



# Pregnancy in European bison (*Bison bonasus*) with generalized tuberculosis – no evidence of vertical transmission

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## Abstract

**Introduction and objective.** One of the main health threats to the endangered European bison (*Bison bonasus*) is bovine tuberculosis, the pathogenesis of which in this species is not fully known. The aim of the study was to confirm a possible case of vertical transmission from a pregnant European bison with generalized tuberculosis to its 12-week-old foetus.

**Materials and method.** During the autopsy it was found that the bison had become pregnant, despite an advanced stage of tuberculosis. Material collected from the organs and foetus was placed on Lowenstein and Stonebrink media and incubated at 37 °C for 12 weeks.

**Results.** Mycobacteria were isolated from the lungs and lymph nodes; however, the tissue of the foetus and fragments of the reproductive system were negative.

**Conclusion.** Vertical transmission was excluded, although it cannot be ruled out that infection could occur as pregnancy progresses.

## Key words

pregnancy, intrauterine infection, foetus, bovine tuberculosis, *Mycobacterium caprae*, European bison

## INTRODUCTION

The European bison (*Bison bonasus*) is a protected species, and due to difficulties in population management [1], and in the face of numerous environmental [2, 3] and health threats [4–7], it requires active protection [8]. Bovine tuberculosis (BTB) has proven to be one of the most dangerous diseases facing the European bison in recent decades [9] and one that poses a threat to the restitution of the species. While BTB is caused by both *Mycobacterium bovis* and *Mycobacterium caprae* belonging to the MTBC (*Mycobacterium tuberculosis* complex), the latter species has been predominantly isolated from European bison in Poland [10]. BTB is an infectious, debilitating disease, as well as a dangerous zoonosis. In European bison, due to the close phylogenetic relationship with cattle, knowledge about the sources, transmission and pathogenesis of BTB is often derived from observations on cows. Each case of natural infection in European bison

presents a unique opportunity for study, which so far has generally been restricted to serological sampling [11].

## OBJECTIVE

The aim of the study was to present the case of pregnancy in European bison naturally infected with *M. caprae*, with generalized tuberculosis, and to confirm possible intrauterine transmission.

## MATERIALS AND METHOD

The 10-year-old European female bison described in the present study came from a herd in which BTB had been repeatedly confirmed in subsequent individuals since 2013 by the Smardzewice Bison Centre, located in central Poland, in the Łódź Province, Tomaszów County, in the Smardzewice Forest District. The diagnosis was based on a positive gamma-interferon test result in 2017 [12]) and a positive *M. caprae* culture result from the material collected *ante-mortem* [13]. Two days before euthanasia, a simplified clinical examination

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was conducted from a distance of about two meters. Rapid breathing and shortness of breath, indicative of dyspnea, were observed. No other symptoms had been previously reported by the animal keepers; however, this may have been due to the environmental conditions: it would have been difficult to notice any fatigue, weakness, hunger or thirst characteristic of BTB in the studied animal as it was supplied with food and water. In natural conditions, European bison habitually walk several kilometers a day in search of food and drinking water [14]. So far, clinical symptoms of BTB (coughing, purulent discharge from the nose, diarrhea and weight loss) have only been described once in European bison [15].

Due to the BTB-positive *ante-mortem* results, the European bison female was euthanized in accordance with the decision of The Polish Ministry of the Environment (No. DOP-WPN.286.219.2018.MS).

Tissue samples of the lymph nodes, lungs, as well as the entire foetus, uterus and ovaries were collected for microbiological studies. The culture was conducted as described by Krajewska-Wędzina et al. [16] has proved to be more accurate in European bison than direct MTBC DNA detection [12]. If colonies appeared on the media, their DNA was isolated using Genolyse Isolatin Kit (Hain Lifescience, Germany) and the mycobacteria species were differentiated using GenoType® MTBC assay (Hain Lifescience, Germany) as described previously [9].

## RESULTS

Necropsy revealed extensive tuberculous gross lesions in the lungs and lymph nodes (Fig. 1), as well as serosal tubercles on the peritoneum. During the autopsy, the cow was found to be pregnant (Fig. 2). Foetal age was assessed to be about 12 weeks.

Mycobacteria were isolated from lung and lymph node material and identified as *M. caprae* according to Didkowska et al. [11]. No mycobacteria were isolated from the uterine, ovarian or foetal specimens.



Figure 2. European bison foetus visible during autopsy

## DISCUSSION

Despite the very advanced stage of disease, the study revealed no vertical transmission into the foetus, nor any mycobacteria in the maternal reproductive system. However, it cannot be ruled out that an intrauterine infection could have occurred as the pregnancy progressed. Some cases of infection with bovine mycobacterium in the womb or during birth have been noted in cattle, but these cases are sporadic [17]. It is possible that congenital tuberculosis may occur following the involvement of the genital tract or placenta; in such cases, infection may spread via the umbilical veins or amniotic fluid [18]. However, in cattle, the most common route of *Mycobacterium* spp. infection remains the aerogenic route, followed by the alimentary route [19], and it can be assumed that this would also be the case in European bison, with a similar frequency.

It should be emphasized that the fact that she became pregnant and maintained the foetus indicates that the studied European bison was otherwise in relatively good



Figure 1. Tuberculous granulomas forming conglomerates

condition. Such cases have been described before in humans [20]. It should be noted that tuberculosis was considered to follow a more aggressive course during pregnancy [21].

To the best of the authors' knowledge, the youngest MTBC-positive European bison identified so far was a 6-year-old cow [12]. However, not all cases, especially those from the Bieszczady Mountains, have been thoroughly documented in the literature, so it should not be ruled out that mycobacterial infection may also have been present in younger animals of this species.

The fact that the placenta and amniotic fluid were not included in the present study may be considered a limitation. However, we deliberately chose to focus on the foetal tissues because tuberculous placentitis does not always lead to congenital infection [22]. Additionally we have found the organs of the cow reproductive system to be MTBC negative.

A key value of the present study is the fact that tuberculosis in bison in Poland is a current problem, and has been observed in many locations around the country, including a farm in Bukowiec, Kujawska-Pomorski Province, northeast Poland [23], in free-living herds in Bieszczady, Sub-Carpathian Province of southeast Poland [24], in Warsaw zoo [25], in the closed breeding center in Smardzewice [8], and also in the 'Wolisko' homestead in the Borecka Forest, Warmian-Mazurian Province, northeast Poland [25]. Hence, tuberculosis remains a serious problem for the endangered European bison, and there is a pressing need to learn about its pathogenesis to allow better monitoring and disease control.

## CONCLUSIONS

An individual with such advanced tuberculous gross lesions becoming pregnant may be considered a rare phenomenon. In addition, no vertical transmission of mycobacteria was found, despite generalized tuberculosis. However, further studies are needed to confirm this, as it is extremely difficult to conduct such research under natural conditions, which further emphasizes the uniqueness and value of the present research. It is therefore planned to take such action in any further cases requiring the elimination of a BTB-positive pregnant female European bison.

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