



Bovine tuberculosis in a Reeves's muntjac (*Muntiacus reevesi*) in a private animal collection in Poland – management and legal implications

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Abstract

Introduction and Objective. Bovine tuberculosis (BTB) remains an emerging problem in animals, and particular care is required in zoos to protect the health of the public visitors (zoonosis) and the unique animals kept in them, which may be endangered species. In larger zoos, the problem is controlled to a greater extent than in private animal collections. Such places pose a significant risk as visitors make direct contact with animals. The article presents the management, diagnostics of a case of BTB in a 'private animal collection', and discusses the legal implications.

Materials and method. Briefly, a post-mortem examination was performed in two Reeves's muntjacs (*Muntiacus reevesi*) in the private animal collection in Poland. Due to the suspicion of BTB, microbiological examination on Lowenstein-Jensen and Stonebrink media and subsequent genotyping was performed.

Results. Post-mortem examination revealed BTB-like lesions. Tests showed that the animals were infected with *Mycobacterium bovis*.

Conclusions. Taking into account the lack of obligation to monitor infectious zoonotic diseases, the often unknown origin of animals and, frequently, very close contact between people and animals in private animal collections, there is a need to clarify the legal obligations of owners of these private animal collections in Poland to ensure public health protection. The findings of this investigation demonstrate that due to the lack of precise epidemiological data for BTB, it is difficult to define the epidemiological status of private animal collections, and further management depends on the owner's cooperation with veterinarians.

Key words

bovine tuberculosis, zoological garden, *Mycobacterium bovis*, animal health law (AHL), *Muntiacus reevesi*

INTRODUCTION AND OBJECTIVE

Tuberculosis is an infectious disease in humans and animals caused by eleven species of the *Mycobacterium tuberculosis* complex (MTBC) [1, 2]. Of these, *Mycobacterium bovis* and *Mycobacterium caprae* are known to be etiological agents of bovine tuberculosis (BTB), found in animals [3]. The most common etiological factor of tuberculosis in humans is *Mycobacterium tuberculosis* [4]; however, BTB has also been noted in humans and has been described recently in Poland [5]. The World Health Organization (WHO) classifies BTB as a direct zoonotic disease: an infectious factor, transmitted from an infected vertebrate (animal) to humans, the latter being a sensitive species, without intermediate hosts [6].

Therefore, to ensure public health and animal welfare, animals kept together in places such as zoos should be subjected to regular testing to minimize the risk of disease, as well as those intended for purchase.

BTB is also one of the infectious diseases occurring among zoo-kept animals [7, 8, 9]. With a large number of zoo visitors, BTB may pose a threat to public health. The main route of transmission of BTB between animals is through the respiratory tract [10]. Indeed, cases of animal-human BTB transmission have been confirmed in zoos, which suggests an airborne transmission [11]. In humans, the most common route of BTB infection is via the consumption of unpasteurized milk; however, increasing attention is being paid to animal-human air-borne transmission, as supported by recent literature reports [12, 13, 14].

Since Poland's accession to the European Union (EU), the measures taken to combat BTB have been based on international law. According to Council Directive 64/432 / EEC, a country is officially tuberculosis free (OTF) if the

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percentage of infected cattle herds does not exceed 0.1% per year, and at least 99.9% herds are officially bovine tuberculosis free each year for six consecutive years. Poland was granted the status of an OTF country following Commission Decision No. 2009/342 / EC of 23 April 2009. Obtaining this status made it possible to reduce the surveillance scheme from 1/3 to 1/5 of cattle herds in a district annually, i.e. all cattle herds in the district are now subjected to testing every five years, with animals over 42 days of age being subject to testing. The official test is carried out by comparative intradermal tuberculin test in the neck area.

BTB is still being recorded in both livestock and wildlife in Poland, with 372 outbreaks recorded in cattle in the past 10 years. In addition, BTB has also been described in free-living and captive wildlife species, most recently in 2018 in captive European bison [15]. Currently, there is no law implemented that would mandate testing for BTB in free-living and captive wild animals in Poland.

