

Serological survey of epizootic hemorrhagic disease in European bison in Poland in 2017-2018*

ANNA DIDKOWSKA¹, MARCIN PTAK¹, KATARZYNA OPALCZYŃSKA¹,
BLANKA ORŁOWSKA¹, MARTA GAŁĄZKA¹, WANDA OLECH², KRZYSZTOF ANUSZ¹

¹Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 166, 02-787 Warsaw, Poland

²Department of Animal Genetics and Conservation, Institute of Animal Sciences, Warsaw University of Life Sciences (SGGW), Ciszewskiego 8, 02-786 Warsaw, Poland

Received 23.03.2021

Accepted 30.06.2021

Didkowska A., Ptak M., Opalczyńska K., Orłowska B., Gałazka M., Olech W., Anusz K.

Serological survey of epizootic hemorrhagic disease in European bison in Poland in 2017-2018

Summary

The European bison (*Bison bonasus*) is an endangered species whose restitution requires systematic health monitoring. The most practical way to test clinical material from large wildlife is by serological methods. Recently, the most serious health hazard faced by the European bison population was tuberculosis. However, climate change and globalization have resulted in the arrival of more exotic diseases, one of which is epizootic hemorrhagic disease (EHD), caused by the epizootic hemorrhagic disease virus (EHDV). EHD is an arthropod-transmitted viral disease noted mainly in ungulates, especially ruminants. Clinical signs vary depending on the virus serotype and host. The aim of this study was to determine the prevalence of EHD in European bison by serological testing for antibodies to EHDV. Serum samples were collected from 90 European bison living in different regions of Poland and tested with PrioCHECK™ EHDV Ab Serum Kit (ThermoFisher, US). One of the bison (from the Knyszyńska Forest) had a positive result. It cannot be excluded, however, that the result was a false positive. There is a need for further EHDV serology monitoring in European bison and other wildlife, particularly in the Knyszyńska Forest.

Keywords: epizootic hemorrhagic disease, European bison, serological survey

Since the reintroduction of European bison (*Bison bonasus*), their global population continues to grow: in 2019, the population in Poland, one third of the global population, exceeded 2200 animals (32). However, despite this growth, the species is still regarded as protected, according to the International Union for Conservation (IUCN), and efforts to support its restitution are continuing. Hence, the species requires active protection (26), especially because of the growing size of the free-living population. Although the main health threats seem to be infectious and parasitic diseases (7, 31), new ones, such as pesticide contamination, are also emerging (17). Recent years have seen the increased occurrence in European bison of viral and bacterial diseases, such as tuberculosis (28), particularly those transmitted by insects (16).

One such potential insect-borne disease is epizootic hemorrhagic disease (EHD), caused by epizootic hemorrhagic disease virus (EHDV), a member of the

Reoviridae family, which exist as seven serotypes (4). It is transmitted by arthropod vectors of the genus *Culicoides* (30). EHDV was first described in white-tailed deer (*Odocoileus virginianus*) (36), but it has since also been noted in other wild ungulates and in cattle (9, 15, 22). EHD is mostly asymptomatic in domestic ruminants, but manifests as a hemorrhagic disease in wildlife (22). This variation in clinical symptoms is further influenced by serotype (10-12), herd immunity (1) and host sustainability (37).

A recent EHDV-7 epidemic in cows resulted in significantly decreased milk production, but this was accompanied by clinical disease (1). In white-tailed deer, EHD manifests as fever, weakness, facial oedema, and inappetence (5), and vaccines have recently been developed (3, 39).

Climate change and globalization have increased the risk of pathogens spreading to new and unexpected geographic areas (21). With this in mind, and considering the acute course of the disease in some wild ruminants and the potential threat to endangered species, the aim of this study was to determine whether European

* The study was supported by the "Complex project for the conservation of European bison by State Forests", financed by the Forest Fund (Poland), contract no OR.271.3.10.2017.

bison in Poland have come into contact with EHDV and whether the disease can pose a real threat to their restitution.

