Mycobacterial infections in European wild boar (*Sus scrofa*) in the Czech Republic during the years 2002 to 2005

I. Trcka[1], J. Lamka[1,2], R. Suchy[2], M. Kopecna[1], V. Beran[1], M. Moravkova[1], A. Horvathova[1], M. Bartos[1], I. Parmova[1,3], I. Pavlik[1]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]Faculty of Pharmacy, Charles University, Hradec Kralove, Czech Republic
[3]Veterinary Diagnostic Institute, Prague, Czech Republic

ABSTRACT: A total of 842 wild boar of differing ages, originating from 29 (37.7%) of the 77 districts in the Czech Republic, were examined during the hunting seasons from 2002 to 2005. Of them, 274 (32.5%) of the animals were wild specimens and 568 (67.5%) from game parks. Out of 786 animals, the following were included in the study: 668 piglets, 61 juveniles, 32 adult males and 25 adult females. A total of 2 704 samples from various tissues and faeces were examined: 309 separately collected faecal samples from 309 (36.7%) animals, 2 332 samples from various tissues and 63 faecal samples from 533 (63.3%) animals. Mycobacteria were isolated from 75 (8.9%) animals from 11 of the districts. Neither a causative agent of bovine tuberculosis, nor any other members of *Mycobacterium tuberculosis* complex were isolated from any of the animals. From one (0.1%) animal, *M. avium* subsp. *paratuberculosis* of IS*900* RFLP type A-C10 was isolated from intestinal lymph nodes, which was also isolated within the same district during other studies of cattle and free living ruminants. The causative agent of avian tuberculosis, *M. a. avium* (IS*901*+ and IS*1245*+), was isolated from 7 (0.8%) animals; among them tuberculous lesions were detected in intestinal lymph nodes, with gross tuberculous lesions visible on two animals. The causative agent of avian mycobacteriosis *M. a. hominissuis* (IS*901*– and IS*1245*+) was detected in lymph nodes without gross lesions in one (0.1%) animal. From 45 (5.5%) animals without lesions, atypical mycobacteria of the following nine species were isolated from pulmonary lymph nodes, small and large intestine, intestinal mucosa, and faeces: *M. fortuitum, M. chelonae, M. scrofulaceum, M. triviale, M. terrae, M. phlei, M. abscessus, M. flavescens,* and *M. smegmatis*. Due to a high density of wild boar and their large migration radius, they can be viewed as a potential source for mycobacterial infections as well as other infectious agents.

Keywords: wild animals; tuberculosis; zoonosis; epidemiology

Bovine tuberculosis is one of the diseases being controlled in the majority of countries with developed agriculture (Thoen and Steele, 1995; Pavlik et al., 2005a,b; Pavlik, 2006). Epizootiological situations developed complications in countries where the causative agent of bovine tuberculosis, affected wild animals before its eradication. Several animal species became a reservoir for the causative agent of bovine tuberculosis for both domestic and wild animals (De Lisle et al., 2001):

– possum (*Trichosurus vulpecula*) above all for cattle and farmed red deer in New Zealand;
– badger (*Meles meles*) particularly for cattle in Great Britain and Ireland;

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– african buffalo (*Syncerus caffer*) and lechwe antelope (*Kobus leche)* for the other wild ruminants including predators in Africa;
– bison (*Bison bison*) and white-tailed deer (*Odocoileus virginatus*) for other wild animals and cattle in North America (USA and Canada);
– water buffalo (*Bubalus bubalis*) for cattle in Australia.

In continental Europe, only one population of European bison in Poland was infected from cattle (*Bison bonasus*; Zorawski and Lipiec, 1997, 1998; Pavlik et al., 2002b, 2005b; Pavlik, 2006). Due to culling of more than 30 animals from the population, a further spread of infection among other animals, including cattle, was prevented (Welz et al., 2005). In other wild animals in Europe, namely red deer (*Cervus elaphus*), bovine tuberculosis was described in Spain (Vicente et al., 2006) and in Austria (Glawischnig et al., 2003).

