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Further epidemiological investigation of tuberculosis in European bison (*Bison bonasus*) in Borecka Forest

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Abstract: European bison (*Bison bonasus*) is very prone to infection with mycobacteria tuberculosis, which is proven by outbreaks of TB observed in this species since 1996. The International Union for Conservation of Nature (IUCN) has classified the wisent as a threatened species, which is why the monitoring of infectious diseases has to be carried out especially carefully. Because two TB cases have been reported in the European bison of Borecka Forest, the population should be continuously controlled in this respect. Actions have been taken to preliminarily specify the epidemiological situation of tuberculosis in the Borecka Forest wisent population. In total 22 individuals were examined using a commercial interferon-gamma release assay (IGRA) that relies on delayed-type hypersensitivity. Blood collected to lithium heparin tubes was stimulated with bovine and avian tuberculin, as well as with PBS (control). The results were interpreted based on the optical density measured for individual samples, as it is proportional to the amount of interferon-gamma released by T lymphocytes (INF-g - a protein characteristic of the immune response in tuberculosis). To verify negative results of the IGRA, material (mandibular and retropharyngeal lymph nodes) was collected *post mortem* from 10 of the 22 examined European bison to be tested microbiologically on specific Stonebrink and, additionally, Löwenstein-Jensen media. Although the results of the IGRA were negative, further monitoring of tuberculosis in the Borecka Forest wisent population is considered as necessary in the future.

Keywords: interferon-gamma release assay, European bison, *Bison bonasus*, tuberculosis

Introduction

Bovine tuberculosis (bTB) is a zoonosis; thus, controlling it is important for public health protection. Additionally, it is of substantial economic importance, as it may cause major losses in dairy and beef cattle production, as well as lead to barriers in trade. In recent years, an increasing numbers of reports have arisen that free-living animals play a significant role in the epidemiology of tuberculosis, because they may act as a reservoir (Delahay *et al*. 2001; Gortazar *et al*. 2011).

Intravital diagnostics of tuberculosis in farm and free-living animals is currently based mostly on tuberculin skin tests. To assess test results, a topical inflammatory reaction (mainly swelling) is observed after an intradermal tuberculin PPD (purified protein derivative) injection. Animals reacting positively show the strongest reaction after approx. 72 hours. Another test that may be performed as part of intravital TB diagnostics in E. bison is the interferon-gamma release assay (IGRA). It has been recommended by the World Organisation of Animal Health since 1996 as supplemental to the tuberculin test. In the European Union, according to Commission Regulation No. 1226/2002/EC, it was authorised as an ancillary assay to the tuberculin test. In the IGRA, T lymphocytes are stimulated in vitro with bovine and avian tuberculin, as well as with a control antigen (PBS – phosphate buffered saline), and subsequently, the amount of interferon-gamma released in the three plasma samples is compared. Currently, the market also offers "peptide cocktails" which help achieve greater specificity with slightly lower sensitivity. Just like the tuberculin test, the IGRA is based on delayed-type hypersensitivity. It should be noted that the results of this test may be affected by many factors such as, above all, the immunological status of the animal. For instance, false negative results are possible in animals which have been treated with anti-inflammatory drugs (Kita, Anusz 2009).

The objective of our research was to discover the epidemiological situation of tuberculosis in the European bison of Borecka Forest, where cases of the disease have been reported. Additionally, the material collected *post mortem* from 10 individuals will allow to compare the IGRA results with microbiological culture results that remain a gold standard for tuberculosis diagnostics in animals. The study will also let us preliminarily assess the usefulness of the IGRA for intravital tuberculosis diagnostics in Borecka Forest wisents.

Material and methods

The research was carried out in Borecka Forest, where at the end of 2017 the population of 120 European bison roamed. Blood to be tested was collected from 22 individuals; 12 intravitally and 10 *post mortem*. The study was conducted using BOVIGAM® assay (Prionics, Switzerland). The blood was collected to 6 ml lithium

heparin tubes. It was then gently mixed by rotating the tubes several times and transported to laboratory at 22°C. A mobile car incubator was applied for the transport, which ensured the appropriate incubation temperature immediately after the collection (it is particularly important in autumn and winter). This, in addition to adhering to the duration of all test stages, is crucial for the survival of lymphocytes. Lymphocyte viability is critical to achieve reliable test results, in particular, to avoid false negatives.

