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Infectious Disease Monitoring of European Bison (*Bison bonasus*)

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Abstract

In 2019, the 90th anniversary of the restitution of European bison (*wisent*) will be celebrated. Therefore, the chapter discusses the past, present, and future health threats of the *Bison bonasus* species that was on the edge of world extinction at the beginning of the twentieth century and was restituted with great efforts from many researchers, breeders, forestry workers, and caretakers. Due to the dramatic genetic “bottleneck” that depleted the gene pool, increasing the inbred of today’s European bison, the breeding may face problems of decreased fertility, deficiency in growth, and increased susceptibility to diseases. While the increasing numbers of European bison may be enjoyed by breeders, the suitable habitat for the largest herbivore in Europe shrinks with increasing human population density, forestry, and agricultural activity. Additional threats include inappropriate management based on animal farming rather than sylvatic ecosystems, need for supplementary winter feeding, and establishment of breeding of related species such as American bison (*Bison bison*) in Europe. The control of European bison exposure to pathogens through passive and active surveillance is a key component of the species conservation. Hereby, the current knowledge on the epidemiology of the most significant infectious diseases in European bison is presented.

Keywords: wisent, surveillance, health, infection, pathogen

1. Introduction

The purpose of this work was to show the real and potential health risks of the European bison, which have or can influence the general condition of the population and affect the restitution effects of this endangered by extinction species. From the species history, through the old, however recently increasingly re-emerging, threats until the new challenges for the species conservation, we have tried to present the most complete picture of the veterinary aspect of the species protection. Not without significance is also the manner in which these tasks are implemented, i.e., active species protection, not always accepted, but effective, which is best evidenced by the continuous increase in the number of the world’s European bison population. Today, when the number of the species exceeded 7000 individuals, it is not of key importance to protect individual European bison at all costs but to improve the welfare of the whole population, e.g., by minimizing the risk of infectious diseases by eliminating sick animals. Health threats can be removed or minimized through implemented prophylaxis and monitoring of pathological conditions in individual populations. Moreover, the exchange of experience and cooperation between breeders at national and international levels is extremely important. Another important

aspect is the sanitary control of the animals in movement, especially of the greatest health threats such as tuberculosis and bluetongue disease and conducting quarantine for the introduced animals. One should also not underestimate the potential health hazards that can be assessed by postmortem diagnostics of fallen and selectively eliminated animals followed by discriminatory laboratory testing. The future of the European bison depends on the wise care of the herd managers and veterinary medicine specialists based on the best scientific and practical knowledge.

2. Species history and restoration

The European bison (*Bison bonasus*) is currently the wild land mammal of Europe, belonging to the cloven-hoofed ruminants, the family *Bovidae*. The males may reach up to 1000 kg (average 700–800 kg) of weight, while female up to 650 kg (average 400–500 kg) [1]. At the beginning of the last millennium, lowland European bison lived widely in the vast forests covering most of Europe and were highly valued hunting prize. In Europe, the wild bison survived the longest at the Białowieża Forest while it was within the borders of the Grand Duchy of Lithuania, the Kingdom of Poland, and the Polish-Lithuanian Commonwealth. In 1919, the last free-living European bison in the Białowieża Forest was killed. In 1927, the scientific expedition to Caucasus has also failed to find any living European bison there. The period between 1919 and 1929 was the time when not a single European bison was present at the Białowieża Forest. When in 1924 a decision was taken to try to save the species, only 54 European bison were still alive worldwide; however only a part of those animals participated in the species restitution. Currently, all European bison living in the world originate from 12 animals, referred to as the founding group. The European bison belongs to two lines, i.e., Lowland (derived only from seven individuals) and Lowland-Caucasian. As part of the restitution breeding in September 1929, the first European bison returned to Białowieża, but it was not until 1952 that two individuals were released into the wild [2]. Currently, over 1200 animals live in the entire Białowieża Primeval Forest (560 on the Belarusian side); there are almost 1900 of them all over Poland and over 7000 in the whole world [3]. In Poland, European bison appear in 5 free-living populations and over 20 enclosures (**Figure 1**). European bison are protected animals and are inscribed in the IUCN Red List of Threatened Species [4] under the vulnerable category. In Poland and Belarus, European bison have the status of animals under species protection, while in other countries they are treated as free-living animals kept in closed breeding. Since they need a space composed of a forest environment in which they spend most of their time, as well as grasslands (pastures) for feeding, only few places in Europe meet the conditions without causing losses in agricultural crops and forestry [1]. An efficient way to minimize conflicts is to carry out winter supplementary feeding of these animals with roughage (hay), which provides an existential minimum for European bison and may protect the crops [5]. For veterinarians, breeders, and carers of the free-living bison population, winter gathering is the appropriate occasion to overview the herd. All European bison are counted, and the sexual (male, female) and age (calves, 0–1 year; youth, 2–3 years; adult, 4 years and more) structures of the herd are determined. The individuals are entered into the European Bison Pedigree Book [3]. In addition, the winter overview of the herd allows to identify any diseased or weak animals, which may constitute a potential epizootic threat to other European bison [1, 6]. Because European bison remain endangered and rare species, as well as attractive because of their uniqueness as a show species, breeding restitution of this species is also carried out in enclosures and reserves in Poland and Europe. All European bison kept in captivity have a