Nowadays, European wild boar (*Sus scrofa*) appears to be a high risk as a potential reservoir of bovine tuberculosis in continental Europe. Circulation of obligatory pathogenic members of *Mycobacterium tuberculosis* complex *M. bovis* and *M. caprae* were found in wild boar populations in West and Central Europe (Mignone et al., 1991; Biolatti et al., 1992; Aranaz et al., 1996; Bachvarova et al., 1996; Serraino et al., 1999; Bollo et al., 2000; Parra et al., 2003; Aranaz et al., 2004; Erler et al., 2004; Gortazar et al., 2005).

Wild species of animals do not reach the status of maintenance host for *M. bovis* in all countries, in most cases they become infected when the challenge level is high. However, when infection is eliminated from the natural host (especially cattle); it also disappears from the reservoir animal species. Consequently, the risk that these reservoirs of infection pose for domestic animals and humans is quite variable depending on the specific epidemiological situation for the species and the environment (Morris et al., 1994).

Other health affecting mycobacterial species were also isolated from the wild boar, such as the causative agent of paratuberculosis: *M. avium* subsp. *paratuberculosis* (Machackova et al., 2003; Alvarez et al., 2005), avian tuberculosis: *M. avium* subsp. *avium* (Corner et al., 1981; Von Weber, 1982; Serraino et al., 1999; Machackova et al., 2003) and avian mycobacteriosis: *M. a. hominissuis* and *M. intracellulare* (Corner et al., 1981). *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. scrofulaceum*, *M. simiae*, *M. smegmatis*, *M. szulgai*, *M. terrae*, *M. vaccae*, *M. xenopi* and other atypical, or conditionally pathogenic mycobacteria (CPM), were isolated from wild boar as described in detail in a study dealing with mycobacterial infections in wild boar (Machackova et al., 2003).

However, the source of CPM in wild animals may be from the various constituents of external environment such as water (Horvathova et al., 1997; Matlova et al., 2003), kaolin (Matlova et al., 2003, 2004a; Trckova et al., 2004), peat (Matlova et al., 2003, 2005; Trckova et al., 2005), invertebrates (Fischer et al., 2001, 2003a,b, 2004a,b, 2005; Machackova et al., 2004), various constituents of wood (Matlova et al., 2003, 2004b), dust and other contaminated components found in the environment (Horvathova et al., 1997; Matlova et al., 1998; Kazda, 2000; Matlova et al., 2003).

Wild boar are viewed as a "spill-over-host", and a potential source of bovine tuberculosis in New Zealand (De Lisle et al., 2001). Under favourable conditions, the wild boar might become a reservoir of the causative agent of bovine tuberculosis in continental Europe in the near future. This was confirmed by the isolation of the causative agent of bovine tuberculosis from wild boar in some countries of Central Europe since 1990, such as Hungary, Slovakia, Croatia, and Bosnia and Herzegovina (Pavlik et al., 2002b, 2005b; Machackova et al., 2003; Pavlik, 2006). Continual increase in the population density of wild boar in Europe may also contribute to this situation (Kern et al., 1999; Sodeikat and Pohlmeyer, 2002, 2003; Fernandez-Llario et al., 2003). Since 1980 the population of wild boar has naturally increased in number and wild boar herds now colonise new locations where they did not live before (Goulding, 2003). The increase in wild boar populations in some countries causes losses of hundreds of thousands of USD for the damage to agricultural crops (Mazzoni-Della-Stela et al., 1995; Vassant, 1997).

This trend was indicated by the number of wild boar killed in the Czech Republic, which increased by 56% in comparison to the previous year, and reached 121 956 animals in 2004 (Zizka, 2005). From an epizootiological aspect, it is necessary to mention that the last outbreak of bovine tuberculosis in cattle in the Czech Republic was diagnosed in 1995 (Pavlik et al., 2002a, 2003), and in other animals in 2001 (Pavlik et al., 2005a; Pavlik, 2006). Accordingly, the Czech Republic was declared a bovine tuberculosis-free state on 31st March 2004 (Anonymous, 2004).