BTB has also been previously described in zoos in Poland [16, 17]. However, the present study instead concerns animals kept on a private estate, officially known as a *private animal collection*. Such collections are not currently subject to specific rules in Polish legislation.

This difference between large zoos and private animal collections is a legal distinction: large zoos were under District Veterinary Supervision while private animal collections were not required to be registered. This is a huge legislative gap, as the origin and the epizootic status of animals kept in private animal collections often remains unknown, and their movement is often not subject to any restrictions or rules preventing the spread of infectious diseases. Most importantly, as animals kept in private animal collections are used as tourist attractions, and are often in direct contact with humans, including children, and can pose a threat of zoonotic transfer. Therefore, the present research examines a case of BTB in a private animal collection, describing the procedures undertaken by the owners and attending veterinarians, and attempts to highlight the legal aspects of the case.

MATERIALS AND METHOD

Animals and samples. The two Reeves's muntjacs (*Muntiacus reevesi*) described in this case study (one male and one female, both aged 4 years) presented similar clinical signs at different time intervals. In February 2021, a veterinarian was summoned to the muntjac deer with signs of general weakness, lack of appetite, and apathy. During the clinical examination, a significant degree of inhalation-expiratory dyspnea was found. It was decided to chemically immobilize the deer for a physical examination, and then to facilitate treatment and administer antibiotics and anti-inflammatory drugs. Unfortunately, the animal died during capture for the examination.

A few weeks later, the owner reported similar clinical signs in a second Reeves's muntjac in the herd. The veterinarian decided to euthanize the animal after the clinical examination, and a *post mortem* examination was carried out. For premedication of the animal, xylazine (1 mg/kg, Sedazin, Biowet Puławy, Puławy, Poland) and ketamine (5.75 mg/kg, Ketamine, Biowet Puławy, Puławy, Poland) were administered intramuscularly, Pentobarbital sodium

(133.3 mg/kg) and pentobarbital (26.7 mg/kg) were used intravenously for euthanasia (Morbital, Biowet Puławy, Puławy, Poland)

Post-mortem examination. The autopsy was conducted according to Annex 3 to the Ordinance of the Minister of Agriculture and Rural Development of 4 July 2017 on the method of keeping documentation related to combating infectious animal diseases [18]. During the *post mortem* examination, fragments of lungs were collected from the first muntjac, and varied material from the second (thoracic, submandibular, mesenteric lymph nodes and fragments of lungs).

Culture and molecular examination. The material was subjected to standard mycobacterial culture as described previously [19]. Each tissue was examined as three replicates. Briefly, after homogenization in 5% oxalic acid (Sigma-Aldrich, Burlington, MA, USA), the sediment was flushed in saline solution (Sigma-Aldrich, Burlington, MA, USA). After centrifugation, the sediment was placed on Lowenstein and Stonebrink media (Becton Dickinson, Franklin Lakes, NJ, USA). Three Lowenstein and Stonebrink media were used in this study for each type of tissue to increase the sensitivity of the test. The media were incubated at 37°C for 12 weeks and checked every 7 days. The DNA was isolated using a Genolyse isolation kit (Hain Lifescience, Germany). Strains were identified with a GenoType®MTBC assay (Hain Lifescience, Germany). Both tests were performed in accordance with the manufacturer's instructions [19].

RESULTS

Herd history. The research was carried out in a private animal collection located in northern Poland. The animals were introduced in 2017 as an exchange; however, their exact origin remains unknown. According to the information obtained from the owner, the animals originated from the vicinity of Kraków, southern Poland. The animals arrived at the facility without any previous disease screening. The described private animal collection is part of an agritourism facility with an area of 6 ha. On the day that the second Reeves's muntjac was euthanized, the facility also housed kangaroos (*Wallabia*), zebras (*Equus quagga*), camels (*Camelus dromader*), sheep (*Ovis aries*), goats (*Capra hircus*), llamas (*Lama guanicoe*), guanacos (*Lama guanicoe*), alpacas (*Vicugna pacos*), domestic donkeys (*Equus asinus*), fallow deer (*Dama dama*), Nilgau antelopes (*Boselaphus tragocamelus*), Sitatunga antelope (*Tragelaphus speki*), Patagonian mara (*Dolichotis patagonum*), peacocks (*Pavo cristatus*), emu (*Dromaius novaehollandiae*), Rhea (*Rhea americana*), and an exotic species of chicken. The animals had no direct contact with muntjacs.

