Faculty of Science
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in České Budějovice

Seroprevalence of *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus in zoo animal species in the Czech Republic

RNDr. Thesis

Mgr. Jana Širmarová

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Annotation:

We investigated the prevalence of antibodies against *Borrelia burgdorferi* (Bb) s.l. and tick-borne encephalitis virus (TBEV) in zoo animals in the Czech Republic. We collected 133 serum samples from 69 animal species from 5 zoos. A high antibody prevalence (60%) was observed for Bb s.l. Only two animals had TBEV-specific antibodies: a markhor (*Capra falconeri*) and a reindeer (*Rangifer tarandus*), both from the same zoo, located in an area endemic for TBEV. Both of these animals were also positive for Bb s.l. anti-bodies. These data confirm prevalence of Bb s.l. and TBEV in *Ixodes ricinus* ticks in Central Europe.

Declaration (in Czech):

Prohlašuji, že svoji rigorózní práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své rigorózní práce, a to v nezkrácené úpravě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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Mgr. Jana Širmarová

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Declaration of author's contribution to the study:

I declare that I had a significant contribution to the following paper. I performed majority of experiments and data analysis. I participated in design of the experiments and coordination to draft the manuscript.

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Short communication

Seroprevalence of *Borrelia burgdorferi* sensu lato and tick-borne encephalitis virus in zoo animal species in the Czech Republic

Jana Širmarová[a], Lucie Tichá[b], Marina Golovchenko[c], Jiří Salát[a,c], Libor Grubhoffer[b], Nataliia Rudenko[c], Norbert Nowotny[d,e], Daniel Růžek[a,b,c,*]

[a] Department of Virology, Veterinary Research Institute, Hudcova 70, CZ-62100 Brno, Czech Republic
[b] Faculty of Science, University of South Bohemia, Branišovská 31, CZ-37005 České Budějovice, Czech Republic
[c] Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-37005 České Budějovice, Czech Republic
[d] Department of Pathobiology, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria
[e] Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

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ABSTRACT

This study was conducted to evaluate the prevalence of antibodies against *Borrelia bugdorferi* (Bb) s.l. and tick-borne encephalitis virus (TBEV) in zoo animals in the Czech Republic. We collected 133 serum samples from 69 animal species from 5 zoos located in different parts of the country. The samples were obtained from even-toed ungulates ($n = 78$; 42 species), odd-toed ungulates ($n = 32$; 11 species), carnivores ($n = 13$; 9 species), primates ($n = 2$, 2 species), birds ($n = 3$; 2 species), and reptiles ($n = 5$; 3 species). A high antibody prevalence (60%) was observed for Bb s.l. On the other hand, only two animals had TBEV-specific antibodies: a markhor (*Capra falconeri*) and a reindeer (*Rangifer tarandus*), both from the same zoo, located in an area endemic for TBEV. Both of these animals were also positive for Bb s.l. antibodies. Our results indicate that a high number of animal species in the Czech zoos were exposed to Bb s.l. and that TBEV infection occurred at least in one of the investigated zoos. Considering the pathogenic potential of these two tick-borne pathogens, clinical and serological monitoring should be continued, and therapeutic and preventive measures should be taken when necessary.

Introduction

The tick *Ixodes ricinus* is the major vector of a variety of pathogens in Europe including *Borrelia* spp., *Ehrlichia* spp., *Rickettsia* spp., *Babesia* spp., *Bartonella* spp., *Anaplasma phagocytophilum*, *Coxiella burnetii*, *Francisella tularensis*, and various viruses, like tick-borne encephalitis virus (TBEV), Louping ill virus, and Tribeč virus (Stanek, 2009). Lyme borreliosis (LB) and tick-borne encephalitis (TBE) are the 2 main tick-borne infectious diseases of humans and animals in Central Europe (Süss, 2011).

