The wildlife hosts of *Mycobacterium avium* subsp. *paratuberculosis* in the Czech Republic during the years 2002–2007

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ABSTRACT: The objective of this study was to determine the wildlife hosts of *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) in the Czech Republic. A total of 8 796 wildlife animals were examined by culture of faecal or tissue samples during the years 2002–2007. *MAP* was isolated from 12 (0.5%) out of 2 296 red deer (*Cervus elaphus*), two (0.2%) out of 835 roe deer (*Capreolus capreolus*), 78 (5.7%) out of 1 381 fallow deer (*Dama dama*), 28 (3.2%)out of 866 mouflons (*Ovis musimon*), four (2.5%) out of 162 chamois (*Rupicapra rupicapra*) and from one (0.1%) out of 805 wild boar (*Sus scrofa*). *MAP* was not cultured from 82 badgers (*Meles meles*), 55 martens (*Martes foina*), one pine marten (*Martes martes*), 25 brown hares (*Lepus europaeus*), five rabbits (*Oryctolagus cuniculus*), nine European polecats (*Mustela putorius*), two steppe polecats (*Mustela eversmannii*), two American minks (*Mustela vison*), four raccoon dogs (*Nyctereutes procyonoides*) and four Eurasian otters (*Lutra lutra*). *MAP* was isolated from three (2.0%) out of 149 small terrestrial mammals: one (5.9%) out of 17 brown rats (*Rattus norvegicus*), one (1.7%) out of 59 common voles (*Microtus arvalis*) and one (2.6%) out of 39 lesser white-toothed shrews (*Crocidura suaveolens*). Culture examinations of 34 house mice (*Mus musculus*) and 2 113 pigeons (*Columba livia* f. *domestica*) were negative. All 123 *in vitro* growing *MAP* isolates from wild ruminants were of IS*900* RFLP type B-C1. One mouflon infected with a *MAP* strain which did not grow on the tested media was after IS*1311*-PRA-PCR assessed as being infected with a "sheep" strain. The RFLP type of the *MAP* isolate from the wild boar was of the RFLP type A-C10. Although the detection of *MAP* in wildlife in the Czech Republic was not very high, their role as a potential risk factor for cattle should be considered.

Keywords: Johne's disease; epidemiology; cattle; non-ruminant species; IS*900* PCR

Domestic and wild ruminants are naturally susceptible to *Mycobacterium avium* subspecies *paratuberculosis* (*MAP*), which is the causative agent of a chronic granulomatous enteritis known as paratuberculosis or Johne's disease. *MAP* is highly resistant to extreme weather conditions, and can therefore survive for a long time in environments such as pastures or stables. The most usual route of *MAP* infection is faecal-oral, meaning that an environment with infected faeces may then serve as a potential source of infection for animals living in this area for a long time. *MAP* has been isolated from different free-living ruminant species, which grazed on pastures that were concurrently or previ-

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ously used for domestic ruminants. The wide range of such infected free-living ruminants includes red deer (*Cervus elaphus*; Riemann et al., 1979; Jessup et al., 1981; Nebbia et al., 2000; Pavlik et al., 2000a; Deutz et al., 2005), fallow deer (*Dama dama*; Marco et al., 2002; Deutz et al., 2005), mouflons (*Ovis musimon*; Mayer and Weiss, 1986; Deutz et al., 2005), roe deer (*Capreolus capreolus*; Pavlik et al., 2000a; Machackova et al., 2004; Deutz et al., 2005), chamois (*Rupicapra rupicapra*; Deutz et al., 2005), ibex (*Capra ibex*; Deutz et al., 2005) and others. As the numbers of infected ruminants has been increasing in Europe and worldwide, the host spectrum of this pathogen has also been extended.

The role of non-ruminants in the epidemiology of *MAP* is unclear. While non-ruminant species do not usually exhibit the classical clinical signs of paratuberculosis, *MAP* has been isolated from their tissues or faeces. Among non-ruminant wildlife, *MAP* has been detected in herbivores (Greig et al., 1999, Beard et al., 2001; Machackova et al., 2004; Deutz et al., 2005; Raizman et al., 2005), carnivores (Beard et al., 1999, 2001; Deutz et al., 2005), omnivores (Beard et al., 2001; Machackova et al., 2003; Alvarez et al., 2005; Kopecna et al., 2006) and even birds (Beard et al., 2001; Deutz et al., 2005). The infected wildlife has originated predominantly from localities with infected ruminants.