The causative agent of bovine tuberculosis was not detected in wild boar in the Czech Republic either during the last monitoring performed in 1986–2001, or in the previous period (Machackova et al., 2003). However, bovine tuberculosis was diagnosed in wild animals in neighbouring countries (Austria, Germany, Poland, and Slovakia) within the past two years (Zorawski and Lipiec, 1997, 1998; Machackova et al., 2003; Erler et al., 2004; Prodinger et al., 2005; Pavlik et al., 2005b, Pavlik, 2006). Due to this, the risk of the causative agent of bovine tuberculosis spreading still exists in the Czech Republic today.

The purpose of the present study was to investigate whether the Czech Republic remains free of the causative agent of bovine tuberculosis, according to measurements of the population of wild boar, and whether causative agents of other serious mycobacterial infections occur in these animals. Further motives of this study were to investigate the prevalence of tuberculous lesions in animals which are infected by mycobacterial species other than *M. tuberculosis* complex members, the distribution of mycobacteria in the organs, and prevalence of mycobacteria in different age categories of wild boar. All the information that will be acquired by this investigation should contribute to the assessment for the risk of spreading by various causative agents of mycobacterial infections, both in the population of wild boar and other wild or domestic animals.

MATERIAL AND METHODS

The monitored region of the Czech Republic

Monitoring of mycobacterial infections in wild boar was performed in the Czech Republic during the hunting seasons of 2002 to 2005. The examined animals originated from 30 hunts and five game parks situated in 29 (37.7%) of 77 districts of the Czech Republic (Figure 1).

Origin, sex, and age of wild boar

A total of 842 wild boar were examined (Table 1): 274 wild animals and 568 animals from game parks. Out of 786 animals, whose age and sex were determined, the following were included in the study: 668 piglets (under the age of one year), 61 juveniles (aged above one year), 32 adult males, and 25 adult females (aged above 3 years).

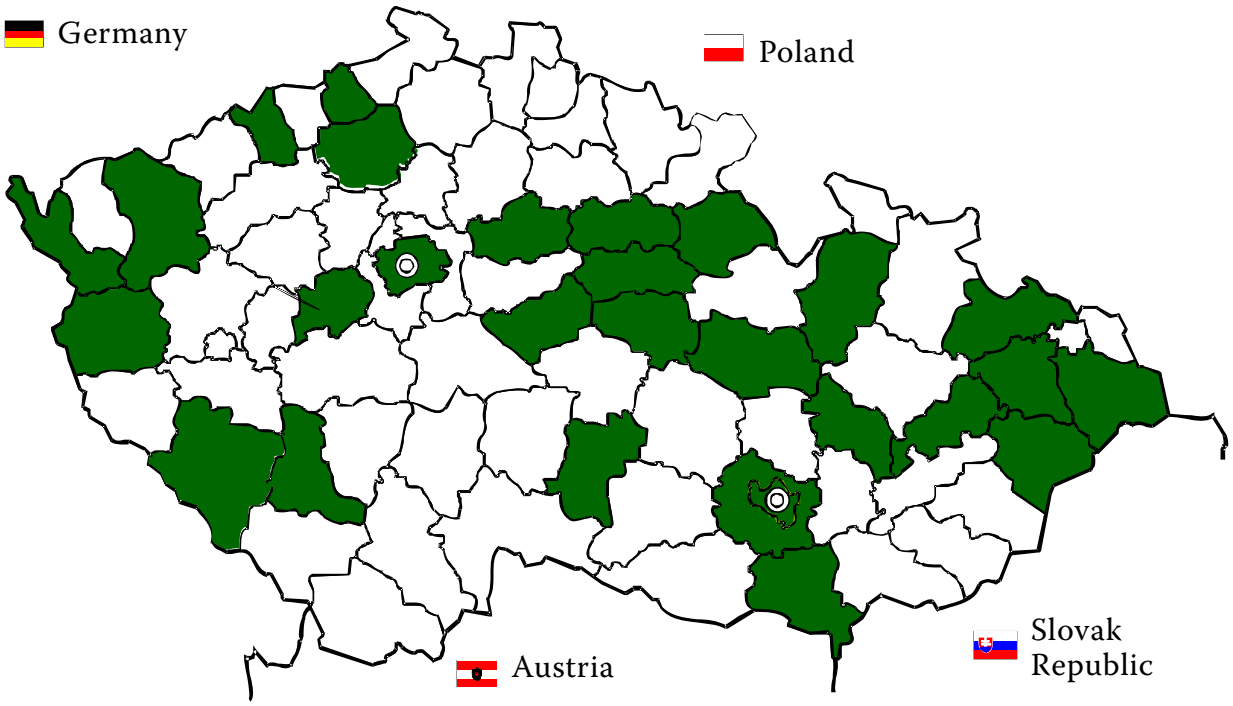

Figure 1. Districts of the Czech Republic where wild boar were examined

Table 1. Age structure, origin and types of collected samples from examined wild boar