I. ricinus is often considered a generalist that has been found on more than 240 different vertebrate species including insectivores, rodents, carnivores, artiodactyls, and birds (Gern, 2008; Cadenas et al., 2007). Such a diversity and quantity of hosts may contribute to tick dispersal and lead to intensive colonization of new areas and establishment of new enzootic LB or TBE foci. The ability of *I. ricinus* ticks to feed on a large variety of hosts has important consequences for animal populations in zoos. Zoo animals that are housed in open-fenced enclosures are likely to encounter local tick populations and their tick-borne pathogens.

Clinically manifested TBE has been predominantly reported in humans, occasionally in dogs, and rarely in horses (Süss et al., 2007). For continuous circulation of TBEV in natural foci, the reservoir hosts (small rodents and insectivores) are of primary importance. However, for the evaluation of natural foci, accidental hosts and indicator animals are also important. Although TBEV infection in indicator animals does not result in a significant level of viraemia, it does induce an immunological response. Thus, a serological survey on indicator animals, including exotic animals kept in zoos, may be used as a diagnostic tool to identify natural foci of infection (Grešíková, 1972). Only very few reports have been published on the prevalence of tick-borne pathogens in zoo animals. In 2007, a severe TBEV infection was observed in a monkey (*Macaca sylvanus*) kept in an outdoor monkey park in a TBEV-endemic area in Germany. The monkey developed staggering paresis of the hind legs, incoordination, and intermittent opisthotonus (Süss et al., 2007). A subsequent serological survey demonstrated that 2.6%

* Corresponding author at: Department of Virology, Veterinary Research Institute, CZ-62100 Brno, Czech Republic. Tel.: +420 38 777 5451; fax: +420 38 531 0388.
E-mail address: ruzekd@paru.cas.cz (D. Růžek).

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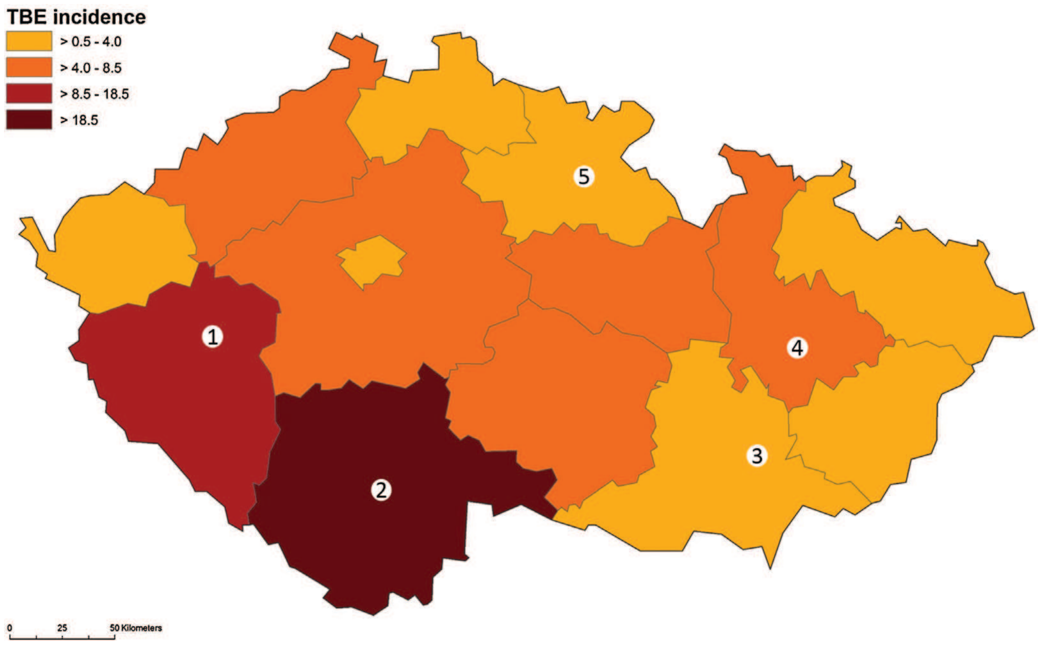

