canine infectious cyclic thrombocytopenia, and the probable vector of this pathogen in Europe is *R. sanguineus*. *A. platys* DNA was recently detected in humans (Arraga-Alvarado et al., 2014; Maggi et al., 2013). Canine infectious cyclic thrombocytopenia has been registered in dogs from Mediterranean countries including Croatia (Huber et al., 2017; Dyachenko et al., 2012), and also Romania (Andersson et al., 2013). There is insufficient data on the prevalence of *A. platys* infection of dogs in Europe based on molecular methods (Sainz et al., 2015). The first and only data, for now, from Serbia comes from the research by Kovačević Filipović et al., who found one dog seroreactive for *A. platys* (Kovačević Filipović et al., 2018).

RICKETTSIA SPP.

Two types of tick-borne rickettsiae are important for the clinical pathology of dogs: *R. rickettsii* in America that causes Rocky Mountain spotted fever (ticks from genera *Dermacentor*, *Amblyomma*, and *Rhipicephalus* are the vectors), and *Rickettsia conorii* ssp. *conorii* in Europe (the vector is the *Rhipicephalus sanguineus* tick). Both diseases are zoonoses. Serologic studies indicate a high prevalence rate of exposure to *R. conorii* among dogs from Mediterranean countries (Pennisi et al., 2012; Alexandre et al., 2011). In accordance with this, in a previous seroepidemiological study, we registered high seroprevalence (44.83%) to *R. conorii* antigens in dogs from Vojvodina, using IFAT (MegaScreen® FLUO RICKETTSIA conorii, MEGACOR Diagnostik GmbH, Austria) (Spasojević-Kosić et al., 2015). Radulović et al. previously identified *Rickettsia helvetica* and *Rickettsia monacensis* in *I. ricinus* ticks, with infection rates of 7.7% and 15.4%, respectively (Radulović et al., 2011). In our later research, we confirmed the presence of *Rickettsia monacensis* in *I. ricinus*. Furthermore, we detected, in ticks, the presence of *Rickettsia raoultii* and *Rickettsia massiliae* in Serbia for the first time (Potkonjak et al., 2016a), but it is still not known if dogs are naturally infected with these *Rickettsia* species from the group of spotted fevers.

HEMOTROPIC *MYCOPLASMA*

So far, two species of hemotropic mycoplasmas are known to infect dogs. These are the *Mycoplasma haemocanis* (*Mhc*) and "*Candidatus* Mycoplasma haematoparvum" (*CMhp*). Clinical signs are rarely apparent in non-splenectomized dogs. *Mhc* has been reported in both North American and European dogs (Kenny et al., 2004; Messick, 2004). A novel small hemoplasma, *CMhp* was isolated from the blood of a splenectomized dog with hematopoietic neoplasia (Sykes et al., 2005; Sykes et al., 2004). Canine hemotropic *Mycoplasma* are transmitted by fleas and ticks, and *Rhipicephalus sanguineus* s.l is most likely a vector (Messick, 2004). Kenny at al. registered 15.4% of dogs were positive for hemotropic *Mycoplasma* by PCR, with *CMhp* predominance in France (Kenny et al., 2004). A similar overall prevalence of hemotropic *Mycoplasma* infection (15.3%) was found in dogs from Turkey (Aktas and Ozubek, 2018). To our knowledge, there is insufficient research and data on these infections in dogs and vectors in Serbia. Our previous research, conducted in 2008, resulted in the first published finding of *CMhp* in dogs from Vojvodina. Scanning electron microscopy revealed the presence of haemoplasmas of 290 nm and 269 nm in diameter on the erythrocyte surface (Figure 1) and partial sequence analysis of the 16S rRNA gene indicated 100% homology between the PCR amplicons of the positive samples and *CMhp* (Lako et al., 2010). More extensive research is needed to establish the actual epidemiological situation and clinical significance of these infections in dogs in Serbia.

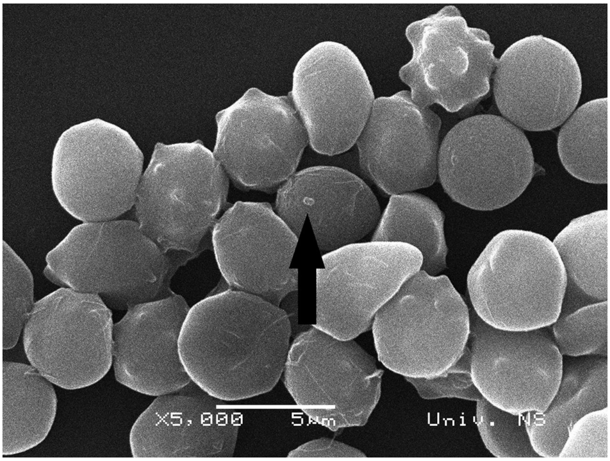

Figure 1. Presence of "*Candidatus* Mycoplasma haematoparvum" on the dog erythrocyte surface