Material and methods

Study preparation. Blood samples were collected *ante mortem* or *post mortem* from 90 European bison between September 2017 and April 2018. The research was conducted as part of the “Complex project for the conservation of European bison by State Forests” in Poland. All procedures were performed with due attention to animal welfare, in accordance with Polish regulations. The age of the animals ranged from eight months to 25 years (Tab. 1). The animals lived as free-ranging and enclosed-breeding herds in various regions of Poland, including the Bieszczady mountains, Białowieska, Borecka and Knyszyńska forests, and in various European bison breeding centers around Poland (Fig. 1).

Most blood samples were collected from the jugular vein (*v. iugularis externa*) and some from the heart or tail vein (*v. caudalis mediana*). All were taken into 9 ml tubes with clot activator. After collection, the tubes were transported to the laboratory as soon as possible. Serum was obtained by centrifugation. Following this, the samples were frozen and stored at -20°C for further analysis.

Serology. The serum was defrosted, brought to room temperature, and tested with a PrioCHECK™ EHDV Ab Serum Kit (ThermoFisher, US), which is an immunoenzymatic test (blocking ELISA) for the detection of anti-EHDV IgG antibodies in ruminant serum. The test was conducted in accordance with the manufacturer’s instructions. The results were read with an EPOCH spectrophotometer (BioTek Instruments Inc., the U.S.) at 450 nm. The results were interpreted on the basis of percent inhibition (% Inh) calculated in the following way:

$$\% \text{ Inh} = [(OD_{mNC} - OD_{sample}) / OD_{mNC}] * 100.$$

The test results are considered valid if mean $OD_{mNC} > 0.600$ and $\% \text{ Inh}_{pc} \geq 60\%$. If $\% \text{ Inh} \geq 60\%$, the results are considered positive. If $\% \text{ Inh} \geq 55\%$ and $\% \text{ Inh} < 60\%$, the results are considered doubtful. If $\% \text{ Inh} < 55\%$, the results are considered negative.

Results and discussion

To obtain the most representative cross-sectional results, the present study included both free-living and captive European bison from various regions of Poland. Although obtaining a large number of serum samples from wildlife threatened with extinction is



Fig. 1. The location and size of free-ranging (green) and captive (dark blue) European bison populations used in the study. The red plus sign indicates the population with the EHDV-positive individual

a considerable challenge, our findings present an accurate picture of the latest serological status for EHD in European bison. In the present study, only one out of 90 samples yielded a positive result ($\% \text{ Inh} = 61.08$) in the blocking ELISA. The sample was collected from an asymptomatic free-ranging five-year-old European bison female from the Knyszyńska Forest (Tab. 1). Our study not only confirms the first positive identification of EHDV antibodies in European bison in Poland and in the world, but also reports the first usage of the PrioCHECK™ EHDV Ab Serum Kit in the genus *Bison*. Previous studies of EHDV antibodies

Tab. 1. The relationship between the herd type (captive or free-ranging), age, and sex of the animals

Type of herd	Age	Number of bison	
		Female	Male
Captive herds	≤ 1 year	6	0
	2-5 years	16	12
	≥ 6 years	2	2
Free-ranging herds	≤ 1 year	2	0
	2-5 years	17*	10
	≥ 6 years	18	5

Explanation: * indicates the group to which the positive individual belonged

in European bison were conducted with the blocking LSIVet™ Ruminant EHDV Serum ELISA Kit (Live Technologies, Carlsbad, California), which yielded only negative results (18).

The present study used the PrioCHECK™ EHDV Ab Serum Kit (ThermoFisher, US) for testing serum. The World Organisation for Animal Health (OIE) recommends the use of such specific monoclonal antibody-based competitive ELISA tests for the detection of anti-EHDV antibodies in serum samples. Moreover, as the kit is used for all ruminants, there is no need to adapt and optimize it for use in European bison, and, according to the manufacturer, it is characterized by a high level of sensitivity and specificity.