Year	Piglets				Juveniles				Boar				Sows				Non-identified				Total			
	WN		GP		WN		GP		WN		GP		WN		GP		WN		GP		WN		GP	
	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F
2002	15	0	45	15	7	0	6	6	6	0	5	2	4	0	4	2	0	6	0	0	32	6	60	25
2003	21	0	24	124	2	0	5	0	4	0	9	0	2	0	8	0	8	0	7	0	37	0	53	124
2004	133	0	101	100	23	0	6	0	2	0	3	0	1	0	2	0	4	0	0	0	163	0	112	100
2005	30	0	37	23	0	0	6	0	0	0	1	0	0	0	2	0	0	6	0	25	30	6	46	48
Subtotal	199	0	207	262	32	0	23	6	12	0	18	2	7	0	16	2	12	12	7	25	262	12	271	297
Subtotal	199		469		32		29		12		20		7		18		24		32		274		568	
Total	668				61				32				25				56				842			
%	79.3				7.2				3.8				3.0				6.7				100			

WN – wild specimens; GP – game park; T – tissue samples ; F – faecal samples

Examined material

A total of 2 704 samples from various tissues and faeces were examined: 309 separately collected anonymous faecal samples were taken from 309 (36.7%) animals and 2 332 samples of various tissues and 63 faecal samples were collected from 533 (63.3%) animals. Anonymous faecal samples were collected in game parks in fresh condition.

Sample collection

Samples were collected during individual or collective hunts of wild animals, in autumn and winter during respective hunting seasons. Samples of internal organs, faeces or complete entrails of killed wild boar, which were subsequently processed in the laboratory, were collected and transferred into portable plastic bags. Due to the fact that hunters wanted their game animals to be largely undamaged, the head and body lymph nodes were only collected from animals with detected gross lesions. These samples were collected by official veterinarians during the meat inspection of the hunted wild boar. The other samples of biological material were collected by the authors of the present study, with the aid of the employees of game parks and members of hunt clubs.

Clinical examination

The clinical examination of animals, before and after the kill, was performed by hunters, game keepers and/or the laboratory staff. The examination was focused on the status of nutrition, changed behaviour or other clinical signs such as cough, diarrhoea and lameness.

Gross examination

The first gross examination was performed immediately after the killing of each animal. It consisted of an external observation (internal organs were roughly inspected during embowelling). A more detailed gross examination was performed during the collection of tissues in the laboratory.

Laboratory examination for mycobacteria

Microscopic examination. Imprint preparations were made from tissue samples delivered to

the laboratory and frozen at –20°C, or delivered fresh to the laboratory immediately after the killing of the animal, and examined. After fixation by a flame, they were stained by the Ziehl-Neelsen (ZN) method, for the detection of acid-fast rods (AFR). At least 100 fields of view were examined of each sample (Kubin et al., 1986).

Culture examination. Tissue samples from internal organs and faeces were processed by a decontamination method with NaOH and HCl (Fischer et al., 2000). Two tubes of Herrold Egg Yolk Media (HEYM) without antibiotics and Mycobactin J (stimulator for the isolation of causal agent of paratuberculosis) for the detection of all *M. avium* complex members and atypical mycobacteria were used. A third culture medium used was HEYM with Mycobactin J for the detection of Mycobactin-dependent species of *M. a. paratuberculosis* and *M. a. silvaticum.* For the isolation of slow growing strains of *M. a. paratuberculosis,* occurring in sheep (*Ovis aries*) and some wild ruminants such as mouflons (*Ovis musimon*) or fallow deer (*Dama dama*), the time of incubation at 37°C was prolonged from 4 to 12 months.