Fig. 1. Geographical locations of the zoos included in the study shown in a TBE incidence map (2000–2010) of the Czech Republic (ECDC; http://www.ecdc.europa.eu/en/healthtopics/emerging_and_vectorborne_diseases/tick_borne_diseases/tick_borne_encephalitis/country-profiles/PublishingImages/Czech-TBE-incidence-high-res.jpg): (1) ZOO and Botanical Garden Plzeň, (2) ZOO Hluboká nad Vltavou, (3) Zoopark Vyškov, (4) ZOO Olomouc, (5) ZOO Dvůr Králové nad Labem.

of monkeys and 9% of sheep grazing on nearby meadows were seropositive for TBEV (Klaus et al., 2010).

Borrelia burgdorferi (Bb) sensu lato (s.l.) causes a chronic multisystem disease with diverse clinical manifestations. Although clinical symptoms of LB have been reported in only few wild species (e.g., Kazmierczak et al., 1988), this disease might nevertheless directly affect free-ranging as well as captive wild animals (Stoebel et al., 2003).

Since there is only very limited information available on tick-borne infections in wildlife and exotic animal species kept in zoos (Stoebel et al., 2003; Klaus et al., 2010), we performed a seroprevalence study of Bb s.l. and TBEV in zoo animals in the Czech Republic, which is a country highly endemic for both of these pathogens. We aimed to identify potential Bb s.l. or TBEV-active foci in zoological gardens and the potential risk of exposure of exotic animals to these important tick-borne pathogens.

Materials and methods

Five zoos located in different regions of the Czech Republic (ZOO Dvůr Králové nad Labem, ZOO Olomouc, Zoopark Vyškov, ZOO Hluboká nad Vltavou, and ZOO and Botanical Garden Plzeň) were included in the study (Fig. 1). The majority of the zoo animals have been kept in open-fenced enclosures, close to natural areas. We collected 133 serum samples from 69 animal species that included even-toed ungulates ($n = 78$; 42 species), odd-toed ungulates ($n = 32$; 11 species), carnivores ($n = 13$; 9 species), primates ($n = 2$, 2 species), birds ($n = 3$; 2 species), and reptiles ($n = 5$; 3 species) (Table 1). The blood samples had been taken for different veterinary reasons, not for the purpose of this study, following zoo and animal ethics regulations. Blood samples were centrifuged at $2500 \times g$ for 10–15 min, and the sera were collected and stored at −20 °C and subsequently at −80 °C until analysis.

The presence of antibodies against Bb s.l. was investigated using the LYMETOP + Vet test (Promevet, Italy), following the instructions of the manufacturer. LYMETOP VET+ is a rapid qualitative commercial immunochromatographic test for the detection of the total antibodies against Bb sensu stricto, *Borrelia afzelii*, and *Borrelia garinii* in animal sera. This commercial kit does not rely on a secondary antibody and is, therefore, equally effective for all species. The zoo veterinary service confirmed that the animals were free from any infections that could cause cross-reactivity in our analysis.

The IMMUNOZYM FSME IgG all-species kit (Progen GmbH, Germany) was used for the detection of TBEV antibodies. Samples exhibiting less than 63 Vienna Units (VIEU)/ml were considered negative, samples with 63–126 VIEU/ml were considered borderline, and those with more than 126 VIEU/ml positive. The borderline and positive samples were subsequently retested by the 'gold-standard' plaque reduction neutralization test (PRNT) as described by Bárdoš et al. (1983) with slight modifications. Sera (including positive and negative controls) were diluted 1:4 in Leibowitz L-15 medium (Sigma–Aldrich, Germany) supplemented with 1% antibiotics (penicillin, streptomycin, amphotericin B; Sigma–Aldrich, Germany) and 3% foetal bovine serum. After heat inactivation of the sera at 56 °C for 30 min, 2-fold serial dilutions of the samples in L-15 medium were incubated with 10^3 PFU of TBEV strain Hypr (the virus dose was adjusted to cause almost confluent plaques with 90–95% cytolysis) for 90 min at 37 °C. 5×10^4 porcine kidney stable (PS) cells were added to each well. After 4 days of incubation, the cell supernatant was removed, and cells were fixed and stained as described previously (De Madrid and Porterfield, 1969). The highest serum dilution that caused a 90% reduction of plaques was regarded as the endpoint titre.