Figure 1.
European bison population distribution in Poland, 2018 (graphic design by J. Tomana).

unique pedigree number and a name, specific for the country and place of the origin [3]. Keeping breeding centers allows to control the breeding of European bison by selection of animals with known pedigree and least related. Moreover, breeding enclosures provide a genetic and breeding reserve of pedigree animals in case if any depopulating incidence occurs, what the species had already experienced at the beginning of twentieth century. Therefore, the management of the enclosures needs to be supervised by a veterinarian. Each movement of animals between breeding centers should be preceded by a clinical examination of the animal; a laboratory test for the most important infectious diseases and prophylaxis (more often therapeutic in practice) against ecto- and endoparasites should be applied [7].

One of the dangers, often underestimated by ignorance or financial incentives, is the commercial breeding of American bison (*Bison bison*) in Europe, which is a serious genetic and epizootic threat for European species. The European and American bison crossbreed can naturally reproduce, which means that the co-existence of both species in one area could lead to loss of the protected species genetic purity and threaten biodiversity and conservation [8]. Therefore, the establishment of commercial American bison farms disqualifies the immediate vicinity as potential places for the release of European bison into the wild. Moreover, the presence of American bison poses a critical epizootic risk to the European cousin, since they can transmit the pathogens threatening European bison health, which are absent or emerging in Europe [9], for example, *Fasciola magna* invasions [10] or epizootic hemorrhagic disease, caused by EHDV [11]. The close interspecies relationship does not ensure similar susceptibility to diseases. Significant differences in clinical outcome of foot-and-mouth disease virus (FMDV) infection were demonstrated, for instance, between dromedary and Bactrian camels [12]. The burden of FMD epizootics in Polish European bison at the beginning of the twentieth century was much higher than in cattle in the same area [13, 14].

3. European bison health threats of the twentieth century

Despite that the species was just starting to be re-established, European bison were put under epidemic pressure and exposed to many pathogens endemic to domestic ruminants in Poland at the beginning of the twentieth century. After the Second World War, the European bison population in Poland systematically increased, except for several incidents associated with the occurrence of infectious diseases (**Figure 2**). Konrad Wróblewski [14], a vet and researcher, gave the first detailed descriptions of the health problems in European bison in Białowieża at the turn of the nineteenth and twentieth centuries. At that time, the causes of morbidity and pathological changes were not yet fully understood due to the lack of knowledge and research tools. Dr. Wróblewski observed pleuropneumonia caused by *Pasteurella multocida* and connected to the *Strongylidae* invasions of lungs [15]. The problem of purulent pneumonia is still observed in European bison at Białowieża [6]; however the agent, *Pasteurella multocida subsp. multocida* serogroup A was identified just recently [16]. At the time, the highest mortality in European bison was caused by blackleg. The infection with *Clostridium chauvoei* probably caused death of 172 European bison in 1904 [14]. The most common were *Fasciola hepatica* and *Trypanosoma* parasite invasions, which affected health condition causing weakness, weight loss, and secondary infections. Occurrence of *Trypanosoma wrublewski* protozoans in the blood of European bison was reported still in the 1990s [15]. Another problem was FMD, endemic in cattle from the area; nevertheless Dr. Wróblewski described only 5% mortality in European bison in the 1920s. However, FMD was taking its toll in the 1950s at the south of Poland causing almost complete depopulation of European bison reserves in Pszczyna, Niepołomice, and Gorce [17–19]. Staśkiewicz [13] has observed FMD in American bison and European-American bison hybrids bred in Smardzewice reserve in 1938. He has noticed significantly higher resistance to FMDV infection of those animals in comparison to European species. This also contends against the introduction of this invasive to Europe bison species into the areas where European bison are being reared.

In 1980s, due to the occurrence of Q fever in domestic animals in northeastern Poland, several dozen free-ranging European bison from Borecka Forest were

