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Seroprevalence and risk factors for selected respiratory and reproductive tract pathogen exposure in European bison (*Bison bonasus*) in Poland

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ABSTRACT

After the complete extinction from the wild of European bison (*Bison bonasus*) at the beginning of the twentieth century, the worldwide species population was restored to approximately 5500 individuals, with the species however remaining endangered. Despite numerous studies on the ecology and genetics of European bison, the threats of infectious diseases have been largely unexamined. The aim of this study was to screen the exposure of the world's largest population of European bison to the pathogens, which may influence the condition and development of the endangered species. A total of 240 free-ranging and captive European bison from eight main Polish populations sampled were tested for the presence of specific antibodies against ten different viruses, bacteria or protozoan. The samples were collected from chemically immobilized, selectively culled or found dead animals. Based on serology, the exposure to bovine viral diarrhea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), *Mycoplasma* and *Brucella* spp. was determined as rather accidental. Using gamma-interferon assay followed by *Mycobacterium tuberculosis* subs. *caprae* detection in tissues, diagnosis of bovine tuberculosis was made for 6 out of 78 (7.7%) bison from one captive herd. The highest seroprevalence was found for bovine adenovirus type 3 (BAdV-3) −60.2% and bovine parainfluenza type 3 (PIV-3) −34.0%, while the antibodies against bovine respiratory syncytial virus (BRSV), *Toxoplasma gondii* and *Leptospira* spp. were found in 10.4%, 10.4% and 8.7% of samples, respectively. In the multivariable statistical analysis using generalized linear mixed models (GLMMS), the risk factors for PIV-3 seropositivity included population type (free-living/captive), age and health status (apparently healthy/eliminated due to the poor condition). Higher risk of BAdV-3 seropositive result was observed in free-living female European bison. The high BAdV-3 and PIV-3 seroprevalences may suggest involvement of these pathogens in the most frequently observed respiratory disorders in European bison. Moreover, this is the first study demonstrating BAdV-3 exposure in the species.

1. Introduction

The European bison (*Bison bonasus*) has been reintroduced to Poland after its complete extinction from the wild since 1929. The number of European bison founders, which survived in zoos and private parks was limited, therefore present population is largely inbred. Initially, the animals were bred and maintained in captivity, and first released into the wild in 1952 (Dackiewicz, 2009). The present world population of European bison is approx. 5000 individuals. In the restitution breeding of endangered species, monitoring of health threats, especially those of an infectious and invasive nature, in addition to conventional procedures is crucial. First reports on infectious diseases derive from the nineteenth century, when clinical cases or specific lesions characteristic for contagious bovine pleuropneumonia (mycoplasmosis) or pasteurellosis and hemorrhagic septicaemia among European bison were described (Wróblewski, 1927; Kita et al., 2003). The second half of the twentieth century has brought new international and intercontinental threats to the species such as bovine tuberculosis (TB), foot-and-mouth-disease and Q fever (Kita et al., 2003; Krajewska et al., 2015a,b). Over the last 20 years, five TB outbreaks caused by *Mycobacterium caprae* or *bovis* have been confirmed in European bison from different regions of Poland. The eradication of the first outbreak in the

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free-ranging European bison herd in Bieszczady Mountains (south of Poland) took over 16 years (1997–2013) (Krajewska et al., 2015a,b). In 2013, another three outbreaks of TB caused by *Mycobacterium caprae* were identified in European bison (Anusz et al., 2017). Molecular analysis has confirmed the high homology of the strains indicating TB spread between the bison from the three different breeding centres. Interspecies transmission from local cattle kept on pastures adjacent to bison territory is suspected to be the main source of the tuberculosis in bison. Fortunately, the largest population of European bison in Białowieża Primeval Forest remains free of TB, perhaps because of less contact with livestock and rigorous sanitary measures applied.