Invertebrates can also be a potential vector of *MAP* and can transmit it not only on their surface, but also through their digestive tracts. This was documented by the isolation of *MAP* from earthworms (*Lumbricus terrestris*; Fischer et al., 2003a) and the imagoes and larvae of different species of Diptera (Fischer et al., 2001; Machackova et al., 2004; Fischer et al., 2005, 2006). Potential passive transmission of *MAP* by invertebrates was also confirmed by the isolation of *MAP* from experimentally infected blowflies (Calliphoridae; Fischer et al., 2004), cockroaches (Blattidae; Fischer et al., 2003b) and syrphid flies (*Eristalis tenax*; Fischer et al., 2005). *MAP* isolates from invertebrates were of identical RFLP type B-C1 to the isolates from infected cattle or the environment on the farms or in the slaughterhouse, where the invertebrates were collected (Fischer et al., 2001, 2003a; Machackova et al., 2004; Fischer et al., 2005). These hosts can be the source of infection for different predators, especially for small terrestrial mammals. In the Czech Republic, on many infected ruminant farms it is difficult to avoid the transmission of *MAP* among cattle and free living ruminants (Pavlik et al., 2000a; Machackova et al., 2004).

The purpose of the present study was to assess the potential risk factor of wildlife as hosts or vectors of *MAP* on cattle farms or their surroundings in the Czech Republic between the years 2002 and 2007.

MATERIAL AND METHODS

Examined samples

Tissues and/or faecal samples were collected from wildlife in numerous localities across the whole country during the six years. Five thousand five hundred and forty wild ruminants, 1 143 non-ruminant animals of fifteen species and 2 113 pigeons were examined. In this study the 805 examined wild boar included 786 animals that were tested by us in previous work of Trcka et al. (2006) in the years 2002 to 2005 (Table 1). The wildlife originated directly from regions with confirmed outbreaks of paratuberculosis in domestic ruminants or at least from a locality or district with a history of paratuberculosis.

Mycobacterial isolation and identification

One gram of tissue (intestinal mucosa, mesenteric lymph nodes, mediastinal lymph nodes and liver) or faeces was homogenised by a stomacher (Lab Blender, Gehrden, Germany) and decontaminated in 0.75% HPC (Hexadecyl Pyridinium Chloride: N-cetylpyridinium chloride monohydrate, No. 102340 Merck, USA) for 72 hours (Pavlik et al., 2000b). The sediment (0.2 ml) of decontaminated samples was cultured on three slopes of different Herrold egg-yolk medium (HEYM) and incubated at 37°C for 3 to 12 months (Machackova et al., 2004). Mycobacterial isolates were identified by PCR assays according to Moravkova et al. (2008). Differentiation of *MAP* isolates was performed by the standardised IS*900* RFLP method using the restriction endonucleases *Pst*I and *BstE*II (Pavlik et al., 1999).

DNA isolation and IS*1311*-PRA-PCR

Acid fast rods were observed in the intestinal tissues of one mouflon but no growth ensued on media. Therefore, DNA from intestinal tissue was isolated by the DNeasy Blood & Tissue Kit (Qiagen, Germany) and the presence of *MAP* in

the tissue was confirmed by PCR assays according to Moravkova et al. (2008). Isolated DNA was also run on the IS*1311*-PRA-PCR assay (Marsh et al., 1999) which enables the differentiation of "cattle" and "sheep" strains.

RESULTS

MAP was detected in 124 (2.2%) of 5 540 wild ruminants (red deer, roe deer, fallow deer, mouflon and chamois) and from four (0.4%) out of 1 143 non-ruminants: in one (5.9%) out of 17 brown rats (*Rattus norvegicus*), one (1.7%) out of 59 common voles (*Microtus arvalis*), one (2.6%) out of 39 lesser white-toothed shrews (*Crocidura suaveolens*) and one (0.1%) out of 805 wild boar (*Sus scrofa*). In wild ruminants *MAP* was isolated from either faeces or tissue or from both. In four non-ruminants *MAP* was isolated only from liver tissue. The RFLP type of *MAP* isolates in wild ruminants was B-C1 (n = 124; Table 1).