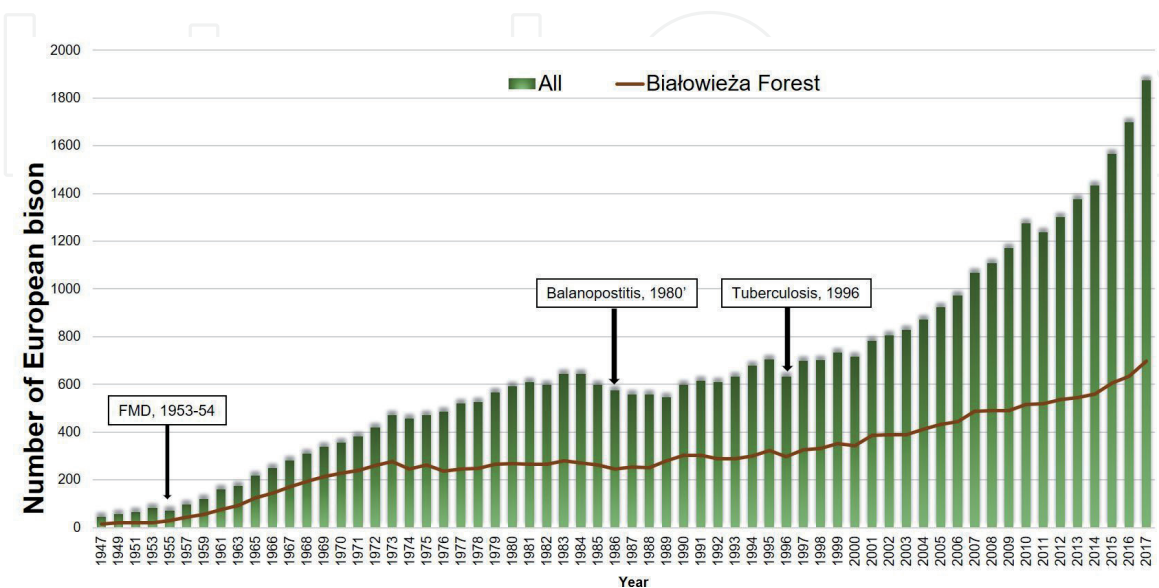


Figure 2. Dynamics of the European bison number in Poland (bars) and Białowieża Forest (curve) between 1947 and 2017 (European Bison Pedigree Book). The cases of diseases which caused significant declines in the population size and dates of their detection are marked.

examined for the presence of specific antibodies [20]. The high seroprevalence (76%) to *Coxiella burnetii* found in European bison indicated that they could be a potential reservoir of Q fever for humans and other animals. The European bison-human transmission was suspected, since the infection was also confirmed in some employees (some hospitalized) of the Białowieża National Park (BNP) staff. Szarek et al. [21] have linked *C. burnetii* infection with the pathomorphological changes of the heart and kidneys observed in European bison specific for Q fever.

The first signs of another important disease in the male European bison, referred to as necrotic posthitis or balanoposthitis (called also pizzle rot), the causes of which still remain unsolved, were observed in Białowieża in 1980 remain unsolved, were observed in Białowieża in 1980 (**Figure 3**) [22]. *Trueperella pyogenes* and some other purulent bacteria have been isolated from the diphtheroid-necrotic changes observed on the prepuce and penis, which may lead to self-mutation of the penis in severe cases causing primarily great pain and immunosuppression, excluding the affected males from breeding [6, 22–24]. Infection with BoHV-1 causing similar symptoms in cattle was excluded from the investigation, since hardly any European bison affected had detectable antibodies to the virus and no isolation from the tissues was possible [1, 22, 25]. Also no association between *Trypanosoma* invasion and balanoposthitis was found [1]. Prior to the prepuce and penis lesions reported in Białowieża, similar symptoms were observed in Pszczyna, in the eastern part of Białowieża Forest (now Belarus), Ukraine, and Russia [1]. The epidemic of balanoposthitis in Białowieża included annually up to 15.2% males in 1993, with the average of 6.1% between 1980 and 2015. Selective culling was found the most effective method to decrease the cases, especially in the recent years [1, 6]. The symptoms are observed in all age groups of males, including calves and 2–3-year-old European bison males, yet not reproductively active [1, 6, 23]; therefore venereal route of transmission may rather be rejected. Since most cases occurred at Białowieża Forest, involvement of some environmental or vectors such as ticks was speculated, however, never verified. Since European bison genetic pool had gone through a dramatic “bottleneck” at the beginning of the twentieth century, high inbred was suspected to play a role; however, little evidence was reported [26, 27].

One of the most devastating bacterial diseases, which remains a current problem in Polish European bison populations, is tuberculosis. The disease was diagnosed in the free-living European bison population in the Bieszczady mountains in the 1990s [28–31], European bison are very susceptible to mycobacterial



Figure 3. Male *Bison bonasus* with clinical signs of balanoposthitis showing symptoms of impeded urination (stranguria) and swelling of the whole external genital area (photo: Ł. J. Mazurek).

infections, and since no vaccination programs or treatment could have been introduced, the only method of disease control and prevention from spreading was the elimination of infected European bison [28, 29, 31–34]. The eradication resulted in a significant depopulation observed in decrease of the numbers of European bison (**Figure 2**). Tuberculosis affecting European bison in Poland is caused by *Mycobacterium caprae* [35]. The management of the diseases is extremely difficult especially in wildlife, since the mycobacteria transmit readily between different wild species such wild boar, deer, and wolves in Bieszczady mountains [31]. American bison bred in Poland are also considered important reservoir of tuberculosis [36].