Results and discussion

Infectious diseases can seriously disrupt efforts to preserve endangered animal species (Stoebel et al., 2003). Information on incidence, distribution, and risk of infectious diseases in captive populations is often limited (Stoebel et al., 2003). Here, we present the first epidemiological study on Bb s.l. and TBEV exposure of zoo animals in the Czech Republic. This country is a region where LB and TBE are highly endemic (Fig. 1).

In the present study, 80 samples were positive for antibodies against Bb s.l., representing 60% of all samples tested. These included samples from even-toed ungulates ($n = 48$; 28

Table 1
Detection of antibodies against *Borrelia burgdorferi* s.l. and tick-borne encephalitis virus in the sera of captive animals from Czech zoos: detailed results.

Animal species				*Borrelia burgdorferi* s.l.		TBEV	
				No. positive	No. negative	ELISA	Neutralization test
Mammals	Even-toed ungulates	White antelope	*Addax nasomaculatus*	5	0	Negative	Negative
		Roan antelope	*Hippotragus equinus*	1	0	Negative	Negative
		Springbok	*Antidorcas marsupialis*	0	1	Negative	Negative
		Sable antelope	*Hippotragus niger*	3	1	Negative	Negative
		Mountain reedbuck	*Redunca fulvorufula*	0	1	Negative	Negative
		Lowland bongo	*Tragelaphus eurycerus*	1	0	Negative	Negative
		African buffalo	*Syncerus caffer*	0	1	Negative	Negative
		Blesbuck	*Damaliscus pygargus phillipsi*	1	0	Negative	Negative
		Dama gazelle	*Nanger dama*	3	2	Negative	Negative
		Thomson's gazelle	*Eudorcas thomsonii*	2	0	1 borderline (95 VIEU/ml); 1 negative	Negative
		Long-tailed goral	*Naemorhedus caudatus*	0	2	Negative	Negative
		Impala	*Aepyceros melampus*	0	3	Negative	Negative
		Elk	*Cervus canadensis*	1	0	Negative	Negative
		Mountain goat	*Oreamnos americanus*	2	0	Negative	Negative
		Domestic goat	*Capra aegagrus hircus*	3	0	Negative	Negative
		Carpathian goat	*Capra aegagrus hircus*	1	0	Negative	Negative
		Cashmere	*Capra aegagrus hircus*	1	0	Negative	Negative
		Markhor	*Capra falconeri*	1	0	Positive (145 VIEU/ml)	Positive (1:16)
		West Caucasian tur	*Capra caucasica*	1	0	Negative	Negative
		Lesser kudu	*Ammelaphus imberbis*	2	0	Negative	Negative
		Greater kudu	*Tragelaphus strepsiceros*	1	0	Negative	Negative
		Guanaco	*Lama guanicoe*	1	0	Negative	Negative
		Eurasian elk	*Alces alces*	1	0	NEGATIVE	Negative
		Nyala	*Tragelaphus angasii*	1	1	Negative	Negative
		Gemsbuck	*Oryx gazella*	2	0	Negative	Negative
		Racka sheep	*Ovis orientalis aries*	1	0	Negative	Negative
		Cameroon sheep	*Ovis orientalis aries*	1	0	Negative	Negative
		Suffolk sheep	*Ovis orientalis aries*	0	1	Negative	Negative
		Valachian sheep	*Ovis orientalis aries*	1	0	Negative	Negative
		German Grey Heath	*Ovis orientalis aries*	0	1	Negative	Negative
		Black wildebeest	*Connochaetes gnou*	1	2	Negative	Negative
