

Original papers

Study on the occurrence of tick-borne encephalitis virus RNA in European bison (*Bison bonasus*) eliminated at Białowieża Primeval Forest (north-eastern Poland) in 2005–2009

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ABSTRACT. Tick-borne encephalitis virus (TBEV) (Flaviviridae, *Flavivirus*) is an arthropod-borne virus, an etiologic agent of tick-borne encephalitis (TBE), an infection involving the central nervous system. The disease is endemic in a large region in Eurasia where it is transmitted mainly by *Ixodes ricinus* in Europe and *I. persulcatus* ticks in Asia. This is the most important tick-transmitted arbovirus of human pathogenicity in Europe. The Białowieża Primeval Forest is a well-known endemic focus of tick-borne encephalitis. The aim of this study was to identify the prevalence of tick-borne encephalitis virus (TBEV) in European bison, the important hosts of ticks in the Białowieża Primeval Forest. In the years 2005–2009, 95 blood samples were collected from European bison and examined for the presence of TBEV using nRT-PCR method. No positive results were obtained. For better understanding of TBEV vertebrate reservoir hosts in Poland, further investigations are needed.

Key words: European bison, tick-borne encephalitis virus, TBEV, nRT-PCR, Białowieża Primeval Forest, Poland

Introduction

The Białowieża Primeval Forest – UNESCO Biosphere Reserve – is a well-known endemic focus of tick-borne diseases: tick-borne encephalitis, Lyme borreliosis and granulocytic anaplasmosis [1,2]. Tick borne encephalitis (TBE) is a viral zoonosis caused by TBE virus (TBEV) belonging to the family Flaviviridae, genus *Flavivirus*. It is the most important tick-transmitted arbovirus of human pathogenicity in Europe. TBE is an endemic disease in a zone extending from central and eastern Europe to Siberia and Japan and corresponds to the distribution of the ixodid ticks which act both as the vectors and the reservoir of TBEV. Genetically, three subtypes of TBEV have been recognized, the European (TBEV-Eu), the Far Eastern (TBEV-Fe) and the Siberian (TBEV-Sb) [3]. The main vector for TBEV-Eu, is the tick *Ixodes ricinus*, whereas TBEV-Fe and TBEV-Sb are transmitted mostly by *I. persulcatus*. In Poland, in ticks *I. ricinus* and *Dermacentor reticulatus* only TBEV-Eu has been recognized [4,5]. *D. reticulatus* is noted as an occasional TBE vector [6–8]. Ticks can be infected in every active stage of development and due to transstadial and transovarial transmission, every stage may transmit infections to mammals. *I. ricinus,* as well *D. reticulatus* ticks commonly attack bison in Białowieża Primeval Forest [9–14], and tick-borne pathogens were recorded in these animals. In bison, there were detected infections with *Anaplasma phagocytophilum* [15,16], *Babesia divergens* [17] and the antibodies to *Borrelia burgdorferi* [18].

European bison and cattle are closely related, therefore share many pathogens and there is the possibility of cross transmission of several bovine pathogens between free-ranging bison and domestic cattle. Among cattle viruses, in bison were diagnosed infections with virus of foot-and mouth disease, IBR/IPV, bovine rhinotracheitis like BoHV, bovine viral diarrhea (BVD), BAPV2 bovine

papillomavirus, bovine viral diarrhea virus (BVD-MD) [19–21]. However, the cases of infections with TBE virus in bison were not recorded so far, although Białowieża Forest is an endemic area of TBE.

Humans acquire the TBEV infection by the bite of an infected tick or by consumption of infected, raw (unpasteurized) milk of goat, less commonly sheep or cow or dairy products [22,23]. Over the past decades, TBE has become a growing public health concern in Europe and Asia and is the most important viral tick-borne disease in Europe [3]. First cases of human TBE in Poland were reported in Białowieża Primeval Forest over 50 years ago [24]. Currently, there are diagnosed in Poland from 101 (in 1999) to 260 (in 2008) new cases of this disease yearly. Over 90% of them were reported in Podlasie, Warmia-Mazuria, Mazovia, Lower Silesia and Opole provinces [25,26].

The aim of our study was to identify the prevalence of TBEV in European bison – natural hosts of *I. ricinus* and *D. reticulatus* ticks in Białowieża Primeval Forest.