TICK-BORNE ENCEPHALITIS VIRUS

The tick-borne encephalitis virus (TBEV) is a single-stranded lipid-enveloped RNA virus that belongs to the tick-borne flavivirus group (genus *Flavivirus*, family *Flaviviridae*) (Gritsun et al., 2003; Mansfield et al., 2009). The disease and/or infection has been registered in humans, dogs, and some other animal species. Dogs can be asymptomatic or can develop encephalitis with neurological signs (Leschnik, 2020). Pfeffer and Dobler indicate that dogs are accidental hosts, and because of that, they cannot spread TBEV further (Pfeffer and Dobler, 2011). Different seroprevalences of TBEV in dogs were reported in European countries: Belgium 0.1% (Roelandt et al., 2011), Czech Republic 3.3% (Klimeš et al., 2001), Denmark 4.8% (Lindhe et al., 2009), Finland 6.7% (Levanov et al., 2016), Germany 2.1% (Balling et al., 2015), Norway 16.4% (Csángó et al., 2004), and Spain 1.7-1.8% (Garcia-Bocanegra et al., 2018). In our previous investigation in Serbia, we identified anti-TBEV IgG antibodies in the sera of 7 dogs (17.5% of the dogs studied) in Vojvodina (Potkonjak et al., 2017). The main vector in Europe is *I. ricinus*, although *Dermacentor reticulatus* is important for the transmission of pathogens to dogs (Leschnik, 2020; Chitimia-Dobler et al., 2019). Several decades after the previous documentation of TBEV in Serbia, we demonstrated the presence of TBEV in *I. ricinus* questing nymphs (with prevalences of 2% and 6.6%

in two different localities) (Potkonjak et al., 2017). The TBEV prevalence in *I. ricinus* ticks from Europe varies between 0.1% and 5% in unfed ticks and increases with development stage (Pettersson et al., 2014; Süss, 2011). Based on the above, it is clear Pfeffer and Dobler's warning that "veterinarians are not considering TBE in dogs and that leads to problems in recognizing and diagnosing this severe infection" is still up-to-date (Pfeffer and Dobler, 2011).

***BABESIA* SPP.**

Canine babesiosis is common and well known in clinical veterinary practice around the world and in Serbia. Both the large and small forms of *Babesia* species (*Babesia canis*, *Babesia vogeli*, *Babesia gibsoni*, and *Babesia microti*-like isolates also referred to as "*B. vulpes*" and "*Theileria annae*") infect dogs in Europe. These species are considered to have no zoonotic potential. Clinical manifestations are well known to veterinary practitioners and vary from subclinical infections to multi-organ failure (Solano-Gallego et al., 2016). Several significant cross-sectional studies have been conducted in recent years in Serbia in order to determine the presence of *Babesia* species in dogs, including their identification based on molecular biological methods. First, Davitkov et al. identified *B. canis* and *B. gibsoni* infections in diseased dogs from Serbia (Davitkov et al., 2015). Then, Gabrielli et al. registered *B. vogeli*, *B. gibsoni*, and *B. microti*-like DNA in the blood of clinically healthy outdoor dogs (Gabrielli et al., 2015). In a recently published study, Kovačević Filipović et al. reported 13.5% of clinically healthy outdoor dogs were *B. canis* PCR positive and 2.7% were *B. gibsoni* PCR positive; the authors consider the Belgrade area is endemic for *B. canis* infection (Kovačević Filipović et al., 2018). The seroprevalence of canine babesiosis was sporadically determined in Serbia. In our previous seroepidemiological studies, we registered a *B. canis* seroprevalence of 26.2% in dogs from Vojvodina, using IFAT (MegaScreen® FLUO BABESIA canis, MEGACOR Diagnostik GmbH, Austria) (Potkonjak et al., 2015). Thereafter, Kovačević Filipović et al. reported 11.7% seroreactivity in dogs to *B. canis*, 1.8% to *B. gibsoni*, and 13.5 % to *B. vogeli* (Kovačević Filipović et al., 2018). Canine babesiosis in Europe is mainly transmitted by ticks (*D. reticulatus* for *B. canis*, and *R. sanguineus* s.l. could serve as potential vectors for *B. vogeli* and *B. gibsoni*). It should be noted that some *Babesia* spp. (primarily *B. gibsoni*) are also transmitted by infected blood (Solano-Gallego et al., 2016). Testing for *Babesia* presence in ticks indicates this genus circulates in several tick species in Serbia. Mihaljica et al. reported a 21.57% prevalence of *B. canis* in *D. reticulatus* ticks and 8.57% in *Haemaphysalis concinna* ticks. In that study, ticks were collected from vegetation at seven localities in northern Serbia (Mihaljica et al., 2012). For the first time in Serbia, Davitkov et al. determined the presence of *B. gibsoni* in *R. sanguineus* ticks, and they also registered the presence of *B. canis* in several tick species (*D. reticulatus*, *R. sanguineus*, and *I. ricinus*) (Davitkov et al., 2016). Potkonjak et al. confirmed the presence of *B. canis* in *D. reticulatus* ticks from dogs, and they also detected *B. venatorum* DNA and *B. microti* DNA in questing *I. ricinus*, for the first time in

Serbia (Potkonjak et al., 2016a). All of the above indicates that babesiosis is the most widespread and most frequently registered tick-borne disease in dogs in Serbia.