Identification of mycobacterial isolates. All the AFR-positive isolates were identified by the PCR method for the detection of specific fragments: IS*900* for *M. a. paratuberculosis*, IS*901* for *M. a. avium*, and IS*1245* for *M. a. hominissuis*. Detection of the specific fragment IS*6110* for *M. tuberculosis* complex members (Bartos et al., 2006) and biochemical tests (Wayne and Kubica, 1986) were used for the identification of *M. bovis*. *M. a. paratuberculosis* isolates were subcultured for the assessment of Mycobactin-dependence on three HEYM with Mycobactin J and on one HEYM without Mycobactin J. A standardized IS*900* RFLP method was used for detailed identification of one *M. a. paratuberculosis* isolate (Pavlik et al., 1999).

Statistical assessment

The Chi^2-test (Stat Plus) was applied for the statistical evaluation (Matouskova et al., 1992).

RESULTS

Clinical, gross, and Z-N microscopic examinations of imprint preparations

No changes in behaviour or clinical signs of any diseases were observed in any of the examined 842 wild boar. Tuberculous lesions were detected by gross examination in only 2 (0.2%) animals (piglets) in their mesenteric lymph nodes. These piglets originated from a game park where the causative agent of avian tuberculosis *M. a. avium* was later isolated from wild boar. AFR were observed by microscopy after Z-N staining in only five animals and mycobacteria were detected by culture from all animals.

Culture examination

Mycobacteria in tissues and in faeces of dissected animals from the wild and from game parks. Mycobacteria were isolated from 75 (8.9%) of 842 animals by culture examination of samples; detection of mycobacteria ranged between 4.1%

Table 2. Prevalence of mycobacterial isolation from examined tissue samples and faeces of wild boar

Year	Examined animals			Examined tissues[1]			Examined faeces only[2]		
	No.	positive	%	No.	positive	%	No.	positive	%
2002	123	5 (0)	4.1 (0)	371	7 (0)	1.9 (0)	31	0	0
2003	214	26 (4)	12.2 (1.9)	350	18 (5)	5.1 (1.4)	124	13	10.5
2004	375	26 (3)	6.9 (0.8)	1 226	29 (4)	2.4 (0.3)	100	3	3.0
2005	130	18 (0)	13.9 (0)	385	17 (0)	4.4 (0)	54	6	11.1
Total	842	75 (7)	8.9 (0.8)	2 395	71 (9)	3.0 (0.4)	309	22	7.1

( ) – animals infected with *Mycobacterium avium* subsp. *avium*

[1]during post mortem, 2 332 tissue samples and 63 faecal samples were collected from wild boar for laboratory examination

[2]separately anonymously collected faecal samples from wild and game park specimens

and 13.9% in respective years. Mycobacteria were isolated from internal organs of 12 (1.4%) animals and from both internal organs and faeces of 2 (0.2%) animals (Table 2). Mycobacteria were detected by culture in 93 (3.4%) of a total of 2 704 samples (2 395 tissue and 309 faecal samples) that were cultured during monitoring.

Seventy one (3.0%) isolates originated from 2 395 samples collected during post mortem of 533 animals; the detection rate in years ranged between 1.9% and 5.1%. A total of 22 (7.1%) mycobacterial isolates originated from 309 individually anonymously collected faeces of animals from game parks where positivity in years ranged between 0 and 11.1%. The detection of mycobacteria in faeces was statistically higher ($P < 0.01$) in comparison with tissue samples (Table 2).

Species identification of mycobacterial isolates. No member from the *M. tuberculosis* complex was isolated from any of the 842 wild boar. *M. a. avium* was isolated from 7 (0.8%) animals between 2003 and 2004: from 4 (1.9%) and 3 (0.8%) animals in 2003 and 2004, respectively (Tables 2 and 3). Three piglets infected with *M. a. avium* originated from a game park and 4 piglets originated from two different localities in the wild. *M. a. hominissuis* was isolated from 1 (0.1%) animal and *M. a. paratuberculosis* of RFLP type A-C10 was isolated from 1 (0.1%) animal. Various species of CPM were isolated from 17 (2.0%) animals during all the monitored years. Identification was unsuccessful in 65 isolates, in which IS*6110* (specific for all members of *M. tuberculosis* complex members), IS*900* (specific for *M. a. paratuberculosis*), IS*901* (specific for *M. a. avium*), and IS*1245* (specific for *M. a. hominissuis*) were not detected by PCR (Table 3).

