Isolation of conditionally pathogenic mycobacteria from the environment of one pig farm and the effectiveness of preventive measures between 1997 and 2003

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ABSTRACT: Between 1997 and 2003, in one herd of breeding pigs with 90 sows and two boars, positive and dubious responses to avian tuberculin were detected in 4 and 72 pigs, respectively. Pigs were examined using the agglutination test for the presence of serum antibodies against corpuscular antigens prepared from various *Mycobacterium avium* complex (*MAC*) members: *M. a. avium* (*MAA*) of serotype 2, *M. a. hominissuis* (*MAH*) of serotype 8 and *M. intracellulare* (*MI*) of serotype 19. Positive skin responses were found in animals with antibodies against *MAH* (18; 23.7%), *MAA* (3; 4.0%) and *MI* (9; 11.8%) antigens. By serological examination of 17 sows with repeated dubious responses for tuberculin skin testing with avian tuberculin, no antibodies against *MAA* were detected; *MAH* antibodies and *MI* antibodies were found in eight and two animals, respectively. By *post mortem* examination of lymph nodes (ln) and organ samples from all 76 animals with responses to avian tuberculin, no tuberculous/tuberculoid lesions were detected. By culture examination of ln and organs from 13 animals, conditionally pathogenic mycobacteria (CPM) were isolated from only one animal (breeding boar): from mesenteric, pulmonary, hepatic ln and from spleen tissue samples. These isolates were identified as *MAH* and CPM by the PCR method and biochemically. By investigation of the external environment (205 samples), 33 (16.3%) CPM isolates were obtained: 13 *MAH*, eight *M. fortuitum,* one *M. nonchromogenicum,* one *M. abscessus*, one *M. scrofulaceum* and nine unidentified isolates, which were non-*MAC* according to the PCR examination. Non-specific responses obtained in the intravital tests (skin and serological tests) caused by CPM present in the environment substantially complicated diagnosis of avian tuberculosis. Based on these findings, animal hygiene measures have been adopted since 2002; resulted in a decrease of environmental contamination with CPM and a reduction in the number of animals giving positive responses to avian tuberculin.

Keywords: IS*901*; IS*1245*; *dnaJ*; zoonosis; avian mycobacteriosis; food safety; ecology

Tuberculosis in pigs causes high economic losses farmers from all over the world. The most consequential causative agents of tuberculosis in pigs at present are members of *Mycobacterium tuberculosis (MTC)* and *M. avium* (*MAC*) complexes. The losses are above all caused by restriction of animal transport from infected farms (with the exception of slaughterhouses), culling of animals positive during the tuberculin testing and the price of meat and organs from slaughtered infected animals (Alfredsen and Skjerve 1993; Dey and Parham, 1993; Morita et al., 1994; Balian et. al., 1997; Cvetnic et al., 1998;

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Komijn et al., 1999; Offermann et al., 1999; Kozak et al., 2003; Pavlik et al., 2003).

Tuberculous lesions in lymph nodes (ln) and parenchymatous organs from animals (above all birds, pigs and cattle) are caused by *M. avium* subsp. *avium (MAA*) of serotypes 1, 2 and 3 and of genotype *dnaJ+*, IS*901+* and IS*1245+*. The main sources of the causative agent of avian tuberculosis are infected domestic and wild birds and small rodents (Pavlik et al., 2000; Fischer et al., 2001; Mijs et al., 2002; Matlova et al., 2003; Bartos et al., 2006; Dvorska et al., 2007).

The most common causative agent found in tuberculous lesions from mesenteric, submandibular and occasionally inguinal ln of animals is *M. a. hominissuis* (*MAH*) of serotypes 4–6, 8–11 and 21 and of genotype *dnaJ+*, IS*901–* and IS*1245+* (Leinemann et al., 1993; Morita et al., 1994; Nishimori et al., 1995; Balian et al., 1997; Ritacco et al., 1998; Dvorska et al., 1999, 2003; Pavlik et al., 2000, 2003; Ramasoota et al., 2001; Mijs et al., 2002). The sources of *MAH*, the causative agent of avian mycobacteriosis, are mostly various components of the life environment: water, soil, bedding, dust and invertebrates (Horvathova et al., 1997; Kazda, 2000; Pavlik et al., 2000; Fischer et al., 2001; Matlova et al., 2003; Trckova et al., 2004, 2005, 2006a,b; Skoric et al., 2007).

