



No evidence of *Mycobacterium tuberculosis* complex infection in samples from cervids in various regions of Poland

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Abstract

Introduction. Tuberculosis (TB) is a widespread disease known to affect livestock and wildlife, as well as humans. However, its incidence in wildlife remains poorly recognized on the global level. In Europe, the majority of TB cases have been confirmed in red deer, badgers and wild boar.

Objective. The aim of the study was to examine the occurrence of TB in Cervidae in Poland, in areas where TB has been detected in cattle and wildlife.

Materials and method Head and thoracic lymph nodes were collected from a total of 76 free-living red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), from nine Polish provinces during a single hunting season (autumn – winter 2018–19). Samples were subjected to conventional microbiological procedures to isolate mycobacteria.

Results. No Mycobacteria was isolated in the material collected from red or roe deer.

Conclusions. There is a need to continue monitoring the presence of TB in cattle and other animal species to ensure the protection of public health.

Key words

red deer, *Mycobacterium tuberculosis* complex, roe deer, *Capreolus capreolus*, *Cervus elaphus*, wildlife, public health protection, *Mycobacterium caprae*

INTRODUCTION

Tuberculosis (TB) is a widespread disease known to affect livestock and wildlife, as well as humans [1]. However, its incidence in wildlife remains poorly recognized on the global level. TB is caused by various bacilli belonging to the *Mycobacterium tuberculosis* complex (MTBC); however, the most widely reported etiological agent of TB in European wildlife is *Mycobacterium bovis* [2]. Infections by *Mycobacterium caprae* have also been reported [3, 4]. In Europe, most TB cases have been confirmed in red deer (*Cervus elaphus*), along with badgers (*Meles meles*) and wild boar (*Sus scrofa*) [1, 5, 6, 7].

The social organization and natural behaviour of cervids favour intra-species transmission, and some infected animals may act as super-shedders [8]. The Cervidae can also act as a reservoir of TB, as described in white-tailed deer (*Odocoileus virginianus*) in the USA [9], and fallow deer (*Dama dama*) in Spain [10]. In Poland, most cases of TB in wildlife have been reported in south-eastern areas, where *M. caprae*

infection has previously been confirmed in wild boar and European bison (*Bison bonasus*). Moreover in this area, cases of *M. caprae* infections, but without macroscopic TB-like lesions, has been found in gray wolves (*Canis lupus*) and a single roe deer (*Capreolus capreolus*) [11, 12, 13, 14].

In Poland, research on MTBC infections among cervids has been carried out only in the Bieszczady Mountains, the most southerly region of Poland bordering with Ukraine and Slovakia. In 2005–2008, 28 red deer and in 2011–2017, 13 red deer and 3 roe deer were tested for MTBC infection [14, 15]. To date, no infection with MTBC in red deer has been found in Poland. *M. caprae* was isolated from one roe deer, as mentioned above [14].

OBJECTIVE

In light of the possibility of snowballing pathogen transmission, and the high prevalence of TB in deer in other European regions, the aim of presented study was to examine the occurrence of TB in Cervidae in Poland, in areas where TB has been detected in cattle and wildlife.

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MATERIALS AND METHOD

Sample collection. Samples were randomly collected from a total of 76 free-living animals, *viz.* 40 red deer (*Cervus elaphus*) and 36 roe deer (*Capreolus capreolus*), from nine Polish provinces (Tab.1, Fig.1) during a single hunting season (autumn-winter 2018/19). The investigated animals were hunted during the game season and delivered by hunters to venison collection points. All necropsies and sample collection procedures were performed by official veterinarians. After collection, the samples, consisting of head and thoracic lymph nodes (mandibular, retropharyngeal, mediastinal, tracheobronchial), were transported to the laboratory under refrigeration. All lymph nodes were then subjected to thorough macroscopic examination and microbiological testing to detect TB. All lymph nodes collected from each given individual were pooled.

Microbiological analysis. The collected deer lymph node samples were subjected to conventional microbiological procedures to isolate mycobacteria [16]. Briefly, the lymph node tissues were cut into small pieces and homogenized in a 5% solution of oxalic acid (OA). The obtained suspension was incubated at 37°C for 10–15 minutes and then centrifuged for 10 minutes at 4,000 rpm (3,000g). The supernatant was then flushed twice in sterile 0.9% sodium chloride to remove the OA. The sediment was then inoculated onto Lowenstein–Jensen and Stonebrink solid media (Oxoid, Germany) and incubated at 37°C for 8 weeks.

RESULTS

All investigated lymph node samples from 40 red deer and 36 roe deer were negative in mycobacteria culture procedure. No mycobacterial growth was observed on either Lowenstein–Jensen or Stonebrink solid media. No anatomo-pathological lesions suggestive of TB were found during carcass necropsies and thorough macroscopic examination of lymph nodes.