Distribution of mycobacteria in tissues. By gross examination of a total of 2 332 samples, tuberculous lesions were detected in only 2 (0.1%) samples of jejunal lymph nodes. AFR were detected by Z-N staining in only 7 (0.3%) samples of various 6 tissues. Mycobacteria were detected by culture in a total of 68 (3.0%) tissue samples; most frequently isolated from hepatic lymph nodes (7; 8.2%). The causative agent of avian tuberculosis *M. a. avium* was detected in 5 (0.2%) jejunal lymph nodes, 3 (0.1%) ileocaecal lymph nodes, and 1 (0.04%)

Table 3. Results of mycobacterial identification in 93 isolates originating from wild boar

Mycobacterium species	Years				Total	
	2002	2003	2004	2005	No.	%
M. a. avium	0	5	4	0	9	9.7
M. a. hominissuis	0	1	0	0	1	1.1
M. a. paratuberculosis	0	1	0	0	1	1.1
M. scrofulaceum	0	1	0	0	1	1.1
M. fortuitum	1	6	1	0	8	8.6
M. phlei	0	2	0	0	2	2.2
M. abscessus	1	0	0	0	1	1.1
M. flavescens	1	0	0	0	1	1.1
M. triviale	0	1	0	0	1	1.1
M. chelonae	0	1	0	0	1	1.1
M. terrae	0	1	0	0	1	1.1
M. smegmatis	0	0	1	0	1	1.1
Mycobacterium sp.	4	12	26	23	65	69.9
Total	7	31	32	23	93	100
%	7.5	33.3	34.4	24.7	100	

Mycobacterium sp. – mycobacterial isolates were negative for IS*6110*, IS*900*, IS*901*, and IS*1245* PCR and were not biochemically identified

Table 4. Mycobacterial distribution in different examined tissues

Examined		GE		ZN		Culture		*M. avium* species			Atypical mycobacteria	*Mycobacterium* sp.
Samples	No.	positive	%	positive	%	positive	%	MAA	MAH	*MAP*		
Lung ln.	261	0	0	1	0.4	11	4.2	0	0	0	2	9
Hepatic ln.	85	0	0	1	1.2	7	8.2	0	0	0	0	7
Jejunal ln.	473	2	0.4	2	0.4	17	3.6	5	1	1	2	8
Ileocaecal ln.	440	0	0	1	0.2	11	2.5	3	0	0	0	8
Coloneal ln.	417	0	0	1	0.2	9	2.2	1	0	0	2	6
Jejunal m.	153	0	0	0	0	4	2.6	0	0	0	1	3
Ileocaecal m.	436	0	0	0	0	6	1.4	0	0	0	0	6
Other tissues*	67	0	0	1	1.5	3	4.5	0	0	0	1	2
Total No.	2 332	2	0.1	7	0.3	68	2.9	9	1	1	8	49

ZN – Ziehl-Neelsen staining; GE Gross examination; *MAA – Mycobacterium avium* subsp. *avium*; *MAH – Mycobacterium avium* subsp. *hominissuis*; *MAP Mycobacterium avium* subsp. *paratuberculosis*
Mycobacterium sp. – mycobacterial isolates negative for IS*901* and IS*1245* PCR and biochemically non-identified
*Other tissues: liver, lungs, spleen, kidney, lymph nodes (submandibular, inguinal, gastric), and muscle
ln – lymph node; m – mucous

coloneal lymph node. *M. a. paratuberculosis* was isolated from 1 (0.04%) jejunal lymph node, as was 1 (0.04%) *M. a. hominissuis*. No statistically significant differences were detected between isolation rates of mycobacteria from mesenteric and pulmonary lymph nodes (Table 4).

Detection of mycobacteria from different age categories of wild boar. Mycobacteria were isolated from 58 (8.6%) of 668 piglets, from 3 (4.9%) of 61 juveniles, from 5 (15.6%) of 32 adult males and from 3 (12.0%) of 25 adult females. Mycobacteria were detected statistically significantly more frequently ($P < 0.01$) in older animals (adult males and females), than in juveniles (Table 5).