4. Telemetric collars and European bison movement monitoring

Free-ranging European bison create herds consisting of cows, their offspring, and youth; solitary bulls live outside of the herd and, however, approach or join in the mixed groups during rutting season and winter (for feeding), while young bulls often create small male groups [37]. Since European bison move over considerable distances during the day (especially the bulls), in order to monitor the distribution of these animals and study their use of space and different ecosystems, telemetric transmitters are being placed on selected individuals. Through such transmitters, we can track the daily rhythm of European bison and the use of various habitats for feeding and resting, divided into seasons and various periods relevant to the behavior of animals (the calving season, mating season) [1]. Such transmitters can use a radio signal emission (the oldest way, currently replaced by GPS); it requires the involvement of a person with a radio receiver together with an antenna to track the animal and record their location. Another way is to connect the transmitter to the server via cellular telephony; coordinates from the GPS system are collected, converted, and transferred to the operator's server, processed and plotted on the map; however, it requires the animal remains within the GSM network, and the reading may be analyzed by the inspector only after some time. The most modern, but also significantly more expensive, way is placing satellite emitters on the European bison, which allows tracking these animals in different conditions (also outside the GSM network) and the ability to record the location of the “marked” animal systematically regardless of the environment in which it is located. By monitoring European bison for a longer period of time, maps can be created for the use of both males and females of different ecosystems, determine the range of existence of individual herds, and determine, for example, the nutritional preferences of these animals. The telemetric coordinates of European bison location are applied to maps and visualized showing land usage of individuals/herds in relation to season, differences in distribution, and behavior of females and males [38]. Same as European bison counting, setting up telemetry transmitters is usually performed during winter concentration at feeding places. For this purpose, the individual is pharmacologically immobilized (**Figure 4**) [7], and the belt with the telemetry transmitter is adjusted to its neck. This is important because a too tight collar may cause difficulties in swallowing and regurgitation, while too loosely attached collar may cause injuries (entrapment of the limb, foreign bodies such as branches). For an adult European bison, a telemetry transmitter that weighs approx. 2 kg may be compared to a watch on a human wrist. Additionally, during the pharmacological immobilization of European bison, samples are collected for veterinary, genetic, and toxicological tests, which is a part of the health monitoring described in paragraph 3.



Figure 4.
Chemical immobilization of free-living European bison (photo: J. Klej).

5. Current major epidemic threats and monitoring of European bison

When considering the problems of infectious and invasive diseases, three aspects (the so-called epidemiological triangle: animal-environment-pathogen) should be considered. The epizootic and epidemiological situation is a resultant of the interactions between the natural environment of the free-living animals, other wild reservoir species and farm animals at the wildlife-livestock interface. An important environmental component will also be access to competent vectors of a given microorganism. In the case of environmental impact, it is also necessary to take into account people who are susceptible to zoonoses, can themselves be a source of infections, or can be a mechanical vector of infectious and invasive agents transmitted to animals, becoming endemic in sylvatic environment [39]. Those aspects meet in the One Health approach, which include wildlife as a key component of the ecosystem [9, 32, 40, 41]. Therefore, in the case of an epizootic, surveillance should be carried out in both domestic and non-domestic populations, both free-living and captive, and the potential role of humans (animal care takers, breeders, vets) as vectors should be taken into account. Climate, environment, socioeconomical changes create alterations also in the distribution of infectious diseases and remind us that animals and humans, environment, and pathogens are the elements of the same ecosystem.

In the last 20 years, the population size of European bison in Białowieża Forest and in the whole country increased more than twice (**Figure 2**). It generated an increasing epidemiological pressure connected to higher animal density, a need for expanding the habitat, and what further increases the frequency of contacts between wildlife, farm animals, and humans. The evident environmental changes may provoke also emergence and re-emergence of new pathogens, change the seasonality of wild species, and induce stress and therefore the immunosuppression leading to increased susceptibility to diseases.

Since the European bison population was on the verge of extinction at the beginning of the twentieth century, each individual was extremely valuable; therefore one of the main aspects of European bison restitution still remains veterinary health protection, especially in terms of the threat of infectious and invasive disease occurrence. The role of European bison veterinarians and keepers include the supervision, health monitoring, understanding disease characteristics, identification of disease risks, provision of information for control, prevention or treatment, and evaluation of the effectiveness of control and its adjustment. One of the effective tools to control the disease is selective elimination of diseased animals and limiting the population size to reduce its density [32]. The selection is made by a panel of experts in the field; however, it should be remembered that the approach presented here is based on veterinary health protection sometimes argued by epidemiologists [42]. In the case of protected species such European bison, enforcement of the veterinary regulations to protect the species by a sacrifice of few diseased individuals, which are reservoirs of the pathogens putting on risk the whole population, becomes often inconvenient decision against some environmentalist protests.