All three infected small terrestrial mammals were captured or trapped on/near to a pasture or in a

Table 1. The isolation of *Mycobacterium avium* subsp. *paratuberculosis* from wildlife in the Czech Republic during the years 2002–2007

Host type	Wildlife species	Examined			RFLP profiles		
		No.	Pos.	%	No.	B-C1	A-C10
Ruminants	red deer (*Cervus elaphus*)	2 296	12	0.5	12	12	0
	roe deer (*Capreolus capreolus*)	835	2	0.2	2	2	0
	fallow deer (*Dama dama*)	1 381	78	5.7	78	78	0
	mouflon (*Ovis musimon*)	866	28	3.2	7	28	0
	chamois (*Rupicapra rupicapra*)	162	4	2.5	4	4	0
	Subtotal	5 540	124	2.2	123	123	0
Non-ruminants	wild boar (*Sus scrofa*)	805[a]	1[a]	0.1	1[a]	0	1[a]
	badgers (*Meles meles*)	82	0	0	0	0	0
	stone marten (*Martes foina*)	55	0	0	0	0	0
	pine marten (*Martes martes*)	1	0	0	0	0	0
	European polecat (*Mustela putorius)*	9	0	0	0	0	0
	steppe polecat (*Mustela eversmannii*)	2	0	0	0	0	0
	American mink (*Mustela vison*)	2	0	0	0	0	0
	raccoon dog (*Nyctereutes procyonoides*)	4	0	0	0	0	0
	brown hare (*Lepus europaeus*)	25	0	0	0	0	0
	rabbit (*Oryctolagus cuniculus*)	5	0	0	0	0	0
	Eurasian otter (*Lutra lutra*)	4	0	0	0	0	0
	small terrestrial mammals[b]	149	3	2.0	0	0	0
	Subtotal	1 143	4	0.4	1[a]	0	1[a]
Birds	pigeon (*Columba livia* f. *domestica*)	2 113	0	0	0	0	0
	Total	8 796	128	1.5	124	123	1

Pos. = culture positive for *Mycobacterium avium* subsp. *paratuberculosis*

[a]786 animals were examined during the period 2002–2005 as published previously (Trcka et al., 2006)

[b]*Mycobacterium avium* subsp. *paratuberculosis* was isolated from one (5.9%) out of 17 brown rats (*Rattus norvegicus*), one (1.7%) out of 59 common voles (*Microtus arvalis*) and one (2.6%) out of 39 lesser white-toothed shrews (*Crocidura suaveolans*). Culture examination of 34 house mice (*Mus musculus*) was negative

stable where an infected cattle herd was present. However, due to the very poor growth of these isolates it was not possible to apply RFLP analysis. The only wild boar infected with RFLP type A-C10 was shot in a district where *MAP* had been detected in wild ruminants (Table 1).

The IS*1311*-PRA-PCR assay applied to DNA from the tissue of one mouflon revealed that the mouflon was infected with a *MAP* "sheep" strain which did not grow *in vitro*.

DISCUSSION

The purpose of the present study was to assess the potential risk factor of wildlife as hosts or vectors of *MAP* on cattle farms or their surroundings in the Czech Republic from 2002 to 2007. In our study, *MAP* was isolated from 124 animals over a six year period; from the tissues and faeces of free-ranging red deer, roe deer, fallow deer, mouflons and chamois. *MAP* was also isolated from the tissues of three small terrestrial mammals and one wild boar (Table 1). The present epidemiological study adds to the so far published studies dealing with the detection of the causative agent of paratuberculosis in ruminant and non-ruminant wildlife (Greig et al., 1999; Beard et al., 2001; Deutz et al., 2005; Raizman et al., 2005; Kopecna et al., 2006).

Faecal contamination of the environment by *MAP* infected ruminants is extensive. It has been documented that clinically ill animals can shed over 10^8–10^{10} CFU/g of *MAP* in their faeces (Chiodini et al., 1984; Crossley et al., 2005). Furthermore, *MAP* is resistant to different physical conditions and can survive for a long time in the environment. Therefore, the most probable route of transmission of infection from cattle to wildlife is indirect contact, i.e., faecal-oral, contaminating pasture being the source of infection (Greig et al., 1999; Beard et al., 1999, 2001; Deutz et al., 2005; Raizman et al., 2005; Kopecna et al., 2006). Furthermore, the detection of *MAP* in wildlife ruminant faeces in our study points to possible transmission from wildlife ruminants to cattle. On the other hand, the detection of *MAP* in only the liver of small terrestrial mammals shows that these animals were the final hosts and did not shed *MAP* in their faeces. This finding did not mirror the results of other authors (Corn et al., 2005; Florou et al., 2007), who furthermore described *MAP* in the faeces of non-ruminant species such as mice, rats, hares, fox, feral cats and birds.