However, other species of conditionally pathogenic mycobacteria (CPM) also known as atypical or opportunistic mycobacteria: *M. fortuitum*, *M. gordonae*, *M. terrae*, *M. chelonae*, *M. smegmatis*, *M. phlei* and *M. scrofulaceum* may participate in the formation of tuberculous lesions in domestic and wild pigs (Wayne and Kubica, 1986; Horvathova et al., 1997; Kazda, 2000; Fischer et al., 2001; Matlova et al., 2003; Pavlik et al., 2003; Trcka et al., 2006). Besides mycobacteria, tuberculoid lesions may be also caused by other microorganisms present in soil, such as *Rhodococcus equi* (Dvorska et al., 1999; Pavlik et al., 2003; Shitaye et al., 2006).

Intradermal tuberculin test with avian and bovine tuberculin is performed with the aim of intravital diagnosis of tuberculosis in cattle and pigs in the Czech Republic (Pavlik et al., 2002; Pavlik, 2006). Since June 1, 1993, in association of the Czech Republic with the European Union, 0.2 ml doses (14 000 TU/ml) of bovine and avian tuberculin were used for skin testing of animals in the Czech Republic. Previously, diluted avian tuberculin with the concentration of 800 TU/ml had been used in pigs in the Czech Republic to reduce the numbers of non-specific reactions (Pavlik et al., 2003). After the introduction of tuberculin testing with a higher TU concentration, the numbers of positive pigs in avian tuberculin tests increased (unpublished data).

Mycobacteria either occur the activities of people as a natural constituent of the ecosystem or are spread to the environment (Masaki et al., 1982; Pavlik et al., 2000). Various mycobacterial species are capable of long-term survival in the external environment where they may propagate in favourable conditions (Kazda, 2000; Matlova et al., 2003).

One of the most commonly notified CPM sources for domestic pigs is drinking water for animals (Matlova et al., 2003). Water may not only become a transport medium, but also the main reservoir to a majority of CPM species. Water plays a key role in the circulation of mycobacteria in the external environment (Horvathova et al., 1997). Water from reservoir tanks, which usually serves for watering pigs, may be contaminated with CPM due to the fact that mycobacteria tolerate wide pH and temperature ranges and various chlorine concentrations (Pelletier et al., 1988; Falkinham, 2003). Accordingly, it is recommended to use pressurized water systems without necessary expansion vessels for watering animals because mycobacteria may propagate in sediments and biofilms (Pavlik et al., 2003; Matlova et al., 2003). In farms, where the gravity flow system, including expansion vessels located in stables, is used for water distribution to drinkers, CPM was isolated from the sediment present in these vessels and the biofilm from pipelines (Matlova et al., 2003).

Contaminated bedding, particularly straw, sawdust, wood shavings, paper cut to pieces and other materials made of wood may be significant CPM sources for pigs (Kazda, 2000; Pavlik et al., 2003; Matlova et al., 2004b; Trckova et al., 2004). Another significant source of CPM may be contaminated feed and water (Dalchow, 1988; Pavlik et al., 2003; Matlova et al., 2003; Trckova et al., 2006a,b), peat and kaolin fed as supplements (Matlova et al., 2004a, 2005; Trckova et al., 2004, 2005, 2006a,b) and soil in pens (Horvathova et al., 1997; Matlova et al., 2003). Mycobacteria may spread through various invertebrate species: earthworms (Fischer et al., 2003a), dipterous insects (Fischer et al., 2001, 2004a,b, 2005, 2006; Machackova et al., 2004), cockroaches (Fischer et al., 2003a), beetles (Fischer et al., 2003b, 2004b) and other invertebrates (Kazda, 2000).

The purpose of the present study was to elucidate the reliability of intravital and *post mortem* diagnosis of tuberculosis in pigs in a breeding herd affected by mycobacterial infection between 1997

and 2003, to investigate sources of mycobacterial infections and to assess effectiveness of preventive measures adopted in the second period of the study between 2002 and 2003.