Since the European bison is closely related to cattle (*Bos taurus*), the starting point for this study was to investigate the exposure to the pathogens known to affect bovids, endemic to Poland and Europe. The respiratory and reproductive infectious diseases remain a problem for domestic and wild ruminants, which may also affect the condition and size of European bison population (Golbert et al., 2013). In the study, the distribution of infections with the key pathogens prevalent to cattle such as bovine viral diarrhea virus (BVDV), bovine herpesvirus type 1 (BoHV-1), parainfluenza virus type 3 (PIV-3), bovine adenovirus (BAdV-3), bovine respiratory syncytial virus (BRSV), *Mycobacterium tuberculosis*, *Mycoplasma* spp. involved in respiratory diseases, as well as *Brucella abortus*, *Toxpolasma gondii* and *Leptospira* spp. producing reproductive losses were investigated. BVDV belongs to a genus of highly variable pestiviruses, which are highly damaging and widely spread in domestic and wild ruminants and pigs all over the world. Due to the immunosuppressive nature of the virus, acute infections often confounded by other infections are manifested by respiratory disease and fertility failure. BVDV persistent infections (PI) of cattle infected *in utero* lead to death of the animal within the first 24 months of life after developing mucosal disease. BoHV-1 is the most important and best characterized member of a large group of alphaherpesviruses (Roizmann et al., 1992). BoHV-1 causes infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), conjunctivitis, fatal multisystemic infection of newborn calves, encephalitis and abortions (Muylkens et al., 2007). Cervid herpesvirus 1 (CvHV-1) and 2 (CVHV-2), alphaherpesvirus species closely related to BoHV-1 are endemic in wild ruminants (das Neves et al., 2010). In Poland, animal-level BoHV-1 seroprevalence in cattle was estimated at 30%, while BoHV-1 infections occurred in over 70% of herds (Rypuła et al., 2012). Worldwide endemic PIV-3, BAdV and BRSV usually occur as co-infections and are described as etiological agents of bovine respiratory disease (BRD), which also involve BVDV, BoHV-1 and *Mycoplasma bovis* (Taylor et al., 2010). Mycoplasmas are conditional pathogens responsible for pneumonia, arthritis and mastitis in cattle also in Poland (Dudek and Bednarek, 2012; Nicholas, 2011). ***Mycoplasma mycoides*** subspecies *mycoides* small colony (*Mmm*SC) causes the most devastating contagious bovine pleuropneumonia (CBPP) OIE-listed hazard for international trade and subject to compulsory eradication. Despite the zoonotic character of *Mycobacterium tuberculosis*, the decision making and eradication of TB in European bison is complicated because of the nature of the species. Since the bacteria may be spread between susceptible species also in sylvatic cycle, the exposure of bison is constant and should be monitored. Other pathogens of possible threat to European bison reproduction include *Brucellae*, which are gram-negative, facultative, intracellular bacteria causing zoonosis of worldwide public health and economic importance (Godfroid et al., 2005; Franco et al., 2007). *B. abortus*, responsible for bovine brucellosis, *B. melitensis*, the main agent of ovine and caprine brucellosis, and *B. suis*, which causes brucellosis in pigs, play the main role in brucellosis epidemiology. While the national brucellosis monitoring program does not include wildlife, the disease is reported in wild animals in Europe (Cvetnic et al., 2004; Szulowski and Pilaszek, 2001). The animal testing is based almost entirely on serological assays, however unequivocal diagnosis of *Brucella* infection can be made only by the culture and identification of the agent. *Leptospira* spp. infection occurring in most mammals may lead to abortions and weak newborns, while ubiquitous and also zoonotic protozoa *Toxoplasma gondii* appears in a variety of warm-blooded animal species, moreover felids are considered the definite host, necessary for the parasite reproduction. *T. gondii* may cause abortions, stillbirths and fetal abnormalities, however bovids are considered rather resistant to *T. gondii* infection (Costa et al., 2011).

The aim of the study was to screen the exposure of European bison from the main populations in Poland to the potentially most threatening pathogens, which may influence the condition and development of the endangered species reared in wild and in captivity. Due to the uniqueness of the research material (the total Polish European bison population is around 1500 animals), such studies bring new insights for the efforts to protect the species of those rare animals. Moreover, since European bison inhabit various environments, they may be also indicate the exposure risk for other wild ruminants in the country.

2. Methods

2.1. Sample collection

A total of 240 serum samples was obtained from European bison between 2011 and 2015. The tested European bison originated from locations: Białowieża Primeval Forest (n = 115); Bieszczady Mountains (n = 14); Gołuchów (n = 5); Niepołomice (n = 26); Pszczyna (n = 46); Borecka Forest (n = 10); Smardzewice (n = 20); and Warsaw ZOO (n = 4) (Fig. 1). The population sizes are presented at Table S1. Captive (kept in fenced reserves) and free-ranging European bison were 150 (62.5%) and 90 (37.5%), respectively. The free-ranging animals originated from Białowieża (n = 75), Bieszczady (n = 7) and Borecka (n = 8; the remaining two individuals were kept in fenced quarantine for few months during the sampling, therefore were included as captive). No vaccinations are given to either free-living or captive European bison in Poland. Slightly more female European bison (n = 127) were sampled than males (n = 108). The age of the animals ranged between a few months and 27 years, with mean of 6.6 years (median 4.0; 95% CI: 5.8; 7.5). For further statistical analysis, the animals were divided into three age categories using the key of Krasińska and Krasiński (2013) as follows: 1) calves ≤ 1 year of age (n = 53); 2) young animals between 2 and 3 years of age (n = 52); and 3) adult animals ≥ 4 years of age which have reached sexual maturity (n = 125). Most of samples (n = 165; 68.8%) originated from European bison pharmacologically immobilized for diagnostic purposes, transportation or collaring according to the previously described protocols (Krzysiak and Larska, 2014). The rest of the samples were collected postmortem from animals which had been selectively eliminated due to poor condition (n = 64); fallen (n = 9); or killed in a traffic accident (n = 2). Due to the limited volume or quality of some samples not all sera were suitable for simultaneous testing for all pathogens, therefore the number of observations was given for each analysis (Tables 1, 2, 5, 6).

Heparinized blood samples from 78 bison immobilized between 2012 and 2015, delivered to the laboratory within 24 h from collection were tested for bovine tuberculosis. The tested European bison originated from eight locations: Bałtów (n = 3); Białowieża Primeval Forest (n = 13); Bieszczady Mountains (n = 3); Gołuchów (n = 5); Niepołomice (n = 7); Pszczyna (n = 14); Wałcz (n = 1); and Smardzewice (n = 32).

2.2. Serological methods

To detect BoHV-1 and BVDV antibodies, blocking IBR gB ×3 Antibody Test kit and indirect IDEXX BVDV Ab Test (IDEXX Laboratories, Inc., Liebefeld-Bern, Switzerland) were used. The tests use cut-off values of S/N = 55% and S/P = 0.3 respectively. According to the manufacturer's brochure, the tests provide 99.8% and 96.3% sensitivity and 100% and 95.0 % specificity respectively. For the detection