		Barbary sheep	*Ammotragus lervia*	5	1	Negative	Negative
		Warthog	*Phacochoerus africanus*	0	1	Negative	Negative
		Scimitar oryx	*Oryx dammah*	3	2	Negative	Negative
		Charolais cattle	*Bos primigenius taurus*	0	1	Negative	Negative
		Dahomey dwarf cattle	*Bos primigenius taurus*	0	1	Negative	Negative
		Reindeer	*Rangifer tarandus*	1	0	Positive (414 VIEU/ml)	Positive (1:64)
		Waterbuck	*Kobus ellipsiprymnus*	0	1	Negative	Negative
		Southern lechwe	*Kobus leche*	1	0	Negative	Negative
		Waterbuck	*Kobus ellipsiprymnus ellipsiprymnus*	0	4	Negative	Negative
		Rothschild's giraffe	*Giraffa camelopardalis rothschildi*	0	2	Negative	Negative
		Reticulated giraffe	*Giraffa camelopardalis reticulata*	0	1	Negative	Negative
	Odd-toed ungulates	Fjord horse	*Equus ferus caballus*	2	0	Negative	Negative
		Shire horse	*Equus ferus caballus*	3	0	Negative	Negative
		Tarpan horse	*Equus ferus ferus*	2	0	Negative	Negative
		Black rhinoceros	*Diceros bicornis*	0	7	Negative	Negative
		Balkan donkey	*Equus asinus asinus*	2	0	1 bordeline (63 VIEU/ml); 1 negative	Negative
		Somali wild donkey	*Equus africanus somaliensis*	1	0	Negative	Negative
		Shetland pony	*Equus ferus caballus*	2	0	Negative	Negative
		Maneless zebra	*Equus quagga borensis*	1	0	Negative	Negative
		Bohmova Grant's zebra	*Equus quagga boehmi*	5	1	Negative	Negative
		Burchell's zebra	*Equus quagga burchellii*	1	0	Negative	Negative
		Hartmann's mountain zebra	*Equus zebra hartmannae*	4	1	Negative	Negative
	Carnivores	Cheetah	*Acinonyx jubatus*	0	1	Negative	Negative
		Spotted hyena	*Crocuta crocuta*	1	0	Negative	Negative
		Indian lion	*Panthera leo persica*	0	1	Negative	Negative
		Lion	*Panthera leo*	0	1	Negative	Negative
		Amur leopard	*Panthera pardus orientalis*	0	1	Negative	Negative
		African wild Dog	*Lycaon pictus*	1	1	Negative	Negative
		Serval	*Leptailurus serval*	0	1	Negative	Negative
		Black-backed jackal	*Canis mesomelas*	1	0	Negative	Negative
		Grey wolf	*Canis lupus*	4	0	Negative	Negative

Table 1 (*Continued*)

Animal species				*Borrelia burgdorferi* s.l.		TBEV	
				No. positive	No. negative	ELISA	Neutralization test
	Primates	Lar gibbon	*Hylobates lar*	1	0	Negative	Negative
		Angola colobus	*Colobus angolensis*	0	1	Negative	Negative
Birds	Flamingos	Greater flamingo	*Phoenicopterus roseus*	1	0	Negative	Negative
	Ostriches	Ostrich	*Struthio camelus*	0	2	Negative	Negative
Reptiles	Squamates	Burmese Python	*Python bivittatus*	0	1	Negative	Negative
	Turtles	Radiated tortoise	*Astrochelys radiata*	0	1	Negative	Negative
	Crocodiles	Siamese crocodile	*Crocodylus siamensis*	0	3	Negative	Negative

species), odd-toed ungulates ($n = 23$; 10 species), carnivores ($n = 7$; 4 species), primates ($n = 1$; 1 species), and birds ($n = 1$; 1 species). All investigated reptiles ($n = 5$; 3 species) were negative (Table 1).