MATERIAL AND METHODS

Anamnestic data

Average yearly populations of 90 to 95 sows and boars were kept in the stock breeding herd of Large White pigs during screening between 1997 and 2003. According to the statistical data obtained from the responsible District Veterinary Administration, recorded during the 5 years (1992 to 1996) preceding the present investigation period, no tuberculous/tuberculoid lesions were detected in ln or parenchymatous organs from these animals. Dubious reactions to avian tuberculin Avitubal (28 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic) were occasionally (in one to three animals) repeatedly observed in the herd between 1992 and 1997. More detailed examinations were performed after the detection of a positive reaction to avian tuberculin in two animals in 1997.

Periods of examination

The examinations in the herd were divided into two periods.

Period A, between 1997 and 2001. Preventive measures were not consistently put into effect to prevent CPM occurrence.

Period B, between 2002 and 2003. The following preventive measures were put into effect:

(i) Introduction of good quality dry straw as bedding for all age categories of pigs.

(ii) Consistent implementation of regular mechanical cleaning by means of hot steam (at least every second month) followed by disinfection, rodents extinction and disinfestations.

(iii) Measures adopted were directed against free living bird access to the herd.

Intravital diagnosis

Tuberculin testing. Tuberculin testing using avian tuberculin was used for the examination of all breeding animals between 1997 and 2003. Each animal was examined 12 times altogether. A total of 1 113 tuberculin tests were performed: 931 and 182 pigs were examined in Period A and Period B, respectively. Avian tuberculin Avitubal prepared from *MAA* strain (D 4 ER) was used in a dose of 0.2 ml according to the manufacturer's instructions (Pavlik et al., 2003). The tuberculin test was performed by intradermal injection of 0.2 ml of Avitubal administered dorsally to auricles at the transition site of the head to auricles at approximately 2 to 3 cm from the auricle base. Forty-eight hours later, a reaction site (characteristic inflammatory swelling usually with concurrent erythema, occasionally central necrosis at the site of inoculation) larger than 10 mm was evaluated as positive, smaller than 9 mm as dubious (Pavlik et al., 2003).

Serological testing. Blood was collected from all 76 animals with a positive reaction to avian tuberculin (4 and 72 animals were positive and dubious, respectively) 1 to 3 days after the appearance of the first signs of reaction. After delivery of blood samples to the laboratory, serological examination was conducted using rapid slide agglutination test with antigens made from three *MAC* members: *MAA* (serotype 2), *MAH* (serotype 8) and *MI* (serotype 19) according to a previously described technique (Pavlas et al., 1993). Within Periods A and B, 75 and one animal were examined, respectively.

Examination of samples from external environment

During screening between 1998 and 2003, two hundred and five samples from the external environment in the investigated herd of pigs with positive skin reactions were collected. All samples were kept at +6°C in a dark room for up to two weeks before examination. Within Periods A and B, 140 and 65 samples from the external environment were examined, respectively. Samples were assigned into five groups:

Group 1 (44 samples): Straw from the stack with soil and moss, stable loft and pig pens.

Group 2 (24 samples): Drinking water and feed (including a mouse trapped in feed stock).

Group 3 (47 samples): Dust, spider nets and scrapings from the stable equipment.

Group 4 (19 samples): Invertebrates (earthworms collected near the straw stack, insects of order Diptera from the stable and from the farm surrounding).

Group 5 (71 samples): Faeces from the pigs, swallows and swallow nests and marten from the stable loft.

Post mortem diagnosis

Gross pathology. Gross pathology of the head and intestinal ln and parenchymatous organs was performed by a previously described method in all animals with a positive reaction in the tuberculin test after slaughter in the slaughterhouse (Pavlik et al., 2003).

Sample collection for laboratory examinations. Tissue samples for laboratory examinations were collected from the following animals:

(i) Breeder boar (on 17th March, 1997, positive reaction with avian tuberculin and with antigen *MAA* during serological examination by agglutination test): 17 tissue samples were taken for laboratory analysis after slaughter: one submandibular ln, six mesenteric ln, one gastric ln, two samples of small intestine mucosa, three pulmonary ln, one sample of each of pulmonary, tonsil and spleen tissues.

(ii) The remaining animals with positive reactions to avian tuberculin; samples of submandibular and mesenteric ln were examined by culture.