Pathology. *Post-mortem* examination revealed extensive tuberculous gross lesions in both muntjac deer (Fig. 1, 2). Granulomas with caseous necrosis and mineralization lesions were found in the liver (Fig. 1, animal No 1, male) lungs (Fig. 2, animal No 2, female), lymph nodes as well as on the serosal surface of the peritoneum. The lesions resembled small gray-white nodules, ranging from 3–5 mm in size. No histopathological examinations were performed.



Figure 1. Anima No 1, male. Granulomatous lesions in internal organs. The figure shows the liver, correct consistency and color. Granulomatous lesions from 3–5 mm in size

Bacteriology and molecular identification. The material collected *post-mortem* was positive for culture. On both types of media, growth was visible in the 4th week of incubation. Based on the GenoType MTBC (based on the polymorphism of the gyrB gene) test, the isolated strains were classified as *Mycobacterium bovis*.

DISCUSSION

The occurrence of BTB in zoo animals should be treated as a public health problem in the area where it occurs. This is particularly important in countries where there is little public health awareness of the disease, as animals can be the source of infection for zoo visitors, and present a threat to zoo staff in first line. In addition, in the absence of animal testing and the exchange of animals between private animal facilities, may also allow easy transmission of mycobacteria. The transboundary nature of BTB was well illustrated by a case of a group of alpacas shipped from the United Kingdom (UK) to Poland. Although *ante mortem* tuberculin skin testing found them to be free from tuberculosis, some had been infected with *M. bovis* prior to transfer, which resulted in transmission of the disease to animals on Polish farms [19]. Another example was the transport of 2 European bison (*Bison bonasus*) to another European bison breeding herd in the country; the bison had not received any BTB testing, and it was later found that one animal was TB-positive [20]. There is clearly a strong need for stricter legislation to avoid such situations, as illustrated by the case described herein, where the epizootic investigation was able to establish the source of the muntjac deer infection.

Due to the legal status of private animal collection-type facilities, they often lack procedures regarding their registration, and they are generally not subject to monitoring for infectious disease. The animals are not subject to compulsory inspection during movement, and their circulation and transport is not governed by the same rules as for zoos. Therefore, it is likely that many cases of BTB infection have been misdiagnosed or have remained undetected. Interestingly, due to the fact that BTB is not monitored *ex officio* in animals other than cattle, the sick animals may not have received pharmacological therapy. In Poland, a single case of BTB treatment has been reported in a male reticulated giraffe (*Giraffa camelopardalis*) in the Silesian Zoological Garden [16]. However, the use of such treatment is very controversial due to the risk to other animals



Figure 2. Animal No 2, female. Granulomatous lesions in internal organs. The figure shows the lungs with bloodshot, correct consistency. Granulomatous lesions from 3–5 mm in size.

and humans posed by interacting with the treated animal; such treatment was not even considered in the presented case.

Regulation (EU) 2016/429 of the European Parliament and the Council of 9 March 2016 on transmissible animal diseases, amending and repealing certain acts in the field of animal health, also known as the 'Animal Health Law' (AHL), was passed on 21 April 2021 [21]. The regulation includes rules for all animals on the territory of the Republic of Poland, as in the territories of all other countries belonging to the European Union, whether they are kept for production breeding, live in zoos, or are used in circuses.

Poland is required to implement the AHL by 21 April 2022, and it is hoped that this will result in better official control of BTB in centres like the one described in the presented study. Currently, the only legislative option is Commission Implementing Regulation (EU) 2018/1882 of 3 December 2018 on the application of disease prevention and control rules; this regulation provides categories of listed diseases and establishes a list of species and groups posing a considerable risk for their spread. Its content changed the rules of categorization and methods of combating tuberculosis [22]. The AHL classifies MTBC infections as category B diseases, i.e. as dangerous bacterial diseases, only for *Bison*, *Bos* and *Bubalus*. As such, these infections are subject to mandatory control in all Member States, and each Member State should adopt compulsory eradication programs.

