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Invited lecture

AN OVERVIEW OF THE RECENT STUDIES ON TICK BORNE PATHOGENS IN SERBIA

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Abstract

Tick borne diseases are one of the key issues within a „One Health“ concept. A triangle between humans, animals and ticks has been a challenge for researchers for some time now. In Serbia, research on ticks as vectors, tick borne pathogens and tick borne diseases has started approximately a decade ago. The first studies were on the presence of *Borrelia burgdorferi sensu lato* starins in ticks, followed by research on *Babesia spp*. During the last few years a lot of work has been done, not only in Serbia, but in the whole region – on the topic of the presence of *Anaplasma phagocytophilum, Coxiella burnetii* and *Francisella tularensis* in ticks.
Tick species found in different regions of Serbia so far are the following: *Ixodes ricinus, Dermacentor marginatus, Dermacentor reticulatus, Rhipicephalus sanguineus, Hemaphysalis punctata, Haemaphysalis concinna*
For *B.burgdorferi s.l.* more genospecies were found in ticks during the last few years: *B. afzelii, B,burgdorferi s.s, B. lusitaniae, B. garinii* and *B. valaisiana*. Monitoring of the prevalenece of *B.burgdorferi s.l.* in ticks is constant, but it differs among different regions in Serbia (Vojvodina, Belgrade region and middle Serbia). The prevalence for B.burgdorferi in ticks varies from 23% to 42.5%.
Babesia spp is found to be present in ticks with two different methods, in different tick species with different prevalence varies from 10.61% to 46.40% in different regions. In another study, *Anaplasma phagocitophylum* has recently been found in 13.9% ticks in Serbia. *Coxiella burnetii* was found in less than 7% in ticks in different regions of Serbia. The prevalence for *Francisella tularensis* microorganisms has also been found as 3.8% in ticks.
All of the monitored pathogens are causative agents for more or less dangerous zoonozes, which can be found in the region of Serbia and almost all of them have already been reported in humans.

Key words: tick borne pathogens, zoonozes, borreliosis, anaplasmosis, ricketiosis, babesiosis

Introduction

Arthropods as vectors of pathogens are causing vector-borne diseases, an important threat to public health. The pathogen carried by the arthropod vector is transferred during blood feeding, the closest interaction between the arthropod vector and the vertebrate host. Whether or not the transferred pathogen is actually causing a disease, depends on a complex interaction of many factors, most important the pathogen, the host and the host’s immune system (Mencke, 2013).

A research on vector borne infections and pathogens has started two decades ago. Vector borne pathogens are often known as emerging pathogens. Emerging pathogens include infectious agents that were previously un recognized and have been identified and associated with a new disease or an illness with a previously unknown aetiology; organisms that have been described in other regions and imported into areas where they were previously unknown, or agents that were constantly present in the affected area at a low level or in a different host and due to some change have become more widely spread in the population under concern (Harrus and Baneth, 2005). Important tick-borne zoonoses such as Lyme disease, human granulocytic anaplasmosis (HGA) caused by *A. phagocytophilum*, human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis*, and human babesiosis caused by *B. microti* were all described in the US during the second half of the 20th century and later in other countries and continents. Candidates *Neoehrlichia mikurensis* is an intracellular bacterium member of the *Anaplasmataceae* family which causes severe disease with fever and septicaemia in humans. It is an example of an "emerging" tick borne infection that has been known only for a decade and appears to be dispersed in multiple regions. It has been identified throughout Europe, in patients in Sweden, Switzerland, Germany, Czech Republic, China (Foldvari, 2014). Similarly to other tick borne infections agents, it is likely to have been circulating among wildlife animals and ticks long before it emerged as a recognised clinical cause of human disease.

Tick borne diseases are one of the key issues within a „One Health" concept. A triangle between humans, animals and ticks within the environmental conditions, has been a challenge for researchers for some time now.