DISCUSSION

No bacteria from the MTBC were isolated from the examined samples collected from 76 free-living red and roe deer. These findings are consistent with those of previous studies conducted in the Bieszczady Mountains, where no MTBC infections were found in cervids, apart from a single case of *M. caprae* infection in a roe deer [14, 17].

Following European Commission Decision 2009/342/EC of 23 April 2009, Poland was declared officially free of bovine TB. Despite this, new outbreaks of animal TB are continually noted in various species [18, 19], and TB is recorded in a mean number of 79 cattle each year [20]. Among free-living animals in Poland, almost all reported cases of TB (less than 100 cases of TB caused by *M. caprae* during the last decade) have concerned European bison and wild boar inhabiting the Bieszczady Mountains (Podkarpackie Province, Southeast Poland). However, among the free-living cervids in Poland, MTBC bacteria have only been isolated from 2 roe deer, one in 1956 and another in 2013 [12, 14, 21].

While countermeasures can be employed in many cases in which the source of the infection is known [18], sometimes

no epidemiological investigation is possible, such as in cases involving private animal collections with unknown sources [19]. TB is an insidious disease, and despite being highly contagious, it is often the case that no disease symptoms are noted in many species; indeed, live mycobacteria have been isolated from the lymph nodes of animals even in the absence of macroscopic lesions [14]. As such, the disease requires careful monitoring.

In Poland, a pilot study on the prevalence of TB in various animal populations was carried out by Lipec et al. (2018) and Didkowska et al. (2022); however, its presence in badgers and sheep has not been confirmed [14, 22, 23], even though TB has been found to cause deaths in these species in other countries [24, 25, 26, 27].

Surprisingly, MTBC were not isolated from deer from the Bieszczady region (Podkarpackie Province), where TB has been identified in wildlife. This may be due to the presence of wolves in the area: they are believed to consume almost the whole deer after hunting, and studies indicate that deer constitute 70% of their diet [28, 29]. Red deer are known to be susceptible to TB infection and may act as maintenance hosts for TB [4, 30, 31, 32]. The susceptibility of cervids to MTBC infection is further illustrated in a recently described case of TB, caused by *M. bovis*, in Reeves' muntjack (*Muntiacus reevesi*) in a private collection of animals in Poland [19].

A key limitation of this study is the relatively small number of deer samples tested. The red deer population in the studied area (9 provinces) was estimated at about 132,000 heads, and roe deer at 504,000 (*Statistical Yearbook of Forestry*, Statistics Poland, Warsaw 2020). In Poland, there are no official government programmes for testing free-living animals for TB. In 2021, the Animal Health Law [33] came into force, and is in the process of being implemented by Polish veterinary state authorities. However, it is important to note that red deer and roe deer are game animals in Poland, and all game undergo macroscopic inspection at the venison collection point by official veterinarians. The presented findings represent the first report of microbiological examinations of samples from deer hunted in Poland, in regions other than the Bieszczady Mountains.

CONCLUSION

No MTBC were found in cervids from selected regions of Poland in the 2018–2019 hunting season. Considering the fact that TB occurs in free-living animals and cattle in Poland (TB Low-risk Area), and that deer are susceptible to MTBC infection, there is a clear need to monitor the health condition of Polish cervids. It is important to note that MTBC infected animals or food products obtained from them may be a source of infection for human [1, 34, 35]. Data from the European Food Safety Authority and the European Centre for Disease Prevention and Control (EFSA and ECDC) indicates 147 confirmed human cases of tuberculosis due to *M. bovis* or *M. caprae* in Europe in 2019 [36].

It is noteworthy that the Covid-19 pandemic of 2020–2022 has disrupted human TB diagnosis services at every level of the healthcare system. Hence, there is a strong need to continue monitoring the presence of TB in cattle and other animal species to ensure the protection of public health.

Animal rights statement: All animal samples used in this study originated from legally hunted deer in accordance with Polish legislation (Hunting Law DzU 1995 Nr. 147 poz. 713). The study did not require the approval of an Ethics Committee because all samples were collected *post mortem*.



Figure 1. Number of deer tested in 2018–2019 in nine provinces in Poland

Table 1. Numbers of red deer and roe deer tested in Poland in 2018–2019 according to province

Province	Number of investigated animals		
	Red deer	Roe deer	Total
Podkarpackie	8	7	15
Dolnośląskie	7	3	10
Małopolskie	1	1	2
Śląskie	12	8	20
Lubelskie	3	6	9
Lubuskie	7	6	13
Warmińsko-Mazurskie	2	3	5
Mazowieckie	0	1	1
Łódzkie	0	2	2
Total	40	36	76

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