Due to farming conditions in the Czech Republic, small terrestrial mammals that may be found in stables the whole year round and can thus permanently contaminate livestock feed are likely to be a risk group for paratuberculosis transmission. Provided the domestic ruminants are kept in stables, they have to ingest the feed given to them. Accordingly, each of the animals ingests different amounts of contaminated feed (Daniels and Hutchings, 2001). If the infected livestock are kept on pastures, they become a source of *MAP* infection for wildlife. Similar results were obtained for wild ruminants by Riemann et al. (1979), Jessup et al. (1981) and Marco et al. (2002).

However *MAP* was also documented in nine birds (European starling, house sparrow, common snipe) trapped in cattle farms in Wisconsin and Georgia (Corn et al., 2005). We did not isolated any *MAP* from 2 113 pigeons (*Columba livia* f. *domestica*) trapped in localities with *MAP* infection in cattle. This result may be due to the fact that pigeons are known to be more resistant to mycobacterial infection than, e.g., the European starling or house sparrow (Hejlicek and Treml, 1995).

By RFLP analysis, it was discovered that all wild ruminants except one mouflon had the RFLP profile B-C1 (Table 1). This is the prevailing profile detected in cattle in the Czech Republic (Pavlik et al., 1999, 2000a) and was detected in cattle isolates in the studied localities (data not published in this article). These results indicate that *MAP* could probably be transmitted from cattle to wild ruminants or vice versa through the sharing of contaminated pasture. The RFLP type of *MAP* isolated from the wild boar was C10, which was identical with cattle isolates that originated from neighbouring districts (Trcka et al., 2006). However, *MAP* RFLP type B-C1 was also detected in wild ruminants in the same district where the wild boar was captured (data not shown). Accordingly, we assume that this wild boar was infected in the neighbouring districts.

Using direct PCR and IS*1311*-PRA-PCR analyses for one mouflon tissue positive for acid fast bacteria by microscopy, but for which no growth was observed on (HEYM) media, revealed that the mouflon was infected with a "sheep" strain of *MAP* (in some studies designated as group I/III). This is in agreement with the observation of Marsh and Whittington (2007) that sheep *MAP* strains produced colonies on modified 7H10 medium but not on (HEYM) medium. Sheep strains are slow growing, pigmented or not pigmented and have usually

been detected in sheep and goats, and occasionally in cattle in Australia, New Zealand and Spain (Marsh et al., 1999; de Juan et al., 2006). This is the first report of a mouflon harbouring a "sheep" type strain in the Czech Republic.

Generally, according to the number of the examined wildlife and the number of infected animals originating from localities with outbreaks of paratuberculosis, the impact of wildlife as a risk factor for the spread of infection does not appear to be very high in our country. The population size of different wildlife species should also be taken into account. In this regard, wild boar are an overpopulated species throughout Central Europe and might play a more significant role than for example hares, as their population is quite low in the Czech Republic. The population size of some small terrestrial mammal species varies significantly from year to year, according to their population dynamics in the last few decades (Elton, 1924; Krebs, 1996). Accordingly, during periods of overpopulation, the impact of these animals on the spread of infection might be more significant. However, this hypothesis could not be confirmed in our country, because *MAP* was only rarely isolated from these animals and because all rodents were captured in stables and not on pastures (Table 1).

CONCLUSION

The present study extended the knowledge of known hosts and vectors of *MAP*. This epidemiological study based on the RFLP analysis of *MAP* isolates confirmed that the RFLP type B-C1 is the most predominant RFLP type in the Czech Republic in free living ruminants and non-ruminants.

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REFERENCES

Alvarez J., de Juan L., Briones V., Romero B., Aranaz A., Fernandez-Garayzabal J.F., Mateos A. (2005): *Mycobacterium avium* subspecies *paratuberculosis* in fallow deer and wild boar in Spain. Veterinary Record, 156, 212–213.

Beard P.M., Henderson D., Daniels M.J., Pirie A., Buxton D., Greig A., Hutchings M.R., McKendrick I., Rhind S., Stevenson K., Sharp J.M. (1999): Evidence of paratuberculosis in fox (*Vulpes vulpes)* and stoat (*Mustela erminea*). Veterinary Record, 145, 612–613.

Beard P.M., Daniels M.J., Henderson D., Pirie A., Rudge K., Buxton D., Rhind S., Greig A., Hutchings M.R., McKendrick I., Stevenson K., Sharp J.M. (2001): Paratuberculosis infection of non-ruminant wildlife in Scotland. Journal of Clinical Microbiology, 39, 1517–1521.