European bison monitoring in Poland consists of four elements: (1) passive surveillance, (2) active (targeted) monitoring, (3) sanitary control of transported animals, and (4) habitat monitoring (**Figure 5**) [43]. The first approach is based on one of the most suitable ways of health control, the postmortem examination as an element of mortality and morbidity investigations and sampling of the material for laboratory testing [6, 23, 44].

The most recent study involved the analysis of necropsy findings of over 230 fallen or selectively culled European bison from Białowieża Forest between 2008 and 2013 [6]. Changes in the male reproductive tract such as posthitis and balanoposthitis remained the most common pathological changes observed in European bison bulls; however, it should be kept in mind that those changes are the main reason for bull elimination (**Figure 6**). Moreover, the decrease in the proportion of males with prepuce and penis lesions of the whole Białowieża male population in regard to the last century was significant [1, 44].

The next most common problem included pneumonia (45% necropsied animals) and pulmonary emphysema (33%) [6], which were also reported in earlier studies [44]. Respiratory problems were associated with *Dictyocaulus viviparus* invasion of the lungs observed macroscopically; however, the characteristic picture of interstitial or catarrhal-purulent pneumonia suggested also involvement of some infectious agents, what was confirmed for *Pasteurella multocida* [16]. The study has shown that the liver fluke *Fasciola hepatica* still remained endemic in European bison in Białowieża [6]. The presence of the parasite is associated directly with the habitat of European bison in Białowieża [45]. The most frequent cause of death of European bison under the age of 6 months were body injuries caused by other European bison or less often as a result of other accidents. An important finding was that the frequency of pathological changes was higher in the selectively culled European bison than those that were found dead in the wild. This confirms the purposefulness of selection and elimination of diseased individuals as a tool to improve the health and welfare of the population of the species and allows to limit the number



Figure 5.
 The four main elements of the European bison health monitoring scheme in Poland.

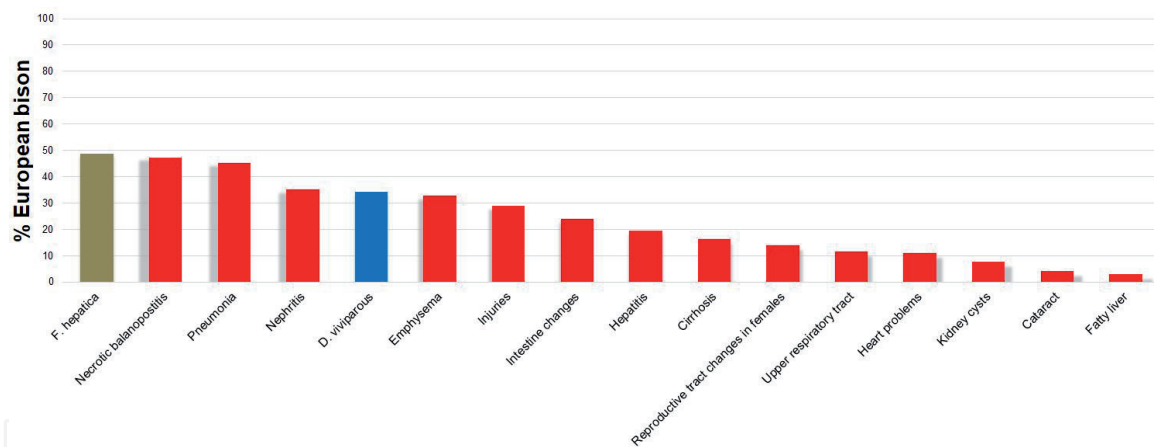


Figure 6.
 Percentage of European bison with individual pathological changes and parasitic invasions observed at postmortem examinations performed between 2008 and 2013 in Białowieża Forest [6].

of animals that constitute a potential reservoir of infectious and invasive diseases [32]. Additionally, unlike farm animals, European bison are protected animals and live to a mature age; the pathological changes characteristic of elderly individuals, such as kidney cysts, fatty liver, and cataracts may be observed [6]. However, their frequency in the studied European bison did not exceed several percent.

Another large serosurvey has revealed the possible involvement of some respiratory viruses in the pathogenesis of the frequent changes observed in the lungs or in the upper respiratory tract such as bovine adenovirus type 3 (BAdV-3), bovine parainfluenza type 3 (PIV-3), and bovine respiratory syncytial virus (BRSV) [46]. The high seroprevalences observed especially for BAdV-3 (60%) and PIV-3 (34%) were surprising, also because they have not been studied too intensively before [47]. Interestingly, the association between BAdV-3 and PIV-3 infections and health status of European bison were demonstrated, with significantly lowest seroprevalences in the apparently healthy animals. Whether the high BAdV-3 and PIV-3

infection rates were due to the circulation inside the European bison population or it is connected to the transmission of those viruses from domestic ruminants remains unsolved. However, higher infection rates in the free-living European bison suggested that it might have been associated with a spillover from farm animals. The European bison kept in the enclosures were possibly protected from a direct contact with domestic species, not sharing grazing areas as observed in Białowieża [48, 49]. The studies on the characterization of the viral strains in European bison, what may explain the transmission source, are ongoing.