According to the OIE, the gold standard for serotype identification is the use of virus neutralization (VN) tests, which are used to detect and quantify serotype-specific antibodies. In addition to serology, EHDV infection can also be diagnosed by detecting the virus itself, and, although virus isolation is more difficult, its RNA can indeed be amplified and detected by PCR. In fact, both RT-PCR and real-time RT-PCR are recommended by the OIE for this purpose (<https://www.oie.int/app/uploads/2021/03/epizootic-haemorrhagic-disease.pdf>).

In the monitoring of European bison, serological methods are both cheaper and easier to use. However, it may be worth introducing RT-PCR testing if more individuals are found to be positive (20, 41). It would also be reasonable to conduct RT-PCR testing for ELISA-positive European bison from the Knyszyńska Forest, but no appropriate material (whole blood, tissues) is available. We therefore propose that the serological survey be extended from European bison to other free-living animals, especially in the area of the Knyszyńska Forest where the EHDV-seropositive European bison was identified.

It cannot be excluded that the result obtained in this study was a false positive. Although the PrioCHECK™ EHDV Ab Serum Kit manual emphasizes that the test is capable of detecting different EHDV serotypes without detecting serotypes of Bluetongue virus, these two viruses are very similar (24, 33), and false diagnoses have been reported (1, 2). The suspicion that it was a cross-reaction stems from previous studies indicating a BTV seroprevalence of 24.7% in European bison in Poland. What is more, BTV-seropositive individuals have also been detected in herds from eastern Poland (18), and European bison are known to be susceptible to BTV infection (34). BTV has also been found in Poland in cattle near the Polish-Belarusian border, but, interestingly, the virus showed a close similarity to the vaccine strain from Lithuanian and Russian animals (27). It should be emphasized that BTV antibodies have also been reported in European bison in Russia (29) and in other animals in Europe (6, 19).

It is difficult to predict the course of EHD in European bison, but it should be regarded as a potential

threat to its restitution. The EHDV epidemic of 2012 among American bison resulted in 7% morbidity, demonstrating that it can significantly affect population numbers (38). Interestingly though, many of the EHDV-infected American bison did not show any clinical sign of disease (2).

Globally, EHDV has been reported in both domestic cattle and wildlife in North and South America, Africa, and Asia (8, 9, 13, 14, 23, 25, 40). It has also been found in countries neighboring Europe, such as Turkey (3). Although EHD is generally regarded as an exotic disease in Poland, globalization and climate change have brought it closer, and its appearance in Polish wildlife is becoming increasingly likely, particularly since *Culicoides punctatus* and *C. obsoletus*, reported to be the main transmitters of EHDV in North America and Japan, are also highly dispersed in Poland (35). It has been suggested that subclinical infection of cattle may play a significant role in the emergence of EHD in Europe (22). If this is true, European bison may also play a similar role among wildlife in Poland.

In conclusion, our findings are the first confirmation of EHDV antibodies in European bison. It cannot be ruled out, however, that it is a false positive result. To be certain, further EHDV testing, including other methods, such as PCR, is recommended in European bison, with a particular emphasis on animals of the Knyszyńska Forest. Future research should also include other free-living animals from this area.