The next step of the procedure was initiated not later than 30h after blood collection. The blood was divided in three parts and, subsequently, stimulating antigens (avian and bovine tuberculin PPD) and blank control (PBS) were added to respective tubes. The blood with antigens was incubated at 37°C. In accordance with other authors' remarks on obtaining more reliable results, our aim was to collect plasma after the maximal incubation time possible (Krajewska *et al*. 2014a), although at this stage, the test allows an incubation of 16 to 24h. The plasma was collected after 24h and used as material for ELISA (enzyme-linked immunosorbent assay). The test we used was a double-antibody sandwich ELISA. In this case, antigens are immobilized between the primary and the secondary antibody.

The optical density was measured at 450 nm. A test was considered diagnostic if the absorbance value was under 0,130 for negative control and above 0,700 for positive control. To interpret the results, we calculated the differential between the absorbance for bovine tuberculin-stimulated plasma and the absorbance for plasma treated with PBS. Our second step was to calculate the differential between absorbance values for bovine and avian tuberculin-stimulated plasmas. If both differentials were under 0,1, the test result was interpreted as negative.

Results

Negative results were obtained for all 22 individuals included in the study, for both avian and bovine tuberculin stimulation.

Discussion

Intravital diagnostics in free-living animals poses a major challenge because of the difficulty in collecting material and carrying out tuberculin tests. For this reason, carefully planned actions are needed to monitor infectious diseases, especially with regard to threatened species such as the European bison. Currently, modified tests for intravital tuberculosis diagnostics in animals are being developed (Maas *et al*. 2013) that should be considered for use in European bison. It is also possible to apply new, enhanced serological tests (Aagaard *et al*. 2006), as well as an interferon-gamma release assay using more specific antigens (Pollock *et al*. 2003). This could increase the reliability of results. For disease diagnostics in threatened species, economic

considerations play a lesser role than for farm animals, which makes it possible to use a greater array of various tests.

Interferon-gamma release assay is only authorized as a supplementary test for tuberculosis diagnostics in animals. It requires very strict conditions of blood transport and the procedure is so complicated that errors may occur at many stages. However, its major advantage over the tuberculin skin test is that it does not require another immobilization of the animal to measure results, which is particularly important with free-living animals.

Another advantage of the IGRA is that results are obtained faster than in the tuberculin test (intravital diagnostics) and microbiological cultures (*post mortem* examination). IGRA sensitivity is lower if blood is collected postmortem than when it is collected intravitally (Rothel *et al*. 1992).

The first case of tuberculosis in Polish population of European bison was reported in 1997, when a cow in the Bieszczady mountains was affected (Żórawski, Lipiec 1997). By 2013, a total of 45 tuberculosis cases in European bison were diagnosed in this area (Krajewska *et al*. 2014b). *Mycobacterium caprae* in Bieszczady has been isolated also from other free-living species including the grey wolf *Canis lupus* (Orłowska *et al*. 2017).

Tuberculosis was also diagnosed in the European Bison Breeding Center in Smardzewice, where *Mycobacterium caprae* was isolated from 10 individuals in 2013–2015 (Krajewska *et al*. 2016). In 2016, tuberculosis was reported in two wisents in the area of Borecka Forest, one of which came from the above Smardzewice breeding center (unpublished data). The second individual stemmed from a free-living herd.

Although the interferon-gamma release assay results were negative, further actions are required to monitor tuberculosis in the Borecka Forest European bison population. Lymph nodes should be collected from all individuals found dead or shot, and cultures should be performed. Additionally, if animals are immobilized, blood should be collected and tested intravitally for tuberculosis (IGRAs, serological tests). It should be considered to perform intravital tests in cattle from this area, covering more individuals and performing the tests more often than it is foreseen in the Instruction of the Chief Veterinary Officer of 8 February 2016 on the proceedings at suspecting, confirming and eradicating bovine tuberculosis in a cattle herd and on conducting control tests for bTB.

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References

Aagaard C., Govaerts M., Meikle V., Vallecillo A. J., Gutierrez-Pabello J. A., Suarez-Güemes F., McNair J., Cataldi A., Espitia C., Andersen P., Pollock J.M. 2006. Optimizing antigen cocktails for detection of *Mycobacterium bovis* in herds with different prevalences of bovine tuberculosis: ESAT6-CFP10 mixture shows optimal sensitivity and specificity. J Clin Microbiol., 44, 4326–35.

Delahay R.J., Cheeseman C.L., Clifton-Hadley R.S. 2001. Wildlife disease reservoirs: the epidemiology of *Mycobacterium bovis* infection in the European badger (Meles meles) and other British mammals. Tuberculosis (Edinb) 81 (1–2), 43–49.