DISCUSSION

The decontaminating method with NaOH-HCl described previously (Fischer et al., 2000) was used in the present study, because it does not cause damage to the majority of conditionally pathogenic mycobacterial species.

The head lymph nodes were not collected due to the limited knowledge of hunters, who taken the samples. The present study followed up the previous monitoring performed in 1986–2001 (Machackova et al., 2003), during which period no *M. tuberculosis* complex members were isolated. This result corresponds to a favourable epidemiological situation in the Czech Republic that has been free of bovine tuberculosis since 2004 (Anonymous, 2004). The occurrence of other obligatory pathogenic mycobacteria (*M. a. avium* and *M. a. paratuberculosis*) in this study, and previous monitoring was the same.

Among other obligatory pathogenic mycobacteria, the causative agent of avian tuberculosis *M. a. avium* was isolated from seven piglets (Table 2). The source of mycobacterial infection for wild boar may be shot or dead animals and birds, originating from the wild, or from captivity (Pavlik et al., 2000b; Matlova et al., 2003). Due to the fact that wild boar are successful predators of small terrestrial mammals (unpublished observations), rodents and insectivores may also be a source of mycobacterial infection; various mycobacterial species including the causative agent of avian tuberculosis have previously been isolated from them (Fischer et al., 2000).

Analysis of all anamnestic data revealed that three piglets infected with *M. a. avium* originating from a game park, were in a close contact with a flock of domestic fowl (*Gallus domesticus*) that were in-

Table 5. Findings of mycobacterial isolations in studied age groups of 786 wild boar

Year	Piglets				Juveniles				Boars				Sows				Total No.			
	tissue		faeces		tissue		faeces		tissue		faeces		tissue		faeces		tissue		faeces	
	No.	+	No.	+	No.	+	No.	+	No.	+	No.	+	No.	+	No.	+	No.	+	No.	+
2002	60	3	15	0	13	1	6	0	11	1	2	0	8	0	2	0	92	5	25	0
2003	45	9	124	12	7	2	0	0	13	2	0	0	10	3	0	0	75	16	124	12
2004	234	18	100	4	29	0	0	0	5	2	0	0	3	0	0	0	271	20	100	4
2005	67	12	23	0	6	0	0	0	1	0	0	0	2	0	0	0	76	12	23	0
Subtotal	406	42	262	16	55	3	6	0	30	5	2	0	23	3	2	0	514	53	272	16
%		10.3		6.1		5.5		0		16.7		0		13.0		0		100		100
Total/positive		668/58				61/3				32/5				25/3				786/69		
%		8.6				4.9				15.6				12.0				8.8		

+ – number of positive animals

fected with avian tuberculosis (unpublished data). The game park owner reported seeing piglets hunting fowl in the game park and these were able to catch and eat the most affected birds (Baumgartner, 2005). The remaining four infected wild boar originated from the wild. The likely sources of infection were carcasses, or faeces of animals with avian tuberculosis (Corner et al., 1981; Von Weber, 1982; Serraino et al., 1999; Fischer et al., 2000; Machackova et al., 2003). The causative agent of avian tuberculosis was isolated from all infected piglets from mesenteric (jejunal, ileocaecal and coloneal) lymph nodes (Table 4), which confirms an oral route of infection.

M. a. paratuberculosis (causative agent of paratuberculosis) was isolated from the jejunal lymph node of a wild boar (Table 4), which originated from a region with an occurrence of paratuberculosis in grazing cattle. The isolate from this wild boar was of identical RFLP type A-C10 to that of the infected cattle (Pavlik et al., 1995, 2000a). With respect to the localisation of the infection (jejunal lymph node; Table 4) we assume that a contaminated external environment (such as field dunghill, placentas of calved cows, and pastures), where the pigs often dug, were the source. Another source of infection might have been the small terrestrial mammals that are usually caught by wild pigs. This route of transmission was also considered in Spain (Alvarez et al., 2005).