References

1. Agüero M., Buitrago D., Gómez-Tejedor C.: False-positive results obtained when bluetongue virus serotype 1 Algeria 2006 was analyzed with a reverse transcription-PCR protocol for detection of epizootic hemorrhagic disease virus. *J. Clin. Microbiol.* 2008, 46, 3173-3174.
2. Ahmed S., Mahmoud M. A. E., Viarouge C., Sailleau C., Zientara S., Breard E.: Presence of bluetongue and epizootic hemorrhagic disease viruses in Egypt in 2016 and 2017. *Infect. Genet. Evol.* 2019, 73, 221-226.
3. Albayrak H., Ozan E., Gur S.: A serological investigation of epizootic hemorrhagic disease virus (EHDV) in cattle and gazella subgutturosa subgutturosa in Turkey. *Trop. Anim. Health Prod.* 2010, 42, 1589-1591.
4. Anthony S. J., Maan S., Maan N., Kgosana L., Bachanek-Bankowska K., Batten C., Darpel K. E., Sutton G., Attoui H., Mertens P. P.: Genetic and phylogenetic analysis of the outer-coat proteins vp2 and vp5 of epizootic haemorrhagic disease virus (EHDV): Comparison of genetic and serological data to characterise the ehdv serogroup. *Virus Res.* 2009, 145, 200-210.
5. Beringer J., Hansen L. P., Stallknecht D. E.: An epizootic of hemorrhagic disease in white-tailed deer in Missouri. *J. Wildl. Dis.* 2000, 36, 588-591.
6. Cappai S., Rolesu S., Loi F., Liciardi M., Leone A., Marzacci M., Teodori L., Mangone I., Sghaier S., Portanti O., Savini G., Lorusso A.: Western Bluetongue virus serotype 3 in Sardinia, diagnosis and characterization. *Transbound. Emerg. Dis.* 2019, 66, 1426-1431.
7. Didkowska A., Krajewska-Wędzina M., Bielecki W., Brzezińska S., Augustynowicz-Kopeć E., Olech W., Anusz K., Sridhara A. A., Johnathan-Lee A., Elahi R., Miller M. A., Ray Waters W., Lyashchenko K. P.: Antibody responses in European bison (*Bison bonasus*) naturally infected with *Mycobacterium caprae*. *Vet. Microbiol.* 2021, 253.
8. Dinh E. T. N., Orange J. P., Peters R. M., Wisely S. M., Blackburn J. K.: Resource Selection by Wild and Ranged White-Tailed Deer (*Odocoileus virginianus*) during the Epizootic Hemorrhagic Disease Virus (EHDV) Transmission Season in Florida. *Animals (Basel)* 2021, 11, 211.
9. Favero C. M., Matos A. C., Campos F. S., Cândido M. V., Costa É. A., Heinemann M. B., Barbosa-Stancioli E. F., Lobato Z. I.: Epizootic hemorrhagic disease in brocket deer, Brazil. *Emerg. Infect. Dis.* 2013, 19, 346-348.
10. Gaydos J. K., Davidson W. R., Elvinger F., Howerth E. W., Murphy M., Stallknecht D. E.: Cross-protection between epizootic hemorrhagic disease virus serotypes 1 and 2 in white-tailed deer. *J. Wildlife Dis.* 2002, 38, 720-728.