The Reeves's muntjac is classified under *other ruminants*. According to Regulation (EU) 2018/1882, tuberculosis in such ruminants, i.e. other than *Bison*, *Bos* and *Bubalus*, belongs to groups D and E, which means that while it is necessary to prevent the spread of the disease and notify its occurrence, the animals are not subject to any eradication programme [22]. The AHL introduces a more universal, but very general, division of all animals into *kept animals*, i.e. animals kept by humans, including those in the private animal collection, and *wild animals*, i.e. those that are not kept in captivity.

Currently, insufficient information is kept on sick zoo or private farm animals and unreported cases, and hence, any conclusive epizootic investigation is difficult with less chance of identifying the source of TB infection. Three articles within the new legislation given above may be of particular value in identifying cases of BTB from private animal collections and for regulating the procedure in the case of infectious diseases. Art. 108 of Regulation 2016/429 imposes on Member States a 'responsibility for establishing a system for the identification and registration of kept terrestrial animals'. In addition, Art. 117 requires operators to comply with regulations regarding individual or group identification and documentation for the identification and tracking of animals, as appropriate for the species concerned; this is needed to keep terrestrial animals other than bovine, ovine, caprine, porcine and equine species [21]. Art. 124 specifies that 'Operators shall take appropriate preventive measures to ensure that the movement of kept terrestrial animals does not jeopardise the health status at the place of destination', and that 'operators shall only move kept terrestrial animals from their establishments and receive such animals if the animals in question fulfil the following condition' of originating from establishments registered or approved by the competent authority or which have been granted a derogation from registration requirements, and fulfill the requirements for the identification and registration of the animals [21].

It should be noted that the management of the studied private animal collection took a very responsible approach in addressing the zoonotic nature of tuberculosis: all animals and workers were tested, the pens disinfected, and the owners cooperated fully with the field veterinarian and research centre. It would seem to be good practice to oblige visitors to wash and disinfect their hands on entry and exit. Animal-to-human and human-to-animal transmission of tuberculosis [12] and other mycobacteriosis [23] has been extensively described, including among animals in zoos [24]; such transmission is particularly suited to the special nature of the private animal collection, where guests can come into close contact with animals, and can pet and feed them [25, 26].

It should be emphasized that the muntjac is an invasive species in some countries with a high prevalence of BTB, and hence has often been found to be positive during local wide screenings [27]. Therefore, its confirmation in another 2 individuals means that this species should be carefully monitored for tuberculosis in zoos.

In recent years, much attention has been paid to the risks associated with the trade in captive wild animals and their presence on farms, either as ornamental or farm animals. When brought into Europe or moved without established control rules, animals can be a source of various pathogens that are dangerous to both other animals and humans. So far, there has been no legislation on the monitoring of infectious and invasive diseases in free-living or kept animals. Pathogens from wild animals pose a direct threat to people visiting private animal collections. Legislation in the field of public health has a solid scientific basis at both the national and local levels. However, there is a pressing need for rules regarding the monitoring of infectious and invasive diseases occurring in animals kept in facilities accessible to humans, and to regulate the rules of movement and circulation of kept wild animals in the EU. The great changes taking place in EU law allow a chance to develop national programmes for controlling infectious diseases, including tuberculosis.

CONCLUSIONS

Taking into account the lack of obligation to monitor infectious zoonotic diseases, the often unknown origin of animals, and the frequent close contacts between guests and animals in private animal collection centres, there is a need to clarify the legal obligations of the owners of these collections with regard to public health protection. As the presented study shows, due to the lack of precise epidemiological data it is difficult to define the epidemiological status of the private animal collection in which BTB appeared, and further management depends on the owner's cooperation with veterinarians. Generally speaking, however, the key roles in prevention are played by Personal Protective Equipment and the awareness of pet owners.

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