(iii) Nine animals with repeated dubious reactions to avian tuberculin with detected antibodies to *MAH* (seven animals: six sows and one boar) and to *MI* (two sows); samples of submandibular and mesenteric ln were examined by culture (Table 3).

Sample storage and gross examination. After collection, tissue samples from pigs were frozen and kept at –18°C for up to three weeks before examination. The tissue samples were rapidly thawed at 37°C and examined for the presence, number and size of tuberculous lesions.

Bacteriological examination of tissues and environmental samples for the presence of mycobacteria

Microscopy of imprints of tissue samples and environmental samples. Before culture, slides with imprints of tissue samples were stained according to Ziehl-Neelsen (Z-N) and examined by light microscopy for the presence of acid-fast rods (AFR). At least 200 fields of view were examined in each sample.

Culture examination. Approximately 1 g of tissue or an external environmental sample was homogenised and decontaminated according to the method described previously by Fischer et al. (2001). A total of 40 µl of suspensions was dispensed into two solid egg based agar media according to Stonebrink, two solid egg yolk agars according to Herrold and two liquid serum media according to Sula. Incubations were performed in parallel, with one agar type at 24°C and 37°C each time. Mycobacterial growth was checked after the first week and then every second week for two months (Matlova et al., 2003).

Identification of mycobacterial isolates. All the AFR positive isolates were examined by the PCR method for the detection of *dnaJ* gene, specific for genus *Mycobacterium* using primers 5'-GGG TGA CGC GAC ATG GCC CA-3' and 5'-CGG GTT TCG TCG TAC TCC TT-3 (Nagai et al., 1990). IS*901* detection primers 5'-GCA ACG GTT GTT GCT TGA AA-3' and 5'-TGA TAC GGC CGG AAT CGC GT-3' (Kunze et al., 1991, 1992) and IS*1245* detection primers 5'-GCC GCC GAA ACG ATC TAC-3' and 5'-AGG TGG CGT CGA GGA AGA-3' were used (Guerrero et al., 1995). In all PCR examinations, internal standards were used (Bartos et al., 2006). All *MAC* isolates were serotyped according to the described system (Wolinsky and Schaefer, 1973) which was later modified (Sussland and Hrdinova, 1976). Mycobacterial isolates that were not classified as *MAC* were assessed by biochemical methods (Wayne and Kubica, 1986).

Statistical analysis of results

The χ^2-test (Stat Plus) was applied to the statistical evaluation of results (Matouskova et al., 1992).

RESULTS

Examination results of the first animal with a positive reaction in intravital tests

After slaughter of a breeding boar with a positive reaction in the avian tuberculin test with detected serum antibodies against *MAA* antigen, only adenopathies (thickened trabeculae and extravasation) of mesenteric ln were observed. AFR were detected by microscopy after Z-N staining in mesenteric and pulmonary ln. Mycobacteria were isolated by culture from 4 (23.5%) of 17 samples: *MAH* was

Table 1. Skin testing results with avian tuberculin[a] during 1997–2003

Animal data		Period A								Period B			Total
Number of animals	Reaction	1997		1998			1999	2000	2001	Subtotal	2002	2003	Subtotal	
		17.3.	27.10.	27.3.	28.5.	23.7.	1.10.	5.5.	1.9.	25.2.	25.1.		12.11.	10.7.		
Examined		92	90	95	93	92	94	92	94	95	94	931	92	90	182	1 113
Reacting	+	1	1	0	1	0	0	0	0	0	0	3	1	0	1	4
%		1.1	1.1	0	1.1	0	0	0	0	0	0	0.3	1.1	0	0.6	0.4
Reacting	±	2	1	6	10	18	7	3	9	7	6	69	1	2	3	72
%		2.2	1.1	6.3	10.8	19.6	7.4	3.3	9.6	7.4	6.4	7.4	1.1	2.2	1.7	6.5
Reacting	Total	3	2	6	11	18	7	3	9	7	6	72	2	2	4	76
%		3.3	2.2	6.3	11.8	19.6	7.4	3.3	9.6	7.4	6.4	7.7	2.2	2.2	2.2	6.8

Period A: 1997–2001; Period B: 2002–2003

[a]skin testing with avian tuberculin (Avitubal, 28 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic): + positive reaction; ± dubious reaction

detected in mesenteric ln, CPM in pulmonary ln, *MAH* in liver tissue and CPM in spleen tissue.