Gortazar C., Vicente J., Boadella M., Ballesteros C., Galindo R.C., Garrido J., Aranaz A., de la Fuente J. 2011. Progress in the control of bovine tuberculosis in Spanish wildlife. Vet. Microbiol. 151 (1–2), 170–178.

Kita J., Anusz K. 2011. Rozpoznawanie gruźlicy u bydła. Życie Wet. 84(6): 467–473.

Krajewska M., Lipiec M., Orłowska B., Anusz K., Szulowski K. 2014. Przydatność testu gamma-interferonowego do przyżyciowej diagnostyki gruźlicy u żubrów. European Bison Conservation Newsletter 7, 29–34.

Krajewska M., Orłowska B., Anusz K., Welz M., Bielecki W., Wojciechowska M., Lipiec M., Szulowski K. 2016. Gruźlica bydlęca w hodowli żubrów w Smardzewicach. Życie Wet. 91, 50–53.

Krajewska M., Welz M., Brewczyński P., Orłowska B., Anusz K. 2014. Gruźlica bydlęca w bieszczadzkiej populacji żubrów. Życie Wet. 89, 148–151.

Maas M., Michel A.L., Rutten V.P. 2013. Facts and dilemmas in diagnosis of tuberculosis in wildlife. Comp Immunol Microbiol Infect Dis. 36(3): 269–85.

Orłowska B., Augustynowicz-Kopeć E., Krajewska M., Zabost A., Welz M., Kaczor S., Anusz K. 2017. *Mycobacterium caprae* transmission to free-living grey wolves (*Canis lupus*) in the Bieszczady Mountains in Southern Poland. Eur J Wild Res (2017) 63, 1–5.

Pollock J.M., McNair J., Bassett H., Cassidy J.P., Costello E., Aggerbeck H., Rosenkrands I., Andersen P. 2003. Specific delayed-type hypersensitivity responses to ESAT-6 identify tuberculosis-infected cattle. J. Clin. Microbiol. 41, 1856–1860.

Rothel J.S., Jones S.L., Corner L.A., Cox J.C., Wood P.R. 1992. The gamma-interferon assay for diagnosis of bovine tuberculosis in cattle: conditions affecting the production of gamma-interferon in whole blood culture. Aust Vet J., 69(1), 1–4.

Żórawski C., Lipiec M. 1997. Przypadek uogólnionej gruźlicy u żubra. Med Weter., 53, 90–92.

Kontynuacja dochodzeń epidemiologicznych w kierunku gruźlicy u żubra w Puszczy Boreckiej

Streszczenie: Żubr (*Bison bonasus*) jest gatunkiem bardzo wrażliwym na zakażenie prątkami gruźlicy, o czym świadczą pojawiające się od 1996 roku przypadki choroby u tego gatunku. Jest on klasyfikowany przez Międzynarodową Unię Ochrony Przyrody (IUCN) jako gatunek zagrożony wyginięciem, co sprawia, że monitoring chorób zakaźnych musi być przeprowadzany ze szczególną uwagą. Ze względu na wystąpienie u żubrów z Puszczy Boreckiej dwóch przypadków gruźlicy wskazane jest kontrolowanie tamtejszej populacji w tym kierunku. Podjęto działania mające na celu wstępne określenie sytuacji epidemiologicznej gruźlicy w populacji żubrów w Puszczy Boreckiej. Zbadano 22 osobniki za pomocą komercyjnego testu gamma-interferonowego, którego zasada działania opiera się na nadwrażliwości komórkowej typu późnego. Krew pobrana do probówek zawierających heparynę litową była stymulowana tuberkuliną bydlęcą, ptasią oraz PBS (kontrola). Wynik interpretowano na podstawie gęstości optycznej uzyskiwanej z poszczególnych próbek, czyli wartości proporcjonalnej do ilości wytwarzanego przez limfocyty T interferonu-gamma (INF-γ – białko charakterystyczne dla odpowiedzi immunologicznej w przebiegu gruźlicy). W celu weryfikacji ujemnych wyników testu gamma-interferonowego od 10 spośród 22 zbadanych żubrów *post mortem* pobrano materiał (węzły chłonne żuchwowe oraz zagardłowe) do planowanych badań mikrobiologicznych na specyficznych podłożach Stonebrinka oraz dodatkowo Löwensteina-Jensena. Pomimo uzyskania ujemnych wyników testu gamma-interferonowego w tym doświadczeniu, w przyszłości konieczny jest dalszy monitoring populacji żubrów z Puszczy Boreckiej w kierunku gruźlicy.