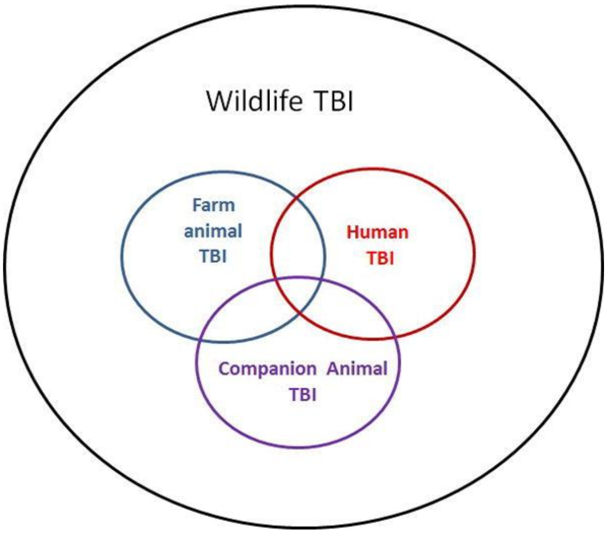

Figure 1 – Overlaping of tick borne infections among different species (Baneth, 2014)

Ticks as vectors

Approximately 900 species of ticks have been described to date, of which more than 700 belong to the *Ixodidae* (hard ticks); approximately 200 belong to the *Argasidae* (soft ticks). The lifestyle of ticks which includes uptake of blood from hosts, secretion of saliva into the host tissues, movement between different hosts and production of eggs from which a new generation of ticks develops, inevitably makes them suitable to host other organisms. A wide variety of pathogens is transmitted from ticks to vertebrates including viruses, bacteria such as rickettsiae and spirochetes, fungi, protozoa and helminths, of which most have a life cycle which requires passage through the vertebrate host (Dantas-Torres et al., 2012).

Several factors contribute to the change in geographic ranges of tick borne infections. Ticks cannot fly or move long distances by themselves and, therefore, their transfer from one location to another depends mostly on movement of hosts including birds which can fly long distances (Mathers et al., 2011 and Hasle, 2013), migrating wild animal species such as jackals, animals introduced into new areas by humans such as farm animals imported into areas where they were not present before, rodents travelling accidently with ships or trucks, or imported pet animals. Climate change may also facilitate migration of vertebrate tick hosts, allowing dispersal of ticks and pathogens into new territories (Estrada-Peña et al., 2012). Transmission of tick borne infections can also occur by contaminated blood transfusions and needles. *Babesia* of different species have been shown to be transmitted by blood transfusion in both humans and dogs (Stegeman et al., 2003). Such artificial transmission may result in spread of tick borne infections to new areas, if competent tick vectors and reservoir hosts are found in those areas. Although the majority of tick borne infections are transmitted via the tick saliva during the course of the blood meal, there are some pathogens of veterinary importance which are transmitted naturally by other mechanisms. For example, *Hepatozoon* spp. is protozoa that belongs to the *Apicomplexa* and infect vertebrates by oral ingestion of an arthropod host containing infective sporozoites. *Hepatozoon* spp. infect dogs that orally uptake and ingest ticks harbouring mature sporozoites.

Ixodes ticks are abundant throughout Europe, but *I.ricinus* nortward shift has been noted, all the way up to Norway and Sweden (Jaenson and Lindgren, 2011). *I. ricinus* has also been recorded at 1200-1300m above sea level in Check Republic and Austria (Daniel et al., 2003; Stuenzner et al., 2006).

Factors that influence the distribution of *I. ricinus* in Europe have been identified as the following: (1) factors directly related to climatic change (affecting the tick, host or habitat), (2) factors related to changes in the distribution of tick hosts (which may be directly or indirectly a consequence of human interventions) and (3) other ecological changes. Considering the large size of the global human population, the high density of humans in some areas, and the surface size of the adult human body, humans would be expected to be one of the most common blood sources for ticks. Certainly most tick borne infections circulate between wildlife animals and ticks, and may affect humans or domestic animals, but do not rely on infecting people for their persistence. For example, Lyme disease circulates mostly among rodents, and humans or domestic dogs are just incidental hosts that could suffer from clinical disease but do not play an important role in the enzootic transmission and epidemiology of this infection (Radolf et al., 2012). Despite the global abundance of humans and their presence in a variety of climates and ecological conditions, they are not major reservoirs for tick borne infections. Tick borne infections of humans, farm animals and companion animals such as dogs and cats, may overlap, and some agents such as *B. burgdorferi* and *Anaplasma phagocytophilum* are able to infect hosts belonging to more than one of these categories, however all of these zoonotic agents are associated with wildlife reservoirs

Tick borreliosis

One of the most prevalent vector borne disease in humans in Europe is borreliosis. In Europe several *Borrelia* species have been described, which are considered to cause human borreliosis with clinical symptoms. These species are referred to collectively as *Borrelia burgdorferi sensu lato*, and *B.afzelii*, *B.garinii* and *B.valesiana*. It is proposed to use term borreliosis or tick borreliosis for *Borrelia* infection in Europe with *B.burgdorferi s.l.* instead of Lyme borreliosis which is used in North America.