Chiodini R.J., Van Kruiningen H.J., Merkal R.S. (1984): Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Veterinarian, 74, 218–262.

Corn J.L., Manning E.J., Sreevatsan S., Fischer J.R. (2005): Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging birds and mammals on livestock premises. Applied and Environmental Microbiology, 71, 6963–6967.

Crossley B.M., Zagmutt-Vergara F.J., Fyock T.L., Whitlock R.H., Gardner I.A. (2005): Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* by dairy cows. Veterinary Microbiology, 107, 257–263.

Daniels J., Hutchings M.R. (2001): The response of cattle and sheep to feed contaminated with rodent faeces and the implications for disease transmission. Veterinary Journal, 162, 211–218.

de Juan L., Alvarez J., Aranaz A., Rodriguez A., Romero B., Bezos J., Mateos A., Dominguez L. (2006): Molecular epidemiology of Types I/III strains of *Mycobacterium avium* subspecies *paratuberculosis* isolated from goats and cattle. Veterinary Microbiology, 115, 102–110.

Deutz A., Spergser J., Wagner P., Rosengarten R., Kofer J. (2005): *Mycobacterium avium* subps. *paratuberculosis* in wild animals and cattle in Styria/Austria (in German). Berliner und Munchener Tierarztliche Wochenschrift, 118, 314–320.

Elton C.S. (1924): Periodic fluctuations in the numbers of animals: their causes and effects. Journal of Experimental Biology, 2, 119–163.

Fischer O., Matlova L., Dvorska L., Svastova P., Bartl J., Melicharek I., Weston R.T., Pavlik I. (2001): Diptera as vectors of mycobacterial infections in cattle and pigs. Medical and Veterinary Entomology, 15, 208–211.

Fischer O.A., Matlova L., Bartl J., Dvorska L., Svastova P., duMaine R., Melicharek I., Bartos M., Pavlik I. (2003a): Earthworms (Oligochaeta, Lumbricidae) and mycobacteria. Veterinary Microbiology, 91, 325–338.

Fischer O.A., Matlova L., Dvorska L., Svastova P., Pavlik I. (2003b): Nymphs of the oriental cockroach, *Blatta orientalis* as passive vectors of causative agents of avian tuberculosis and paratuberculosis. Medical and Veterinary Entomology, 17, 145–150.

Fischer O.A., Matlova L., Dvorska L., Svastova P., Bartl J., Weston R.T., Pavlik I. (2004): Blowflies *Calliphora vicina* and *Lucilia sericata* as passive vectors of *Mycobacterium avium* subsp. *avium*, *M. a. paratuberculosis* and *M. a. hominissuis*. Medical and Veterinary Entomology, 18, 116–122.

Fischer O.A., Matlova L., Dvorska L., Svastova P., Bartos M., Weston R.T., Kopecna M., Trcka I., Pavlik I. (2005): Potential risk of *Mycobacterium avium* subspecies *paratuberculosis* spread by syrphid flies in infected cattle farms. Medical and Veterinary Entomology, 19, 360–366.

Fischer O.A., Matlova L., Dvorska L., Svastova P., Bartos M., Weston R.T., Pavlik I. (2006): Various stages in the life cycle of syrphid flies (*Eristalis tenax;* Diptera: Syrphidae) as potential mechanical vectors of pathogens causing mycobacterial infections in pig herds. Folia Microbiologica, 51, 147–153.

Florou M., Leontides L., Kostoulas P., Billinis C., Sofia M., Kyriazakis I., Lykotrafitis F. (2007): Isolation of *Mycobacterium avium* subspecies *paratuberculosis* from non-ruminant wildlife living in the sheds and on the pastures of Greek sheep and goats. Epidemiology and Infection, 136, 644–652.

Greig A., Stevenson K., Henderson D., Perez V., Hughes V., Pavlik I., Hines M.E., McKendrick I., Sharp J.M. (1999): Epidemiological study of paratuberculosis in wild rabbits in Scotland. Journal of Clinical Microbiology, 37, 1746–1751.

Hejlicek K., Treml F. (1995): Comparison of pathogenesis and epizootiology signification of avian mycobacteriosis in different sorts of domestic and free living synanthropic fowl (in Czech). Veterinarni Medicina, 40, 187–194.

