

Mycobacteria in wild boar (*Sus scrofa*) in the Czech Republic

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ABSTRACT

Bovine tuberculosis in wild boars (*Sus scrofa*) was detected in Central Europe during 1983-2001 in Croatia, Hungary and Slovakia. Accordingly, the purpose of the present study was investigation of mycobacterial infections in our country during 2002-2004 in a total of 2319 samples originating from 766 wild boars of different age categories from 29 (39.2%) of 74 districts of the Czech Republic. Individually collected anonymous faecal samples from 309 animals and 1 947 samples of various tissues from 457 animals were examined. Wild boars originated from both free nature (239 animals), and game parks (527 animals). Fifty boars and sows and 388 piglets and yearlings at the age of 1 to 2 years were included among 438 (57.1%) animals with known age and sex. Small tuberculous lesions in the intestinal lymph nodes were detected in 2 (0.3%) wild boars only. Causative agents of tuberculosis or paratuberculosis were not isolated from any of the animals. Mycobacteria were isolated from 64 (8.3%) wild boars. *Mycobacterium avium* subsp. *avium*, the causative agent of avian tuberculosis was isolated from intestinal lymph nodes of 7 (0.9%) wild boars (tuberculous lesions were detected in two animals). Thirty four infected wild boars originated from free nature and from a game park with occurrence of avian tuberculosis in domestic fowl, respectively. Atypical mycobacteria of 8 species (*M. fortuitum*, *M. chelonae*, *M. scrofulaceum*, *M. triviale*, *M. terrae*, *M. phlei*, *M. smegmatis*, and *M. flavescens*) were isolated from 57 (7.4%) animals. Atypical mycobacteria were isolated from pulmonary lymph nodes, small and large intestines, intestinal mucosa and faeces of wild boars both from free nature and game parks. Due to the high density of wild boars and their large migration radius, they can be viewed as a potential source of a number of infections including the causative agent of avian tuberculosis.

Key words: wild boar, bovine tuberculosis, Czech Republic

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Introduction

During evaluation of occurrence of mycobacterial infections in wild boars (*Sus scrofa*) in Central Europe from 1983 to 2001 the causative agent of bovine tuberculosis was detected in Croatia, Hungary and Slovakia. Overall analysis of the available results obtained by examination of 431 wild boars showed that tuberculous lesions in parenchymatous organs of wild boars were found in 43 (10.0%) animals. *M. bovis*, *M. avium* subsp. *avium*, *M. a. paratuberculosis* and atypical mycobacteria were detected in 22 (5.1%) 2 (0.4%), 1 (0.2%) and 27 (6.3%) animals, respectively (MACHACKOVA et al., 2003).

These findings and the current population growth of wild boars led us to further investigation of mycobacterial infections in our country between 2002 and 2003.

Materials and methods

During 2002-2004 a total of 2 265 samples from 712 wild boars of different age categories originating from 29 former districts of the Czech Republic were examined. Individually collected "anonymous" faecal samples from 309 animals and 1 947 samples of various tissues from 457 animals were examined by culture. Wild boars originated from both free nature (239 animals), and game parks (527 animals). Among 438 animals with known age and sex 50 boars and sows and 388 piglets and yearlings were included. Material for examination was collected by the employees of the Forests of the Czech Republic, Forests of the City of Brno and volunteers from hunting associations. The following methods were used for the diagnosis of mycobacterial infections:

Clinical examination of animals. Clinical inspection was performed by the hunters before shooting animals, gamekeepers and employees of the Veterinary Research Institute. The examination was particularly focused on the state of nutrition, changes in behaviour and other clinical signs.

Gross and microscopic examinations. Gross examination was performed in the field where the shot wild boars were emboweled, or during further processing of samples in our laboratory. Examinations of respiratory and gastrointestinal tracts and their adjacent lymph nodes were considered as most important. Imprint samples of tissues of the examined organs were prepared and stained by the Ziehl-Neelsen technique (DRAZAN et al., 1962). At least 100 fields of each sample were viewed. The finding was assessed according to the number of acid-fast rods (AFR) in one field of view (KUBIN et al., 1986).

Culture examination. Before examination, the samples from wild boars were processed by the decontamination method with NaOH and HCl (FISCHER et al., 2000). This method was used because of its mild effect on all mycobacterial species. Two types

of Herrold agar-yolk culture media without antibiotic and mycobactin J were used for the detection of atypical mycobacteria and members of *M. avium* complex. The third culture medium was Herrold agar with mycobactin J for the detection of *M. a. paratuberculosis*. The samples were cultured at 37°C for five months.

Polymerase Chain Reaction (PCR). All ART positive isolates were identified by the PCR method for the detection of specific fragment IS900 (KUNZE et al., 1992) for *M. a. avium*, IS901 for *M. a. paratuberculosis* and IS1245 for *M. a. hominissuis* (PAVLIK et al., 2000). The detection of specific fragment IS6110 for *M. tuberculosis* complex members was used for identification of *M. bovis* (DVORSKA et al., 2001).

Biochemical methods. Atypical mycobacteria were identified on the basis of the results of biochemical tests (WAYNE and KUBICA, 1986).

Results

Mycobacteria were isolated from 64 (8.3%) of 766 animals (Table 1). *M. bovis* and *M. a. paratuberculosis* were not isolated from any animal. *M. a. avium* and “atypical” mycobacteria were isolated from 7 and 57 wild boars, respectively (Table 2).