The risk of tick bite during outdoor activities for humans and dogs was studied in hunters and compared to humans without association to hunting. The study indicated a higher prevalence of borreliosis in owners of hunting dogs (15%) compared to non hunters (9%), but in more than 90% of hunters, there was no evidence of clinical symptoms. There is an association between occupations such as forestry workers, military personnel, rangers and borreliosis (Nohlmans et al., 1991).

Information on the role of dogs in tick borreliosis, as infected patient or as a sentinel for tick borreliosis in Europe is rather limited. The data for seroprevalence of borreliosis in dogs in Europe vary from only several percentages in Czech Republic, Poland and Romania, to 50% in Slovakia (Pelchalova et al., 2006; Scotarczak and Wodecka, 2003; Kiss et al., 2011; Stefancikova et al., 1998)

Tick-borne rickettsiales

Rickettsiales transmitted by ticks in Europe and causing clinical signs in dogs are *Anaplasma phagocytophilum* and *Anaplasma platys.* There is also *Erlichia canis*, belonging to the *Anaplasmataceae* family. *Anaplasma phagocytophilum* has been found all over Europe in the following countries: Austria, Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, Luxembourg, Netherlands, Norway, Poland, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom (Jahfari et al., 2014)

Rickettsia conorii is the most important tick borne rickettiosis with public health impact in Europe. It is the pathogen of „Mediterranean spotted fever". The main vector for *Rikettsia conorii* is *Rhipicephalus sanguineus*, but also *Ixodes ricinus* and *Dermacentor spp* have been described (Benianti et al., 2002). Dogs have been identified as reservoirs of *Rickettsia conorii* (Levin et al., 2012)

Tick borne babesiosis

In temperate areas of Europe, *Dermacentor reticulatus* is a quite common tick species affecting dogs and is the primary vector of canine babesiosis due to *Babesia canis*

Canine babesiosis is a tick-borne disease caused by intraerythrocytic protozoa of the genus *Babesia* which can cause severe clinical illness. In recent years, the geographic distribution of babesiosis has expanded from western and central Europe toward northern Europe, probably due both to changes in the climate which has increased tick survival and due to an increase in companion animal travels.

Human babesiosis is caused by the intraerythrocytic parasite of the genus *Babesia* (phylum *Apicomplexa*). Humans are commonly infected by the bite of *Ixodid* ticks. Rarely, transmission does occur perinatal or via contaminated blood transfusion. Human babesiosis is a zoonotic disease with a worldwide increasing importance according to the increasing number of immunocompromised patients. Clinical symptoms have a wide range from asymptomatic to severe and letal cases. So far, the detection of the parasites in ticks and seroepidemiological data in Europe identified 3 humanpathogenic species: *B. microti*, *B. divergens* und *B. venatorum* (EU1-3). The relative small number of approximately 50 documented human cases is probably due to the lack of knowledge of the disease and the availability of diagnostic tools. Comprehensive systematic investigations of the prevalence in ticks, seroepidemiological data and improved diagnostic tests are urgently needed to evaluate the importance of the parasite.

Review of current situation in Serbia

The development of molecular diagnostic tools such as conventional PCR, real-time PCR, DNA sequencing and other methods have not only enhanced the capacity of diagnostic laboratories to detect the presence of infection, but have also expanded the capability of detecting new, previously unknown, pathogens and distinguishing between species and strains of microorganisms, which was difficult and sometimes impossible prior to the advent of molecular biological techniques. Furthermore, these molecular capabilities have become accessible and affordable to diagnostic laboratories in the last decade, and not merely restricted to research facilities.

In Serbia, research on ticks as vectors, tick borne pathogens and tick borne diseases has started approximately a decade ago. The first studies were on the presence of *Borrelia burgdorferi sensu lato* strains in ticks, followed by research on *Babesia spp*. During the last few years a lot of work has been done, not only in Serbia, but in the whole region – on the topic of the presence of *Anaplsma phagocytophilum, Coxiella burnetii* and *Francisella tularensis* in ticks.

Tick species found in different regions of Serbia so far are the following: *Ixodes ricinus, Dermacentor marginatus, Dermacentor reticulatus, Rhipicephalus sanguineus, Hemaphysalis punctata, Haemaphysalis concinna* (Tomanović, 2013).