M. a. hominissuis was isolated from a jejunal lymph node (Table 4) from only one wild boar. *M. a. hominissuis* causes great economic losses in herds of domestic pigs; tuberculous lesions are usually found in their mesenteric lymph nodes (Pavlik et al., 2003, 2005a; Matlova et al., 2004b, 2005). Non-comparable findings between the prevalence of *M. a. hominissuis* may be caused by a higher resistance of wild boar to this infection in contrast to domestic pigs. This resistance in wild boar has been described (Acevedo-Whitehouse et al., 2005).

Tuberculous lesions detected by gross examination in previous studies were found in 33% of wild boar in New Zealand (Wakelin and Churchman, 1991) and in 47.7% of wild boar in Australia (Corner et al., 1981). In contrast, during the monitoring performed in the present study, tuberculous lesions were detected in mesenteric lymph nodes from only 2 (0.2%) animals (piglets; Table 4). The low number of tuberculous lesions in this study could be the impact of a low number of collected submadibular and retropharyngeal lymph nodes from which mycobacteria were often isolated (Gortazar et al., 2003).

Mycobacteria were detected more frequently in older animals (adult males and females) than in juveniles. This fact corresponded with other studies (Corner et al., 1981; Wakelin and Churchman, 1991). The frequent occurrence of mycobacteria presence in piglets from this study was previously described in a paper published by Vicente et al. (2006). The conclusion of this is that wild boar is exposed to mycobacterial infection during the initial months of their lives.

During investigation of the respective mycobacterial isolates obtained during the monitored period, mycobacteria were isolated from organs and lymph nodes of the respiratory tract, from organs and lymph nodes of the gastrointestinal tract, and from faeces. It follows that boar may be passive vectors of mycobacteria, or are infected perhaps only occasionally, and generally do not usually produce any gross lesions (Table 4).

Isolation of mycobacteria from organs and lymph nodes of the gastrointestinal tract indicates that the oral route of infection for wild boar is possible after feed ingestion; this was indicated by the isolation of *M. a. avium* and *M. a. paratuberculosis* in the present study (Table 4). The possibility of spreading the infection via respiratory tract (such as digging the ground, ingestion of carcasses etc.) has been confirmed by the isolation of atypical mycobacteria from lymph nodes in the present study and detection of the causative agent of paratuberculosis from a lymph node from one wild boar during a previous monitoring period in the Czech Republic (Machackova et al., 2003). The potential concurrent spread of infection both *per os* and *intra nasam* has been confirmed by the isolation of mycobacteria from three wild boar; mycobacteria were concurrently isolated from their pulmonary and mesenteric lymph nodes (Table 4). No statistically significant differences were detected between the isolation rates of mycobacteria from organs and lymph nodes of the respiratory tract and from organs and lymph nodes of the gastrointestinal tract.

No statistically significant differences were detected between the isolation rates of mycobacteria from piglets, adult wild boar and sows (Table 5). Statistically significant differences ($P < 0.01$) were detected between the isolation rates of mycobacteria from juveniles and adult males, as well as between the isolation rates of mycobacteria from juveniles and adult females ($P < 0.05$). Increased isolation rates of mycobacteria from adult wild boar might have been caused by a repeated infection during the course of the animals' life.

Due to a large area of migration, which ranges between 140 and 424 ha for adult females and up to 700 ha for adult males (Mauget, 1981), these animals pose a threat of spreading various infections. This potential migration of wild boar over great distances increases the above-mentioned risk. Cases of migration of both individual wild boar and entire herds to a distance of up to 200 km, have been described in Poland (Wolf, 1995).

CONCLUSIONS

1. The causative agent of bovine tuberculosis was not detected in the bovine tuberculosis-free Czech Republic during the monitoring period between 2002 and 2005.
2. The causative agent of avian tuberculosis and the causative agent of paratuberculosis were occasionally isolated.
3. Among other CPM, slow growing species were also detected.
4. Mycobacteria were isolated both from tissues (particularly from lymph nodes) and faeces of wild boar, which represent a risk of the spread of mycobacteria to the external environment.
5. With regard to the high density of wild boar and their large area of migration, wild boar may spread mycobacteria to great distances.

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Corresponding Author:

Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 601, fax +420 541 211 229, e-mail: pavlik@vri.cz