11. Gaydos J. K., Davidson W. R., Elvinger F., Mead D. G., Howerth E. W., Stallknecht D. E.: Innate resistance to epizootic hemorrhagic disease in white-tailed deer. *J. Wildlife Dis.* 2002, 38, 713-719.
12. Gaydos J. K., Stallknecht D. E., Kavanaugh D., Olson R. J., Fuchs E. R.: Dynamics of maternal antibodies to hemorrhagic disease viruses (Reoviridae: Orbivirus) in white-tailed deer. *J. Wildlife Dis.* 2002, 38, 253-257.
13. Gordon S. J. G., Bohwell C., Rogers C. W., Musuka G., Kelly P., Guthrie A., Mellor P. S., Hamblin C.: A serosurvey of bluetongue and epizootic haemorrhagic disease in a convenience sample of sheep and cattle herds in Zimbabwe. *Onderstepoort J. Vet. Res.* 2017, 84, e1-e5.
14. Kamomae Y., Kamomae M., Ohta Y., et al.: Epizootic Hemorrhagic Disease Virus Serotype 6 Infection in Cattle, Japan, 2015. *Emerg. Infect. Dis.* 2018, 24, 902-905.
15. Kedmi M., Van Straten M., Ezra E., Galon N., Klement E.: Assessment of the productivity effects associated with epizootic hemorrhagic disease in dairy herds. *J. Dairy Sci.* 2010, 93, 2486-2495.
16. Kęsik-Maliszewska J., Krzysiak M. K., Grochowska M., Lechowski L., Chase C., Larska M.: Epidemiology of Schmallenberg virus in European bison (*Bison bonasus*) in Poland. *J. Wildl. Dis.* 2018, 54, 272-282.
17. Klich D., Lopucki R., Stachniuk A., Sporek M., Fornal E., Wojciechowska M., Olech W.: Pesticides and conservation of large ungulates: Health risk to European bison from plant protection products as a result of crop depredation. *PLoS One* 2020, 15: e0228243.
18. Krzysiak M. K., Iwaniak W., Kęsik-Maliszewska J., Olech W., Larska M.: Serological Study of Exposure to Selected Arthropod-Borne Pathogens in European Bison (*Bison bonasus*) in Poland. *Transbound. Emerg. Dis.* 2017, 64, 1411-1423.
19. Kundlacz C., Caignard G., Sailleau C., Viarouge C., Postic L., Vitour D., Zientara S., Breard E.: Bluetongue Virus in France: An Illustration of the European and Mediterranean Context since the 2000s. *Viruses* 2019, 11, 672.
20. Maan N. S., Maan S., Potgieter A. C., Wright I. M., Belaganahalli M., Mertens P. P. C.: Development of Real-Time RT-PCR Assays for Detection and Typing of Epizootic Haemorrhagic Disease Virus. *Transbound. Emerg. Dis.* 2017, 64, 1120-1132.
21. MacLachlan J. N., Guthrie A. J.: Re-emergence of bluetongue, African horse sickness, and other orbivirus diseases. *Vet. Res.* 2010, 41, 35.
22. MacLachlan J. N., Zientara S., Savini G., Daniels P. W.: Epizootic haemorrhagic disease. *Rev. Sci. Tech.* 2015, 34, 341-351.
23. Merrill M. M., Boughton R. K., Lollis L. O., Saylor K. A., Wisely S. M.: Epidemiology of Bluetongue Virus and Epizootic Hemorrhagic Disease Virus in Beef Cattle on a Ranch in South-Central Florida. *Vector Borne Zoonotic Dis.* 2019, 19, 752-757.
24. Mertens P. P. C., Maan S., Samuel A., Attoui H.: Orbivirus, Reoviridae, Virus Taxonomy, VIIIth Report of the ICTV. 2nd ed., Elsevier Academic Press, San Diego, CA, USA 2005, pp. 466-483.
25. Nol P., Kato C., Reeves W. K., Rhyhan J., Spraker T., Gidlewski T., VerCauteren K., Salman M.: Epizootic hemorrhagic disease outbreak in a captive facility housing white-tailed deer (*Odocoileus virginianus*), bison (*Bison bison*), elk (*Cervus elaphus*), cattle (*Bos taurus*), and goats (*Capra hircus*) in Colorado, USA. *J. Zoo Wildl. Med.* 2010, 41, 510-515.
26. Olech W., Klich D., Perzanowski K.: Development of a new Action Plan for the European bison. *Oryx* 2019, 53, 214-214.
27. Orłowska A., Trezbas P., Smreczak M., Marzec A., Zmudziński J. F.: First detection of bluetongue virus serotype 14 in Poland. *Arch. Virol.* 2016, 161, 1969-1972.