Moreover, other endemic in ruminants infectious agents were considered as potential threats to European bison. Those included pestiviruses with bovine viral diarrhoea virus type 1 (BVDV-1) and alpha- and gammaherpesviruses (bovine herpesvirus type 1—BoHV-1, BoHV-4, and BoHV-6), endemic in Polish cattle herds [46]. While in farm animals those viruses are responsible for economic losses, they may prevent the reproduction of an endangered species and make the restitution program fail. However, very low seroprevalence to those viruses suggests certain resistance or only accidental exposure of European bison and hence little importance for European bison at present [15, 25, 46, 47]. On the other hand, low seroprevalence means that most European bison are naïve and fully susceptible to infections with BVDV-1 and BoHV-1, BoHV-4, and BoHV-6. The involvement of herpesviruses in the etiology of balanoposthitis was also disclosed [22].

Other respiratory agents, which could affect the clinical picture observed frequently postmortem in European bison such as *Mycoplasma* spp. and *Mycobacterium* spp., are probably of limited geographical importance [33, 46, 50, 51]. Infections with *Mycoplasma bovis* in few individuals suggest that the exposure was accidental and, however, might have added to some pathological changes of the respiratory tract observed in one case such as intrabronchial pneumonia and pleurisy [51]. In addition, *Mycoplasma bovis genitalium* was suggested to be involved in balanoposthitis under serological evidence; however, the bacteria have never been detected in the lesions of the male genital tract [50]. Moreover, the occurrence of *Mycoplasma ovipneumoniae* infections was never investigated; notwithstanding this direction is worth considering in the pathogenesis of respiratory disorders [52]. Bovine tuberculosis (bTB) remains one of the major infections putting on risk the whole population. *Mycobacterium* accompanies European bison since the dawn of time. Bacterial DNA has been discovered in the bison fossil dated 17,000 years before the present [53]. One of the major obstacles for tuberculosis control in wildlife is that the mycobacteria spread among different mammalian species and survive in numerous biotopes (soil, water, vegetation) becoming endemic in the wild ecosystem for a very long time. Additionally, the diagnostic test used for European bison is dedicated mostly to cattle; therefore, the sensitivity may vary. Sample quality is critical, and the use of several complementary diagnostic methods is necessary to confirm a bTB case in European bison [54]. Fortunately, the disease has not been observed in European bison from the world largest population in Białowieża for some decades now, probably owing to the elimination of the pathogen from cattle in Poland. The disease stroke, however, in a reserve in the European bison breeding center in Smardzewice [33, 46], what has led to stamping out of the whole herd in 2018. The outbreak as the previous one in Bieszczady mountains was connected to *Mycobacterium caprae* [31, 35]. Reports of tuberculosis in captive European bison caused by *M. bovis* in German and Brazilian zoos underline the need for control in the species being important bTB reservoir worldwide [55–57].

Further studies are concerned with pathogens, which may be depopulating through their influence on European bison reproduction, reducing already fragile number of the species under restitution. Low seroprevalences of *Brucella* spp., *Leptospira* spp., and *Toxoplasma gondii* observed in European bison in

eight main Polish populations sampled between 2011 and 2015 by Krzysiak et al. [46] suggest limited importance of those agents for the species conservation at present. It had been also confirmed by the results of some previous investigations [47, 58]. Only slightly higher *T. gondii* parasite seroprevalence was observed in European bison from Białowieża prior to the study of Krzysiak et al. [46, 59]. It was followed by the report of *T. gondii* isolation from an aborted at 4–5-month-old gestation of European bison fetus in Białowieża in 2014 [60]. Similar to *T. gondii*, European bison pose some potential as *Neospora caninum* and *Chlamydia* spp. reservoirs [58, 61].

Bovine leukemia virus (BLV) and *Mycobacterium avium* subsp. *paratuberculosis* (Johne's disease) infections, endemic in Polish cattle, are subject of official tests performed routinely in the movement of European bison inside the European Union. Only one case of subclinical BLV infection has been reported so far in the 1880s. Just as paratuberculosis [51], bovine leukemia does not pose a serious threat to Polish European bison at present.

Finally, it is worth mentioning European bison susceptibility to malignant catarrhal fever virus (MCFV) infection, which is comparable to cattle's. The reports of clinical picture of MCF in the species kept in zoos are quite old [62, 63]. MCF is considered more emerging and non-endemic in Poland; therefore, no current data exists. However, it was well documented that the exposure of American bison to sheep-associated MCFV is frequently fatal [64, 65]. In 2018, an outbreak of fatal MCF in European bison enclosure in Switzerland was consulted with European Bison Pedigree Book department at the Białowieża National Park; however no report was published officially (Baumgartner, personal communication).