A serological survey of zoo animals in St. Louis, USA, was the first study to evaluate the exposure of a broad range of zoo animals to Bb s.l. This study also demonstrated that exotic animal species kept in open-fenced areas can be found seropositive for Bb s.l. (Feir et al., 1993). In German zoos and wildlife parks, 10.4% of animals were seropositive for Bb s.l. and 11.3% were borderline seropositive (Stoebel et al., 2003). The percentage of the seropositive individuals was related to species and origin (zoo) and increased with age of the animals. Sex and season did not affect seroprevalence (Stoebel et al., 2003).

ELISA and Western blot are the most commonly used tests for the diagnosis of LB. However, the detection of antibodies against Bb does not prove an active spirochaete infection and may only reflect the immune response to past exposure. Even the detection of spirochaete DNA in the host by PCR does not provide definitive proof that a given animal species is a competent reservoir host for Bb and whether the bacteria are alive or viable. DNA fragments from dead bacteria can be detected many months after the pathogen was killed by the host complement (Kurtenbach et al., 2002).

The detection of antibodies against Bb s.l. in 60% of the samples confirms that zoo-housed animals (local and exotic species) have been exposed to tick bites and Bb s.l. similarly to free-ranging wild vertebrates. The zoo staff confirmed that they had occasionally noticed ticks feeding on the animals (personal communication). The reservoir competence for Bb s.l. of each zoo-housed animal species needs to be tested and will be the subject of a separate study.

Very few studies have investigated the TBEV seroprevalence in zoo animals (Klaus et al., 2010). In our study, only 2 individuals showed TBEV-specific antibodies: one markhor (*Capra falconeri*) and one reindeer (*Rangifer tarandus*). Both animals were kept in the same zoo (ZOO Olomouc, no. 4 in Fig. 1), located in a TBEV-endemic area. Since 2 other samples were borderline seropositive for TBEV in the TBEV antibody ELISA, all samples prescreened by ELISA were retested by the neutralization assay (PRNT) to exclude false-positive results (Klaus et al., 2010; Rushton et al., 2013). The neutralization assay confirmed the presence of anti-TBEV antibodies in the 2 samples (Table 1). No TBEV-associated clinical signs have been observed in these 2 animals. It is well known that several species of ruminants are susceptible to TBEV infection, however, TBEV-associated central nervous system disease in ruminants is rare (e.g., Bagó et al., 2002). Both animals were also positive for antibodies to Bb s.l.

While exposure of zoo animals to Bb s.l. seems to be common (60% of animals seropositive for Bb s.l.), only 2 animals were seropositive for TBEV. This is in accordance with the data on prevalence of Bb s.l. and TBEV in *I. ricinus* ticks in Central Europe. Usually less than 1% of questing ticks are positive for TBEV, but 10–25% of ticks are positive for Bb s.l. (Bingsohn et al., 2013).

Transmission of vector-borne pathogens and infectious diseases between wildlife and domestic animals is becoming an issue of major interest. Zoos represent a unique environment, where exotic and native vertebrates, arthropods, and humans interact, providing many opportunities for pathogen transmission or "sharing" infectious diseases. The risk of tick-borne infections to zoo animals was out in the spotlight after the reported severe case of TBE in a monkey (*Macaca sylvanus*) kept in a monkey park in Germany (Süss et al., 2007). The seroprevalences of TBEV and Bb s.l. in zoo animals add further information to the ecoepidemiological status of this unique environment. Preventive measures should aim to minimize tick infestation of zoo animals. The risk of infection can be reduced by avoiding habitats with a high tick density, such as wooded areas with scrub and dense vegetation (Stoebel et al., 2003).

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