Materials and Methods

The studies of European bison were conducted in Białowieża Primeval Forest (N52°29'–52°57', E23°31'–24°21'). The blood samples were obtained from animals eliminated during selection in winter. Material used in the study was gathered during the years 2005–2009. Blood samples were collected into sterile microtubes containing EDTA and frozen at –80°C prior to RNA isolation. The blood samples were obtained from 93 adult bison and 2 fetuses.

RNA extraction. Total RNA was extracted from the blood samples using Viral DNA/RNA kit (A&A Biotechnology) according to the manufacturer protocol and the obtained DNA/RNA samples were kept frozen in –80°C for further investigation.

Nested RT-PCR (reverse transcription-polymerase chain reaction). The reverse transcription reactions were performed as described previously [27]. For nested PCR (nRT-PCR) were used two pairs of primers (1:5'-CTCTTTCGACA CT CGTCGAGG-3', 2:5'-GCGTTTGCT(C,T)CGGA-3' and 3:5'-CCTTTCAG(A,G)ATGGCCTT-3', 4:5'-CGGA(C,T)AGCATTAGCAGCG-3') for the 5'-NCR and the 5'-terminus of the C protein coding region, which are highly conserved among TBEV isolates [28]. In this assay, the size of the first round amplification product was 175 nucleotides and that of the second round amplification was 128 nucleotides. For the second round, 1 µl of PCR mixture from the first reaction was used. All PCRs were conducted in 20 µl volume and under the same conditions: 15 min at 95°C for initial denaturation, followed by 38 cycles: 1 min at 92°C denaturation, 1 min at 37°C annealing, 2 min at 72°C extension and 7 min final extension at 72°C [29]. One positive (TBEV Langat strain) and two negative (sterile RNase-free water instead of bison RNA after the RT reaction and DDW instead of cDNA in nested PCR reactions) controls were run with each PCR reaction. All PCR reactions were carried out in Perkin Elmer GeneAmp PCR System 2400 and 9700 thermocyclers.

Amplification products were analysed by electrophoresis in 1.5% agarose gel stained with ethidium bromide. The presence of the specific products of 128 base pairs [bp] was considered as positive result.

Results

There were investigated in total 95 blood samples from European bison, collected in 2005 (n=42), 2006 (n=9), 2007 (n=13), 2008 (n=17), 2009 (n=12) and from two fetuses. No positive results on the RNA of TBE virus were obtained.

Discussion

Wild and domestic animals are commonly used as sentinels of TBE-endemic areas [30,31,3]. In Poland, TBE virus was noted in the milk of cows, goats and sheep [32]. It was also diagnosed indirectly, by the presence of antibodies in wild mammals in Europe. In Austria 26% antibodies prevalence in roe deer (*Capreolus capreolus*) was recorded, and the areas of high antibody prevalence in roe match those in which humans have been infected [33]. Recently, in the Netherlands, at least two clinical cases of TBE have been confirmed, TBEV antibody was detected in 0.5% sera of red foxes (*Vulpes vulpes*) and 7% sera of wild boar (*Sus scrofa*) [34]. Furthermore 1.1% positive spleen samples were found from red deer were found (*Cervus elaphus*) in Croatia indicating viraemia [35].

Although our results are negative, the potential role of European bison as TBE reservoir is not unlikely. Ticks are the vectors of TBEV in Białowieża Forest [5]. They are also common

parasites of bison, consequently the transmission of TBEV to these animals is very likely. It is possible, that viraemia in bison is short and in winter, when the studies were conducted, it becomes extinct [36]. It is also possible that these animals support the virus transmission between infected and uninfected ticks feeding closely [37].

Conclusions

For better understanding and identification of TBEV vertebrate reservoir hosts in Poland, further investigations are needed, including other animal species, such as small mammals, foxes and deer.

References

[1] Grzeszczuk A., Stańczak J., Kubica-Biernat B., Racewicz M., Kruminis-Łozowska W., Prokopowicz D. 2004. Human anaplasmosis in north-eastern Poland: seroprevalence in humans and prevalence in *Ixodes ricinus* ticks. *Annals of Agricultural and Environmental Medicine* 11: 99-103.

[2] Przesmycki F., Taytsch, Wróblewska Z., Lachmajer J. 1960. Investigation of a natural focus of encephalitis in the „Puszcza Białowieska" National Park. *Journal of Infectious Diseases* 106: 276-283.

[3] Süss J. 2011. Tick-borne encephalitis 2010: Epidemiology, risk areas, and virus strains in Europe and Asia – An overview. *Ticks and Tick-Borne Diseases* 2: 2-15.