6. Emerging, re-emerging, and vector-borne pathogens infecting European bison

The twenty-first century brings new challenges of the protection of animal health, including free living. Social and economic changes, globalization, intensification of intra- and intercontinental trade and travels, and environment and climate change contributed to the observed increase in the risk of emergence and re-emergence of pathogens [66]. The last few years have brought an increase in the importance of new pathogens, completely unknown before or not present in a given geographical latitude [67, 68], which may also have a significant impact on the health of an endangered species such as the European bison. It is related to processes related to human activity or changes in the environment but also to the development of science, improvement of disease diagnostics, and the introduction of new cognitive techniques, such as metagenomics and next-generation sequencing (NGS). Due to the habitat and the maintenance of a part of the European bison population in captivity, they are exposed to pathogens from both wild and domestic animals. Along with climate change, infections caused by pathogens transmitted by vectors such as arthropods (ticks, biting midges, mosquitoes) are more and more frequently reported in our part of Europe. In 2007, the first cases of infection with bluetongue virus (BTV) serotype 8 (BTV-8) causing significant mortality in European bison at an enclosure in Hardehausen occurred. The virus is transmitted by the blood-sucking midges of the family of *Culicoides* spp. (**Figure 7a, b**). BTV infections have never been detected as far north of Europe. During the epizootics more than 30% of European bison kept in Hardehausen died, and the surviving animals received a vaccine containing homologous inactivated BTV-8 [69]. BTV-8 epizootics did not reach Poland; however another strain, BTV-14, was detected almost simultaneously in cattle and in European bison at the northeast of Poland, near the Belarusian and Lithuanian borders [11, 70].

The source remains puzzling, particularly since the virus was highly homologous to South African BTV-14 reference strains used in vaccines, which were never used in Europe. However, closely related BTV-14 was detected in Russia almost at the same time. Therefore, combining this with the dynamics of geographical spread of BTV-14 in Poland, use of some illegal vaccine containing or contaminated with this BTV strain was suggested [71]. Serosurveillance of BTV in European bison showed that the infections occurred only in the eastern populations [11]. Interestingly, despite that the discovered BTV-14 has been described as attenuated strain causing only subclinical infection in cattle, significant association between health status of European bison and the presence of specific antibodies was observed [11]. The exposure rate to BTV in selectively eliminated and fallen animals in respect to healthy ones was clear; however multivariable analysis has not confirmed the health status as a risk factor. Notwithstanding, the most important consequences of the occurring of BT are the culling of diseases or suspected animals and restrictions on the movement of animals, since the disease is notifiable disease listed by the World Organization. Control or eradication of BTV in protected wild species such as European bison is rather doomed, especially that the environment provides more susceptible species such as cervids and favorable conditions for midge reproduction and survival [72, 73]. BTV epidemiological situation remains under continuous supervision (unpublished data). Additionally, the occurrence of epizootic hemorrhagic disease virus (EHDV) infections transmitted by *Culicoides* spp. in European bison in Poland was also ruled out [11]. However, since some diseases of wildlife considered so far as absent in certain geographic areas such as African swine fever in wild boar or chronic wasting disease (CWD) in cervids become endemic in Europe, the threat of EHD in the future should also be considered real [9].

After the bluetongue epizootics (BTV) in 2007 [67], a new pathogen named Schmallenberg virus (SBV) was identified in Europe in 2011 [74], which spread very quickly across the continent. The first SBV infection in cattle in Poland was found in 2012, and in the same year, virus transmission to the ruminants of free-living ruminants in the Białowieża Forest, including European bison, was observed [75]. The first case of acute SBV infection in wild animals was actually confirmed in a fallen elk calf rescued by the animal keepers of the Białowieża National Park [76, 77]. This was the first report on the identification of the SBV virus in a free-living animal that began an international discussion on the importance of free-living animals as a reservoir of this new virus [77]. It proceeded the subsequent surveillance of European bison and other



Figure 7.

BTV, EHDV, and SBV insect vectors: biting midges of Culicoides obsolete species—(a) freshly blood-fed and (b) gravid (containing a mature egg batch in the abdomen). Ultraviolet (UV) light trap (CDC 1212, John W. Hock Company, USA) for Culicoides spp. midges collection set at the Show Reserve in Białowieża National Park (c) (photo: Ł. Mazurek). The midges are active at night; therefore the trap was set on from dusk till dawn once a week during the vector activity season (April–October). UV light attracts the insects, which were trapped and fell down into the jar containing water with detergent [73].

wild ruminants at Białowieża Forest, what allowed to determined that the transmission of SBV occurred during 2012 *Culicoides* activity season [76]. Further studies concentrated on the epidemiology of SBV in European bison and the environment (other susceptible species, vector) [11, 73, 78]. SBV seroprevalence in European bison in all studies was significantly much higher than observed in the cervids sampled simultaneously at the same locations. It is not, however, due to the higher susceptibility of the European bison to SBV infection, rather than higher exposure to midge biting. Similar differences were reported between cattle and small ruminants. Most probably, higher exposure in larger ruminants is associated with their higher production of carbon dioxide, one of the strongest attractants of many arthropods including *Culicoides* spp. [79, 80]. SBV *in utero* infections may lead to congenital malformations of newborns, stillbirths, or abortions. Limited access to the material of aborted fetus in the wild European bison did not allow to draw many conclusions [73]; however some losses in reproduction rate may be expected as observed in cattle.