For *B.burgdorferi s.l.* more genospecies were found in ticks during the last few years: *B. afzelii, B,burgdorferi s.s, B. lusitaniae, B. garinii* and *B. valaisiana* (Tomanović et al., 2014; Potkonjak et al., 2014. Monitoring of the prevalenece of *B.burgdorferi s.l.* in ticks is constant, but it differs among different regions in Serbia (Vojvodina, Belgrade region and middle Serbia). The prevalence for *B.burgdorferi* in ticks varies from 23% to 42.5% in different regions (Milutinović et al., 2008; Savić et al., 2010). *Babesia spp* is found to be present in ticks with two different methods, in different tick species. It was found in Dermacentor reticulatus (21.57%) and Haemophysalis concinna (8.57%) with one group of authors, by molecular detection in ticks collected from the field (Mihaljica et al., 2012). The other group of authors searched for Babesia by microscopic analysis of the midgut content of ticks in R. sanguineus, D. marginatus and D. reticulatus ticks, collected from dogs with clinical sighs of babesiosis. The prevalence in ticks in the second study was quite higher 46.40% (Pavlović et al., 2002). Both studies were done in the same region. In another study, *Anaplasma phagocitophylum* has recently been found in 13.9% ticks in Serbia, including strains with proven pathogenicity for humans (Tomanović et al., 2010; Potkonjak et al., 2013). *Coxiella burnetii* was found in less than 7% in ticks in different regions of Serbia (Tomanović et al., 2013). The prevalence for *Francisella tularensis* microorganisms has also been found as 3.8% in ticks (Milutinovićet al., 2008).

All of the monitored pathogens are causative agents for more or less dangerous zoonozes, which can be found in the region of Serbia and some of them have already been reported in humans (Savić et al., 2012).

Table 1 – Zoonotic tick borne diseases and hosts found in Serbia

Disease	Vector	Detection in Serbia
Borreliosis	Tick	Ticks, dogs, humans
Bartonellosis	Flea, Tick	Cats
Ehrlichiosis	Tick	*
Rickettsiosis	Tick, Flea	Ticks
Anaplasmosis	Tick	Ticks, dogs
Tularaemia	Tick	Ticks
Coxiellosis	Tick	Ticks, sheep, humans
Tick-borne encephalitis	Tick	*
Louping ill	Tick	No

*unpublished data, research still going on

Conclusion

Vector-borne diseases are important infectious disease of human and veterinary medicine across Europe. From all the published data recently, it is concluded that further research is needed and field studies on vector borne diseases in Serbia. The epidemiology of ticks, the dynamics of ticks spreading into new habitats due to climate changes and land use is not enough known.

Veterinary medicine in general, needs to be included in any action of European bodies regarding tick borreliosis and also this disease is an important canine vector borne disease. Infections with *Rickettsiales*, like *Anaplasma phagocytophilum* or *Rickettsia conorii* are emerging and re-emerging vector borne diseases in Europe.

Vector borne diseases shared between humans and dogs, point clearly towards the necessity for a joint effort under the ‘One health’ concept in all aspects, from epidemiology, clinic, diagnostic, therapy and prevention. From a veterinary medicinal perspective the importance of a ‘One health’ concept was recently addressed by the World Small Animal Veterinary Association (WSAVA) by initiating the ‘One Health Committee’ (Day, 2010). It is the aim of this committee to position small animal veterinary medicine as an integral part of the ‘One health’ concept in terms of zoonotic and vector-borne diseases (Day, 2011).

The question at the moment is weather is the global prevalence of tick borne infections increasing or are improvements in the ability to detect infection using sensitive and specific new techniques, and an increased awareness, responsible for more detection of disease? It is most likely that both - an increase in the spread of tick borne infections and an improved capability of detection are the answer for the global rise in reporting of tick borne infections. Human tick borne infections cannot be described without studying and understanding their relationship to animal hosts and reservoirs. Unlike some of the major human vector-borne diseases associated with flying insect vectors, where infection could be independent of association with animals, tick borne infections are zoonoses and control efforts must consider this when programs are developed to limit or eradicate them. Integration of veterinary and human reporting systems, surveillance in wildlife and tick populations, and combined teams of experts from several scientific disciplines such as entomology, epidemiology, medicine, public health and veterinary medicine are needed for the formulation of regulations and guidelines for the prevention of tick borne infections (Baneth, 2014).

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