Results of avian tuberculin test

From 1 113 tuberculin tests performed between 1997 and 2003 in both Periods A and B, 76 (6.8%) reactions were detected: 4 (0.4%) were positive and 72 (6.4%) dubious. Prevalence of positive or dubious reactions to avian tuberculin in respective testing periods ranged between 2 (2.2%) to 18 (19.6%). Significantly more ($P < 0.01$) reactions (72; 7.7%) were recorded during Period A in comparison with Period B (4; 2.2%; Table 1).

Results of serological examination with antigens of *MAC* members

Among 76 sows and boars with reaction to avian tuberculin, antibodies to *MAA*, *MAH* and *MI* were detected in 3 (4.0%), 18 (23.7%) and 9 (11.8%) animals, respectively, using the agglutination test in both Periods A and B. Differences between the frequency of detection of antibodies to *MAA* in four animals with a positive reaction (50.0%) and in 72 (1.4%) animals with a dubious reaction were significant ($P < 0.01$). In animals with a dubious reaction, in Period A, differences in the frequency of detection of *MAH* antibodies in 54 animals with one reaction (18.5%) and in 15 animals with repeated reactions (46.7%) were significant at $P < 0.05$ (Table 2).

Repeated reactions of tuberculin skin tests and serological examination with *MAC* antigens

In both Periods A and B, tuberculin skin test reactions repeated two to four times were noted by separate observations of 17 pigs (15 sows and two boars). Antibodies to *MAA* were detected in none of the animals, antibodies to *MAH* and *MI* were found in eight (47.1%) and two animals (11.8%), respectively. Tuberculous lesions in ln or parenchymatous organs were not observed in any of these 10 pigs with reactions to *MAH* and *MI* antigens. Culture examination of intestinal and head ln for mycobacteria was likewise negative in all animals (Table 3).

Table 2. Serological examination of animals reacting with avian tuberculin during 1997–2003

Period[a]	Skin testing with avian tuberculin						Antibodies against antigens					
							MAA[b]		*MAH*[c]		*MI*[d]	
	reaction	No.	1st	2nd	3rd	4th	No.	%	No.	%	No.	%
A	positive	2[e]	+	nt	nt	nt	2	100	0	0	0	0
		1[e]	±	+	±	nt	0	0	1	100	0	0
	dubious	54	±	nt	nt	nt	1[e]	1.9	10	18.5	7	13.0
		11	±	±	nt	nt	0	0	6	54.6	0	0
		3	±	±	±	nt	0	0	0	0	2	66.7
		1	±	±	±	±	0	0	1	100	0	0
B	positive	1	+	nt	nt	nt	0	0	0	0	0	0
	dubious	2	±	nt	nt	nt	0	0	0	0	0	0
		1	±	±	nt	nt	0	0	0	0	0	0
Subtotal	positive	4					2	50.0	1	25.0	0	0
	dubious	72					1	1.4	17	23.6	9	12.5
Total		76					3	4.0	18	23.7	9	11.8

[a]Period A: 1997–2001; Period B: 2002–2003; nt = not tested

[b]*Mycobacterium avium* subsp. *avium* (serotypes: 1, 2, and 3; genotype: *dnaJ*+, IS*901*+, IS*1245*+)

[c]*Mycobacterium avium* subsp. *hominissuis* (serotypes: 4–6, 8–11, and 21; genotype: *dnaJ*+, IS*901*–, IS*1245*+)

[d]*Mycobacterium intracellulare* (serotypes: 7, 12–20, and 22–28; genotype: *dnaJ*+, IS*901*–, IS*1245*–)

[e]Positively reacting animals were slaughtered within one week after the last skin testing with negative *post mortem* findings and culture examination of mesenteric and head lymph nodes

Examination of samples from the external environment

In both Periods A and B between 1997and 2003, mycobacteria were detected in 33 (16.1%) of the 205 samples from the external environment. In Period A, mycobacterial isolates from the external environment (21.4%) were significantly ($P < 0.01$) more frequently detected than in Period B, where only 4.6% positivity was found (Table 4).