HEPATOZOON CANIS

Autochthonous *Hepatozoon canis* infection is reported mainly from tropical, subtropical, and temperate climate regions where the vector *R. sanguineus* ticks are abundant. In Europe, autochthonous infection is found mainly close to the Mediterranean basin. Infection is often sub-clinical, but it can also manifest as a moderate to severe disease (Baneth, 2011). Recent data indicate the prevalence of *H. canis* infection in dogs from Italy was 14% (Pacifico et al., 2020); and 4.4 % from Germany (Helm et al., 2020). There is insufficient data on this pathogen in Serbia. Gabrielli et al. reported the presence of *H. canis* in a dog (4.5% of studied dogs) from Niš (Gabrielli et al., 2015), while Potkonjak et al. identified *H. canis* DNA in *I. ricinus* ticks collected from dogs in Serbia for the first time. They concluded it is probable the ticks acquired this organism by a blood meal from an infected dog (Potkonjak et al., 2016a), because the main vector for this protozoon is the *R. sanguineus* tick (Baneth, 2011).

CONCLUSION

In this review, the most important studies of tick-borne infections in dogs in Serbia are presented. Most authors have identified this geographical area as endemic to certain tick-borne infections. Lyme borreliosis is certainly the most significant infectious tick-borne disease of humans in our geographical area. Babesiosis stands out as the most common and important disease in dogs. In addition to these pathogens, the presence of *H. canis* and *CMhp* in the blood of dogs was proved using molecular methods. Seroreactivity in dogs has been proven for *B. burgdorferi* s.l. complex, *E. canis*, *E. ewingii*, *A. phagocytophilum, A. platys, R. conorii*, and TBEV. However, clinically ill dogs were not observed, and no DNA of these pathogens was found in their blood. The presence of *B. burgdorferi* s.l. complex, *A. phagocytophilum*, *Rickettsia* spp. from the group of spotted fevers, and TBEV in ticks indicates the geographical distribution and potential for infecting humans, dogs, and other animals in Serbia. Numerous questions about clinical veterinary practice are unknown and open. That is why further systematic, comprehensive, well designed bacteriological, virological, parasitological, epidemiological, clinical (infectological), and acarological research is needed in Serbia. As most of these diseases and infections are naturally focal zoonoses, intersectoral cooperation by veterinarians, epidemiologists and infectologists is needed in order to improve public health according to the principles of “One health” in Serbia.

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Authors' contributions

AP wrote, drafted and revised the manuscript. MŽS provided stylistic/grammatical revisions to the manuscript with technical and reference editing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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KRPELJSKI PRENOSIVE INFEKCIJE PASA U SRBIJI: PREGLED ISTRAŽIVANJA

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Kratak sadržaj

Povećanje vrednosti prevalencije krpeljski prenosivih bolesti kod ljudi i životinja predstavlja značajno pitanje iz oblasti javnog zdravlja. Psi mogu da obole sa ispoljenom kliničkom slikom, budu rezervoari patogenih uzročnika koje prenose krpelji ili sentinel populacija za istraživanje. Svakako, sprovedena su ograničena istraživanja u populaciji pasa u Srbiji i u ovom preglednom radu biće predstavljeni rezultati dosadašnjih istraživanja poznatih i novoiskrslih patogenih uzročnika koje prenose krpelji kod pasa u Srbiji. Babesioza se ističe kao najčešća i najvažnija bolest kod pasa. Pored ovog uzročnika, primenom molekularnih metoda dokazano je prisustvo *Hepatozoon canis* i "*Candidatus* Mycoplasma haematoparvum" u krvi pasa. Seroreaktivnost kod pasa dokazana je na *Borrelia burgdorferi* sensu lato (s.l.) complex, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Rickettsia conorii* i virus krpeljskog meningoencefalitisa (Tick-Borne Encephalitis Virus – TBEV). Nije zapažena pojava klinički obolelih pasa, a ni prisustvo DNK prethodno navedenih uzročnika nije dokazano u njihovoj krvi. Prisustvo *B. burgdorferi* sensu lato (s.l.) complex, *A. phagocytophilum*, *Rickettsia* spp. iz grupe pegavih groznica i TBEV u krpeljima ukazuje na geografsku rasprostranjenost ovih uzročnika i potencijal za zaražavanje ljudi, pasa i drugih životinja u Srbiji. U Srbiji su potrebna dalja sistematska, sveobuhvatna, dobro osmišljena, bakteriološka, virusološka, parazitološka, epidemiološka, klinička (infektološka) i akarološka istraživanja.

Ključne reči: krpeljski prenosivi uzročnici, psi, pregledni rad, Srbija