[4] Wójcik-Fatla A., Cisak E., Zając V., Zwoliński J., Dutkiewicz J. 2011. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). *Ticks and Tick-Borne Diseases* 2: 16-19.

[5] Biernat B., Cieniuch S., Stańczak J. 2014. Detection of TBEV RNA in *Ixodes ricinus* Ticks in North-Eastern Poland. *Annals of Agricultural and Environmental Medicine* (In press).

[6] Kožuch O., Nosek J. 1971. Transmission of tick-borne encephalitis (TBE) virus by *Dermacentor marginatus* and *D. reticulatus* ticks. *Acta Virologica* 15: 334.

[7] Georgiev B., Rosický B., Pavlov P., Daniel M., Arnaudov D. 1971. The ticks of the natural focus of tick-borne encephalitis of sheep and man in the Rhodope mountains (Bulgaria). *Folia Parasitologica* 18: 267-273.

[8] Randolph S.E., Gern L., Nuttall P.A. 1996. Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. *Parasitology Today* 12: 472-479.

[9] Izdebska J.N. 1998. Występowanie *Dermacentor reticulatus* (Acari, Ixodidae) u żubra (*Bison bonasus*) z Puszczy Białowieskiej. *Przegląd Zoologiczny* 42: 219-221.

[10] Izdebska J.N. 2000. Stawonogi pasożytnicze żubra jako potencjalny wektor patogenów. In: *Stawonogi pasożytnicze i alergogenne.* (Eds. A. Buczek, Cz. Błaszak). KGM, Lublin: 57-64.

[11] Izdebska J.N. 2001. The occurrence of parasitic arthropods in two groups of European bison in the Białowieża Primeval Forest. *Wiadomości Parazytologiczne* 47: 801-804.

[12] Kadulski S. 1977. Ectoparasites of the *Bison bonasus* L. from the Puszcza Białowieska (Białowieża Forest). *Wiadomości Parazytologiczne* 3: 227-229.

[13] Kadulski S., Izdebska J.N., Kończyk M. 1996. Stawonogi pasożytnicze żubra *Bison bonasus* (L.) z Puszczy Białowieskiej. *Wiadomości Parazytologiczne* 42: 255-260.

[14] Karbowiak G., Izdebska J.N., Czaplińska U., Wita I. 2003. Przypadki zimowania kleszczy z rodziny Ixodidae na żywicielach w Puszczy Białowieskiej. In: *Stawonogi i żywiciele.* (Eds. A. Buczek, Cz. Błaszak). Liber, Lublin: 77-82.

[15] Grzeszczuk A., Ziarko S., Radziwon P.M., Prokopowicz D. 2004. Evidence of *Anaplasma phagocytophilum* infection of European Bison in the Białowieża Primeval Forest, Poland. *Medycyna Weterynaryjna* 60: 600-601.

[16] Matsumoto K., Grzeszczuk A., Brouqui P., Raoult D. 2009. *Rickettsia raoulti* and *Anaplasma phagocytophilum* in *Dermacentor reticulatus* ticks collected from Białowieża Primeval Forest European bison (*Bison bonasus bonasus*), Poland. *Clinical Microbiology and Infection* 15 (s2): 286-287.

[17] Karbowiak G., Slemenda S., Pieniazek N.J., Rychlik L., Stanko M., Sinski E., Wita I., Czaplinska U. 2004. Babesiosis of wild animals in Poland. Abstracts of IX European Multicolloquium of Parasitology, 18-23 July, Valencia, Spain: 451.

[18] Siński E., Gill J., Rijpkema S. 1996. Prevalence of antibodies to *Borrelia burgdorferi* in European bison (*Bison bonasus*) from Białowieża Primeval Forest. *Roczniki Akademii Medycznej w Białymstoku* 41: 111-116.

[19] Borchers K., Brackmann J., Wolf O., Rudolph M., Glatzel P., Krasińska M., Krasiński A.Z., Frölich K. 2002. Virologic investigations of free-living European bison (*Bison bonasus*) from the Białowieża Primeval Forest, Poland. *Journal of Wildlife Diseases* 38: 533-538.

[20] Kita J., Anusz K. 2006. Infectious diseases in *Bison bonasus* in the years 1910-1998. In: *Health threats for the European bison particularly in free-roaming populations in Poland.* (Eds. J. Kita, K. Anusz). SGGW Publishers, Warsaw: 17-26.