Jessup D.A., Abbas B., Behymer D. (1981): Paratuberculosis in tule elk in California. Journal of the American Veterinary Medical Association, 179, 1252–1254.

Kopecna M., Ondrus S., Literak I., Klimes J., Horvathova A., Moravkova M., Bartos M., Trcka I., Pavlik I. (2006): Detection of *Mycobacterium avium* subsp. *paratuberculosis* in two brown bears in the Central European Carpathians. Journal of Wildlife Diseases, 42, 691–695.

Krebs C.J. (1996): Population cycles revisited. Journal of Mammalogy, 77, 8–24.

Machackova M., Matlova L., Lamka J., Smolik J., Melicharek I., Hanzlikova M., Docekal J., Cvetnic Z., Nagy G., Lipiec M., Ocepek M., Pavlik I. (2003): Wild boar (*Sus scrofa*) as a possible vector of mycobacterial infections: review of literature and critical analysis of data from Central Europe between 1983 to 2001. Veterinarni Medicina, 48, 51–65. http://www.vri.cz/docs/vetmed/48-3-51.pdf

Machackova M., Svastova P., Lamka J., Parmova I., Liska V., Smolik J., Fischer O.A., Pavlik I. (2004): Paratuberculosis in farmed and free-living wild ruminants in the Czech Republic (1999–2001). Veterinary Microbiology, 101, 225–234.

Marco I., Ruiz M., Juste R., Garrido J.M., Lavin S. (2002): Paratuberculosis in free-ranging fallow deer in Spain. Journal of Wildlife Diseases, 38, 629–232.

Marsh I.B., Whittington R.J. (2007): Genomic diversity in *Mycobacterium avium*: single nucleotide polymorphisms between the S and C strains of *M. avium* subsp. *paratuberculosis* and with *M. a. avium*. Molecular and Cellular Probes, 21, 66–75.

Marsh I., Whittington R., Cousins D. (1999): PCR-restriction endonuclease analysis for identification and strain typing of *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *avium* based on polymorphisms in IS*1311*. Molecular and Cellular Probes, 13, 115–126.

Moravkova M., Hlozek P., Beran V., Pavlik I., Preziuso S., Cuteri V., Bartos M. (2008): Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and heavily infected tissues. Research in Veterinary Science, 85, 257–264.

Mayer H., Weiss E.H. (1986): Paratuberculosis in one mufflon in Nordbaden (in German). Tierarztliche Umschau, 41, 112–117.

Nebbia P., Robino P., Ferroglio E., Rossi L., Meneguz G., Rosati S. (2000): Paratuberculosis in red deer (*Cervus elaphus hippelaphus*) in the Western Alps. Veterinary Research Communications, 24, 435–443.

Pavlik I., Horvathova A., Dvorska L., Bartl J., Svastova P., Dumaine R., Rychlik I. (1999): Standardisation of restriction fragment length polymorphism analysis for *Mycobacterium avium* subspecies *paratuberculosis*. Journal of Microbiological Methods, 38, 155–167.

Pavlik I., Bartl J., Dvorska L., Svastova P., Du Maine R., Machackova M., Ayele W.Y., Horvathova A. (2000a): Epidemiology of paratuberculosis in wild ruminants studied by restriction fragment length polymorphism in the Czech Republic during the period 1995–1998. Veterinary Microbiology, 77, 231–251.

Pavlik I., Matlova L., Bartl J., Svastova P., Dvorska L., Whitlock R. (2000b): Parallel faecal and organ *Mycobacterium avium* subsp. *paratuberculosis* culture of different productivity types of cattle. Veterinary Microbiology, 77, 309–324.

Raizman E.A., Wells S.J., Jordan P.A., DelGiudice G.D., Bey R.K. (2005): *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging deer and rabbits surrounding Minnesota dairy herds. Canadian Journal of Veterinary Research, 69, 32–38.

Riemann H., Zaman M.R., Ruppanner R., Aalund O., Jorgensen J.B., Worsaae H., Behymer D. (1979): Paratuberculosis in cattle and free-living exotic deer. Journal of the American Veterinary Medical Association, 174, 841–843.

Trcka I., Lamka J., Suchy R., Kopecna M., Beran V., Moravkova M., Horvathova A., Bartos M., Parmova I., Pavlik I. (2006): Mycobacterial infections in European wild boar (*Sus scrofa*) in the Czech Republic during the years 2002 to 2005. Veterinarni Medicina, 51, 320–332. http://www.vri.cz/docs/vetmed/51-5-320.pdf

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