Since the transmission of arthropod-borne pathogens involves environmental factors, entomological and virological studies of the midges near European bison were performed between 2014 and 2015 [73]. Using specially designed ultraviolet (UV) light traps (**Figure 7c**) placed near European bison resting places at Białowieża National Park abundance, species composition and virus exposure were tested. The wood midges of *Culicoides achrayi* species were the most abundant with almost 280 thousand (over 50% of the whole midge number collected) individual midges of the species trapped over the 2-year period [73]. The presence of SBV was confirmed in some midges trapped in 2015. The work except its originality provided very needed data to study all *Culicoides*-borne infections including BTV in the sylvatic habitat of European bison.

Further studies suggest that European bison may be an important reservoir of tick-borne Lyme disease, since *Borrelia burgdorferi* was detected in blood of several animals in Białowieża [81]. Earlier, the presence of specific antibodies to *B. burgdorferi* was confirmed in European bison in the same area in the 1990s [82]. Other documented in European bison investigations of the infections transmitted by ticks include tick-borne encephalitis (TBE), tularemia, and anaplasmosis. While TBE virus was detected in the ticks collected from European bison [83], no antibodies supported the hypothesis that the species is an important reservoir of the virus [84]. European bison probably have little importance in the transmission of another zoonotic pathogen, *Francisella tularensis*, since none of the 251 individuals from eight Polish populations had antibodies against the bacteria according to Krzysiak et al. [46]. However, it is different when *Anaplasma phagocytophilum* responsible for human granulocytic ehrlichiosis (HGE) is considered. This tick-transmitted bacterium was found in a blood of over 66% of European bison in Białowieża Forest [85]. The pathogen was also confirmed to be present in the ticks collected from the animals [86]. However, whether any of the detailed tick-borne pathogens influences European health remains unexplored.

The last emerging disease with high epizootic potential we would like to discuss shortly is hepatitis E. The pigs including wild boar are the main reservoirs of hepatitis E virus (HEV); however, cases of virus to humans through cervid meat have been also reported. In a recent study, none of the European bison had antibodies against HEV [87]; however, the sensitivity of the methods to ruminants is debatable, and therefore the studies are being continued.

7. Conclusions

European bison survived to the present times, only thanks to human care and protection. Because Europe, unlike the Americas, was significantly more

populated in the Middle Ages, European bison conservation by the Polish kings saved this species from total extinction, as was the case outside the Kingdom of Poland. Successive rulers continued to protect the species, but warfare led to the total depopulation of the last surviving free-living population in the Białowieża Primeval Forest. Thanks to the efforts of scientists and naturalists, the current global population of European bison which was founded by 12 individuals is over 7000. This is undoubtedly a success, but without the supervision of the health of European bison, it would be impossible. European bison, in general opinion, is characterized by a decreased resistance to infections due to their low genetic heterogeneity after passing through the “bottleneck.” Except for *Mycobacterium* spp. and foot-and-mouth disease, there are few threats threatening to total depopulation of the species at the moment. However, many European bison seems to be susceptible to many pathogens which may influence their health status and exclude genetically valuable individuals from the breeding. Monitoring of the exposure is essential for the knowledge but also for taking the necessary steps to protect and control. It requires a sampling plan, which allows collection of representative number of specimen necessary for statistical analysis, which is not always feasible since European bison are few and most live in the wild. Therefore, many discussed above studies do not meet any epidemiological assumption and need to be analyzed with caution. Many retrospective studies suggest that European bison may be a reservoir of pathogens; however, the susceptibility to the infection is arguable, since they do not manifest any clinical symptoms (borreliosis, toxoplasmosis). In contrast to high-production animals, it seems not to be highly susceptible to infection, e.g., with pestiviruses and herpesviruses. At the same time, the possible interspecies transmission between domestic ruminants and European bison may be possibly responsible for the high prevalence of respiratory viruses and *Pasteurella multocida* infections in which clinical manifestations are observed frequently postmortally in European bison. Changing environment brings new challenges to European bison protection too, since the entire population may be at risk due to the emerging and re-emerging diseases observed recently such as bluetongue disease and malignant catarrhal fever. Therefore, maintaining the health monitoring of free-living European bison and eliminating all epizootic threats from the environment are crucial. It is also very important to follow the veterinary procedures and appropriate prophylactic measures when moving these animals. In our opinion, “prevent to protect” should stand for active protection, which proved to be the most effective in European bison restitution so far. Restitution breeding is a long and tedious process, often associated with making difficult and unpopular decisions.

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Conflict of interest

We declare no conflict of interest exists for this publication.

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