[21] Literák I., Tomita Y., Ogawa T., Shirasawa H., Smíd

B., Novotny L., Adamec M. 2006. Papillomatosis in a European bison. *Journal of Wildlife Diseases* 42: 149-153.

[22] Balogh Z., Ferenczi E., Szeles K., Stefanoff P., Gut W., Szomor K.N., Takacs M., Berencsi G. 2010. Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. *Journal of Virological Methods* 163: 481-485.

[23] Grešíková M. 1972. Studies on tick-borne arboviruses isolated in Central Europe. *Biologicke Prace (Bratislava)* 18: 1-116.

[24] Demiaszkiewicz W. 1952. Wiosenno-letnie kleszczowe zapalenie mózgu w Puszczy Białowieskiej. *Polski Tygodnik Lekarski* 7: 799-801.

[25] Stefanoff P., Rosińska M., Zieliński A. 2006. Epidemiologia chorób przenoszonych przez kleszcze. *Przegląd Epidemiologiczny* 60: 151-159.

[26] Stefanoff P., Siennicka J., Kaba J., Nowicki M., Ferenczi E., Gut W. 2008. Identification of new endemic tick-borne encephalitis foci in Poland – a pilot seroprevalence study in selected regions. *International Journal of Medical Microbiology* 298 (S1): 102-107.

[27] Huang C., Slater B., Campbell W. 2001. Detection of arboviral RNA directly from mosquito homogenates by reverse-transcription-polymerase chain reaction. *Journal of Virological Methods* 94: 121-128.

[28] Ramelow Ch., Süss J., Berndt D., Roggendorf M., Schreier E. 1993. Detection of tick-borne encephalitis virus RNA in ticks (*Ixodes ricinus*) by the polymerase chain reaction. *Journal of Virological Methods* 45: 115-119.

[29] Sparagano O.A., Allsopp M.T., Mank R.A., Rijpkema S.G., Figueroa J.V., Jongejan F. 1999. Molecular detection of pathogen DNA in ticks (Acari: Ixodidae): a review. *Experimental and Applied Acarology* 23: 929-960.

[30] Klaus Ch., Beer M., Saier R., Schau U., Moog U., Hoffman B., Diller R., Süss J. 2012. Goats and sheep as sentinels for tick-borne encephalitis (TBE) virus – epidemiological studies in areas endemic and non-endemic for TBE virus in Germany. *Ticks and Tick-Borne Diseases* 3: 27-37.

[31] Leschnik M.W., Kirtz G.C., Thalhammer J.G. 2002. Tick-borne encephalitis (TBE) virus in dogs. *International Journal of Medical Microbiology* 291: 66-69.

[32] Cisak E., Wójcik-Fatla A., Zając V., Sroka J., Buczek A., Dutkiewicz J. 2010. Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and sheep in eastern Poland. *Annals of Agricultural and Environmental Medicine* 17: 283-286.

[33] Gerth H.J., Grimshandl D., Stage B., Döller G., Kunz C. 1995. Roe deer as sentinels for endemicity of tick-borne encephalitis virus. *Epidemiology and Infection* 115: 355-365.

[34] Van der Poel W.H.M., Van der Heide R., Bakker D., de Looff M., de Jong J., Van Manen N., Gaasenbeck C.P.H., Borgsteede F.H.M. 2005. Attempt to Detect Evidence for Tick-Borne Encephalitis Virus in Ticks and Mammalian Wildlife in the Netherlands. *Vector-Borne and Zoonotic Diseases* 5: 58-64.

[35] Jemeršić L., Deždek D., Brnić D., Prpić J., Janicki Z., Keros T., Roić B., Slavica A., Terzić S., Konjević D., Beck R. 2014. Detection and genetic characterization of tick-borne encephalitis virus (TBEV) derived from ticks removed from red foxes (*Vulpes vulpes*) and isolated from spleen samples of red deer (*Cervus elaphus*) in Croatia). *Ticks and Tick-Borne Diseases* 5: 7-13.

[36] Van Tongeren H.A. 1955. Encephalitis in Austria. IV. Excretion of virus by milk of the experimentally infected goat. *Archiv für Gesamte Virusforschung* 6: 158-162.

[37] Randolph S.E. 2011. Transmission of tick-borne pathogens between co-feeding tick: Milan Labuda's paradigm. *Ticks and Tick-Borne Diseases* 2: 179-182.

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