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Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Two Brown Bears in the Central European Carpathians

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ABSTRACT: The incidence of mycobacterial infections was monitored in brown bears (*Ursus arctos*) in the National Park Low Tatras in the central European Carpathians in Slovakia. Tissue samples of 20 brown bears were examined microscopically and by culture for the presence of mycobacteria. Acid-fast rods were detected by Ziehl-Neelsen staining in a smear from the kidney of one brown bear, although the culture was negative for mycobacteria. *Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of paratuberculosis in ruminants, was isolated from the intestinal mucosa of another two brown bears. The isolates were identified by polymerase chain reaction for the specific insertion sequence IS*900*. Using standardized IS*900* restriction fragment length polymorphism (RFLP) analysis, the *M. a. paratuberculosis* isolates were classified as RFLP type B-C1, which also were detected in the infected cattle in surrounding area. This study describes the first isolation of *M. a. paratuberculosis* from a brown bear. Our results confirm that animal species other than ruminants can become infected with *M. a. paratuberculosis* and can act as potential vectors and/or reservoirs of the infection.

Key words: Carnivores, epidemiology, Johne's disease, *Mycobacterium avium*, paratuberculosis, wildlife.

Paratuberculosis, or Johne's disease, is a specific infectious granulomatous enteritis of domestic and wild ruminants (Ayele et al., 2001; Machackova et al., 2004) caused by facultatively anaerobic intracellular acid-fast rods (AFRs) of *Mycobacterium avium* subspecies *paratuberculosis*. Paratuberculosis is mainly a subclinical infection with a protracted incubation period. The major clinical signs of the disease in ruminants (particularly in cattle and red deer) are chronic diarrhea and progressive afebrile weight loss that consequently leads to emaciation (Ayele et al., 2001). Factors that increase the likelihood of acquiring the disease are stress, nonbalanced diets, intercurrent infections, parasitic diseases and others. *Mycobacterium avium* subsp. *paratuberculosis* has been isolated from other nonruminant wildlife such the European rabbit (*Oryctolagus cuniculus*; Greig et al., 1997, 1999), brown hare (*Lepus europaeus*; Beard et al., 2001; Machackova et al., 2004), brown rat (*Rattus norvegicus*; Beard et al., 2001), and long-tailed field mouse (*Apodemus sylvaticus*; Beard et al., 2001).

Mycobacterium avium subsp. *paratuberculosis* was likewise detected in carnivores such as red fox (*Vulpes vulpes*), stoat (*Mustela erminea*), and weasel (*Mustela nivalis*; Beard et al., 1999, 2001), and also in omnivores such as Eurasian badger (*Meles meles*; Beard et al., 2001) and wild boar (*Sus scrofa*; Machackova et al., 2003; Trcka et al., 2006). The infected wild animals originated from localities with paratuberculosis incidence in ruminants (Greig et al., 1997; Beard et al., 1999, 2001; Pavlik et al., 2000a; Machackova et al., 2003, 2004). Isolation of *M. a. paratuberculosis* from a wide range of hosts that constitute respective parts of the food chain supports the suggestion of a more complex epidemiology for paratuberculosis than is currently recognized.

In the literature, there are limited data regarding the isolation of bacteria from bears. Most studies have involved oral bacteria of bears or bacteria associated with bear bite wounds (Parry et al., 1983; Goatcher et al., 1987; Kunimoto et al., 2004). The only reported nontuberculous mycobacterium from any bear species is

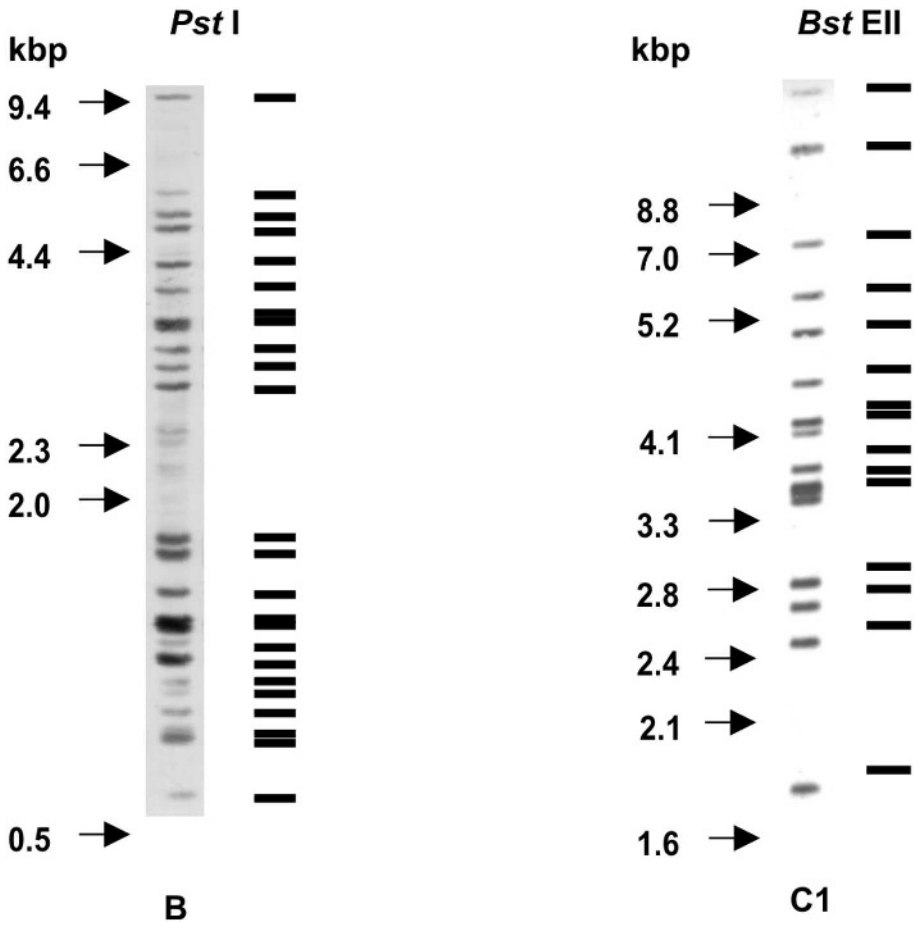

FIGURE 1. The patterns of RFLP types of *Mycobacterium avium* subsp. *paratuberculosis*: C1 (restriction endonuclease *Bst*EII), B (restriction endonuclease *Pst*I) according to Pavlik et al. (1999a).