28. Orłowska B., Krajewska-Wędzina M., Augustynowicz-Kopeć E., Kozińska M., Brzezińska S., Zabost A., Didkowska A., Welz M., Kaczor S., Żmuda P., Anusz K.: Epidemiological characterization of *Mycobacterium caprae* strains isolated from wildlife in the Bieszczady Mountains, on the border of Southeast Poland. *BMC Vet. Res.* 2020, 16, 362.
29. Pavlova E. V., Christopolova M. D., Hernandez-Blanco J. A., Alschinetski M. V., Sipko N. D., Naidenko S. V.: Estimation of hematological parameters and antibodies against diseases in free-living European bison (*Bison bonasus*) in Russia. Wisent in the Miroslawiec bioregion, Walcz, Poland 2013, 4-5.
30. Pfannenstiel R. S., Mullens B. A., Ruder M. G., Zurek L., Cohnstaedt L. W., Nayduch D.: Management of North American Culicoides Biting Midges: Current Knowledge and Research Needs. *Vector Borne Zoonotic Dis.* 2015, 15, 374-384.
31. Pyziel A. M., Björck S., Wiklund R., Skarin M., Demiaszkiewicz A. W., Höglund J.: Gastrointestinal parasites of captive European bison *Bison bonasus* (L.) with a sign of reduced efficacy of *Haemonchus contortus* to fenbendazole. *Parasitol. Res.* 2018, 117, 295-302.
32. Raczyński J.: European Bison pedigree book 2019. Białowieża National Park, Białowieża 2020, 1-83.
33. Ruder M. G., Lysyk T. J., Stallknecht D. E., Foil L. D., Johnson D. J., Chase C. C., Dargatz D. A., Gibbs E. P.: Transmission and Epidemiology of Bluetongue and Epizootic Hemorrhagic Disease in North America: Current Perspectives, Research Gaps, and Future Directions. *Vector Borne Zoonotic Dis.* 2015, 15, 348-363.
34. Sanderson S.: Bluetongue in non-domestic ruminants: experiences gained in EAZA zoos during the 2007 & 2008 BTV8 and BTV1 epizootics, [in:] Kaandorp J., Chai N. and Bayens A. (eds): *Transmissible Diseases Handbook. Infectious Diseases Working Group, European Association of Zoo and Wildlife Veterinarians*, Amsterdam, the Netherlands 2010.
35. Savini G., Afonso A., Mellor P., Aradaib I., Yadin H., Sanaa M., Wilson W., Monaco F., Domingo M.: Epizootic haemorrhagic disease. *Res. Vet. Sci.* 2011, 91, 1-17.
36. Shope R. E., Macnamara L. G., Mangold R.: A virus-induced epizootic hemorrhagic disease of the Virginia white-tailed deer (*Odocoileus virginianus*). *J. Exp. Med.* 1960, 111, 155-170.
37. Stallknecht D. E., Nettles V. F., Rollor E. A., Howerth E. W.: Epizootic hemorrhagic disease virus and bluetongue virus serotype distribution in white-tailed deer in Georgia. *J. Wildlife Dis.* 1995, 31, 331-338.
38. Stevens G., Mc Cluskey B., King A., O'Hearn E., Mayr G.: Review of the 2012 Epizootic Hemorrhagic Disease Outbreak in Domestic Ruminants in the United States. *PLoS One* 2015, 10(8):e0133359.
39. Sunwoo S. Y., Noronha L. E., Morozov I., Trujillo J. D., Kim I. J., Schirtzinger E. E., Faburay B., Drolet B. S., Urbaniak K., McVey D. S., Meekins D. A., Palmer M. V., Balaraman V., Wilson W. C., Richt J. A.: Evaluation of A Baculovirus-Expressed VP2 Subunit Vaccine for the Protection of White-Tailed Deer (*Odocoileus virginianus*) from Epizootic Hemorrhagic Disease. *Vaccines (Basel)* 2020, 8, 59.
40. Toye P. G., Batten C. A., Kiara H., et al.: Bluetongue and epizootic haemorrhagic disease virus in local breeds of cattle in Kenya. *Res. Vet. Sci.* 2013, 94, 769-773.
41. Viarouge C., Breard E., Zientara S., Vitour D., Sailleau C.: Duplex Real-Time RT-PCR Assays for the Detection and Typing of Epizootic Haemorrhagic Disease Virus. *PLoS One* 2015, 10, e0132540.

Corresponding author: Anna Didkowska, PhD, DVM, Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 166, 02-787 Warsaw, Poland; e-mail: anna_didkowska@sggw.edu.pl