Table 1. Mycobacteria in wild boars in the Czech Republic (2002-2004)

Year	Number of animals	Number of organs	Number of faecal samples	Number of isolates
2002	92	371	33	7
2003	90	350	141	23
2004	275	1226	135	34
Total	457	1947	372	64

Table 2. Mycobacterial species isolated from wild boars (2002-2004)

Year	Number of animals	Number of mycobacterial isolates (%)			
		<i>M. bovis</i>	<i>M. a. paratuberculosis</i>	<i>M. a. avium</i>	Atyp. mycob.
2002	92	0	0	0	7
2003	90	0	0	3	20
2004	275	0	0	4	30
Total	457	0 (0%)	0 (0%)	7 (0.9%)	57 (7.4%)

The isolates of *M. a. avium* originated from mesenterial lymph nodes, the other mycobacteria were found in the intestinal, pulmonary and hepatic lymph nodes and intestinal mucosa. Mycobacteria were also detected in faeces and some other tissues (Table 3).

Table 3. Distribution of mycobacteria in cultured tissues

	Lymph nodes			Intestinal mucosa	Other tissues	Faeces	Total
	pulmonary	hepatic	intestinal				
Culture	210	70	1088	512	67	372	2319
Isolates	7	4	25	7	3	18	64
%	0.3%	0.6%	2.3%	1.4%	4.5%	4.8%	2.8%

Discussion

Wild boar (*Sus scrofa*) is an omnivorous species. They feed on vegetables, and also on small vertebrates, young hares, pheasants and roe deer, and dead bodies of free living and wild mammals that may be infected with causative agents of serious mycobacterial and other diseases. This fact has been confirmed by isolation of the causative agent of avian tuberculosis from mesenterial lymph nodes. Another route of transmission of mycobacteria into organisms of wild animals is inhalation by animals seeking feed; this was confirmed by the detection of mycobacteria in pulmonary lymph nodes (Table 3).

Wild boars represent a serious epizootiological problem due to their feed composition, large migration radius and current population growth (annual cull is approximately 80000 head). Based on the results of the examination of wild boars for mycobacteria in the Czech Republic during 2002-2004 (Tables 1, 2, 3) and other Central European countries in the previous period (MACHACKOVA et al., 2003) it is evident that wild boars can be at least vectors of causative agents of mycobacterial infections. At present, after joining the EU, rearing of ruminants and domestic pigs on pastures has been more and more used, and a considerable hazard of transmission of mycobacterial infections by wild boars exists.

Conclusions

During three-year monitoring in the Czech Republic, causative agents of bovine tuberculosis and paratuberculosis were not detected. The isolation of the causative agent of avian tuberculosis from various tissues of 7 (0.9%) wild boars indicated that they might be sources of *M. a. avium* for other wild animals or animals kept on pastures.

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SAŽETAK

Tuberkuloza divljih svinja uzrokovana bakterijom *Mycobacterium bovis* utvrđena je na području središnje Europe (Hrvatska, Mađarska i Slovačka) u razdoblju od 1983. do 2001. godine. Slično navedenim istraživanjima, svrha predmetnog rada bilo je određivanje prisutnosti mikobakterioza u Češkoj u razdoblju od 2002. do 2004. godine na ukupno 2319 uzoraka podrijetlom od divljih svinja različite dobi. Uzorci su potjecali s 24 (39,2 %) od ukupno 74 lokaliteta u Češkoj. Pregledano je 309 pojedinačno prikupljenih uzoraka izmeta te 1947 uzoraka različitih tkiva podrijetlom od 457 životinja. Od toga je 239 svinja potjecalo iz slobodne prirode, a 527 iz ograđenih uzgajališta. U istraživanje je bilo uključeno 438 (57,1%) životinja poznate dobi i spola, odnosno 50 odraslih svinja i 388 prasadi i nazimadi. Tuberkulozna žarišta slabije jačine uočena su na mezenterijalnim limfnim čvorovima samo dviju svinja (0,3%). Uzročnici tuberkuloze goveda ili paratuberkuloze nisu izdvojeni iz prikupljenih uzoraka. Preostale mikobakterije izolirane su iz 64 (8,3%) svinje, pri čemu je bakterija *Mycobacterium avium* subsp. *avium*, uzročnik avijarne tuberkuloze, izolirana iz mezenterijalnih limfnih čvorova 7 (0,9%) svinja (tuberkulozne lezije su utvrđene u samo dvije životinje). Trideset i četiri zaražene divlje svinje potjecale su iz slobodne prirode ili ograđenih uzgajališta s područja na kojima je utvrđena avijarna tuberkuloza domaće peradi. Iz uzoraka medijastinalnih limfnih čvorova, tankoga i debeloga crijeva, crijevne sluznice i izmeta 57 (7,4%) životinja izolirane su ove bakterije: *M. fortuitum*, *M. chelonae*, *M. scrofulaceum*, *M. triviale*, *M. terrae*, *M. phlei*, *M. smegmatis*, i *M. flavescens*. Zbog visoke gustoće populacije divljih svinja i njihove sklonosti većim migracijama, iste se moraju promatrati i kao možebitni izvor brojnih bolesti, uključujući i avijarnu tuberkulozu.

Ključne riječi: divlja svinja, tuberkuloza goveda, Češka