M. fortuitum that was isolated from a brown bear (*Ursus arctos*) bite wound (Lehtinen et al., 2005).

The incidence of bacterial infections and parasites in different wildlife species was monitored in the National Park Low Tatras in central European Carpathians in Slovakia. Twenty brown bears were examined within the 4-yr period (2002–2005). There were 15 males and five females of various age and size with the average body weight of about 130 kg. Tissue samples (Table 1) were examined microscopically after staining according to the Ziehl-Neelsen method for AFR detection. Subsequently, 1 g of tissue was homogenized by a stomacher (Kleinfeld Labortechnik, Gehrden, Germany) and decontaminated in 0.75% hexadecyl pyridinium chloride (HPC):*N*-cetylpyridinium chloride monohydrate (no. 102340 Merck, Whitehouse Station, New Jersey) for 72 hr (Pavlik et al., 2000b). Sediment (200 µl) of each decontaminated sample was cultured on three slopes of different Herrold egg yolk media with Mycobactin J and incubated at 37 C for 12 mo (Machackova et al., 2004). Isolates of *M. a. paratuberculosis* were identified by IS*900* polymerase chain reaction (PCR; Bartos et al., 2006). Standardized IS*900* restriction fragment length polymorphism (RFLP) by using restriction endonucleases *Pst*I and *Bst*EII was used to further differentiate *M. a. paratuberculosis* isolates (Pavlik et al., 1999a).

TABLE 1. Mycobacteria detection in the organs of 20 brown bears (*Ursus arctos*).

Examined organ	No.	Microscopy[a] positive	Culture positive
Lungs	2	0	0
Liver	10	0	0
Spleen	10	0	0
Kidney	3	1	0
Mesenteric lymph node	11	0	0
Intestinal mucosa	16	0	2[b]

[a] Detection of acid-fast rods by Ziehl-Neelsen staining.

[b] *Mycobacterium avium* subsp. *paratuberculosis* of restriction fragment length polymorphism type B-C1 was identified and typed by IS*900* polymerase chain reaction.

Gross examinations did not reveal any lesions in gastrointestinal tract that are pathognomonic of paratuberculosis. Mycobacteria in the form of long AFRs were detected by microscopy of a smear prepared from the kidney of one brown bear; however, isolation from this sample was unsuccessful. Mycobacterial isolates were obtained from the intestinal mucosa of another two brown bears (Table 1), which were classified by IS*900* PCR as *M. a. paratuberculosis of* RFLP type B-C1 (Fig. 1).

Mycobacteria detected by microscopy in the bear kidney could not be isolated because of loss of viability during processing, or they were already dead, or uncultivable. These pathogens were probably environmentally derived mycobacteria, and they are usually killed by HPC during sample processing (Machackova et al., 2002; Beran et al., 2006).

Infected brown bears included a male (120 kg) and a 5-yr-old female (90 kg). The male bear originated from a location in the National Park where a red deer (*Cervus elaphus*) with small intestinal lesions that were pathognomonic for paratuberculosis previously had been reported. The bear was killed in a collision with a truck on the main road; hence, the health status of the bear was not determined. The female bear was in good body condition and was shot on the other side of the National Park Low Tatras.

It is commonly known that brown bears leave their den in spring (after 90- to 120-day winter rest) and feed on carcasses of animals that died during the winter (Nowak, 1999). The population density of brown bears in the Carpathians is approximately one bear per 20 km^2. Other subspecies of brown bear are generally known to roam hundreds of kilometers toward major food sources during seasonal movements. Conversely, at feeding sites, density may reach one bear per 0.05 km^2 (Nowak, 1999). Because of potential long-distance movements bears may associate with livestock, and both infected brown bears in the present study were killed in the buffer zone of the National Park where it is permitted to graze domestic ruminants. Sheep are mostly grazed at this area, but the status of paratuberculosis in sheep is unknown. Nevertheless, paratuberculosis was detected in cattle herds in this area in previous years (Pavlik et al., 1999b). It is therefore possible that these brown bears were infected by consuming the corpse of an infected ruminant.

Isolation of *M. a. paratuberculosis* from intestinal mucosa supports the suggestion that brown bears can be passive carriers of *M. a. paratuberculosis*. The RFLP type B-C1 is the most common RFLP type of *M. a. paratuberculosis* detected in domestic ruminants in Slovakia and other central European countries (Pavlik et al., 1999a, b), and the RFLP type B-C1 of *M. a. paratuberculosis* has been detected in

cattle in this area. Although *M. a. paratuberculosis* has not been isolated from wild ruminants in this area, they cannot be excluded as an alternative source of these infections (Machackova et al., 2004).

The present study described the first isolation of *M. a. paratuberculosis* from a brown bear, and these findings support an increasing body of evidence that indicates that a wide diversity of wildlife species as well as domestic ruminants can become infected with *M. a. paratuberculosis*. The significance of brown bears as a potential reservoir is difficult to assess because they are strictly protected; but based on these results, it may be reasonable to evaluate other species and populations of bears in other European countries or worldwide.

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