Percentages of detected mycobacteria in groups 1 to 4 were decreased in Period B in comparison with Period A. Groups 2 and 4 were reduced to 0%, Group 1 was reduced from 25.0% to 6.3% and Group 3 was reduced from 33.3% to 7.1%. Increased percentages of detected mycobacteria (from 8.3% to 14.3%) were only found in Group 5 (animal faeces; Table 4).

By the PCR and serotyping methods, 13 (39.4%) isolates were identified as *MAH* serotypes 4 ($n = 1$), 8 ($n = 6$) and 9 ($n = 1$) and genotype *dnaJ*+, IS*901*– and IS*1245*+. Biochemically, 8 (24.2%) isolates were identified as *M. fortuitum* and one isolate (3.0%) of each of *M. nonchromogenicum, M. abscessus* and *M. scrofulaceum* was detected. The remaining 9 (27.4%) slowly growing isolates were not *MAC* members after the PCR examination and they could not be identified biochemically (Table 4).

DISCUSSION

Due to a positive result during skin testing with avian tuberculin on 17th March, 1997 and serological detection of antibodies to *MAA* by the agglutination method, the breeding boar was slaughtered. However, subsequent laboratory examination of 17 tissue samples did not reveal *MAA*, but only CPM (two *MAH* isolates and two isolates that were not *MAC* members by the IS*901* and IS*1245* PCR method and could not be identified biochemically). Due to the fact that a positive reaction was detected in another animal in the same year (27th October, 1997), an external environment examination was

Table 3. Serological examination of animals repeatedly reacting to skin testing with avian tuberculin during 1997-2003

Animal identification		Skin testing with avian tuberculin[a]												Antibodies[b] against members of *M. avium* complex		
		Period A										Period B				
		1997		1998				1999		2000	2001	2002	2003			
ID	Gender	17.3.	27.10.	27.3.	28.5.	23.7.	1.10.	5.5.	1.9.	25.2.	25.1.	12.11.	10.7.	*MAA*	*MAH*	*MI*
1	♂[c]	±	–	–	±	±	±	–	–	–	–	nt	nt	–	+	–
2	♀[c]	±	±	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	–	+	–
3	♀	–	–	±	–	±	–	nt	nt	nt	nt	nt	nt	–	–	–
4	♀[c]	–	–	±	+	±	nt	nt	nt	nt	nt	nt	nt	–	+	–
5	♀[c]	–	–	±	–	±	–	–	±	–	nt	nt	nt	–	–	+
6	♀	–	–	–	±	±	–	nt	nt	nt	nt	nt	nt	–	–	–
7	♀	–	–	–	±	±	–	–	–	–	nt	nt	nt	–	–	–
8	♀[c]	nt	–	–	–	±	–	–	±	nt	nt	nt	nt	–	+	–
9	♀[c]	nt	nt	–	–	±	±	–	–	–	–	nt	nt	–	+	–
10	♀[c]	nt	nt	–	–	±	–	–	–	±	–	nt	nt	–	+	–
11	♀[c]	–	–	–	–	±	–	±	nt	nt	nt	nt	nt	–	+	–
12	♀	nt	–	–	–	±	±	–	–	±	nt	nt	nt	–	–	–
13	♀[c]	–	–	–	–	–	±	–	±	nt	nt	nt	nt	–	+	–
14	♀	–	–	–	–	–	±	–	±	–	nt	nt	nt	–	–	–
15	♂	nt	–	–	–	–	–	–	±	±	–	nt	nt	–	–	–
16	♀[c]	nt	nt	nt	nt	nt	–	–	±	±	±	nt	nt	–	–	+
17	♀	nt	nt	nt	nt	nt	nt	nt	nt	nt	–	±	±	–	–	–

[a]skin testing with avian tuberculin (Avitubal, 28 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic): + positive reaction; ± dubious reaction; – negative reaction

[b]serological examination was carried out one to three days after the first positive or dubious reaction after the skin testing with Avitubal (28 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic); *MAA* = *Mycobacterium avium* subsp. *avium* (serotype 2); *MAH* = *Mycobacterium avium* subsp. *hominissuis* (serotype 8); *MI* = *Mycobacterium intracellulare* (serotype 19)

[c]slaughtered animals with detected antibodies against *MAH* and *MI* antigens

nt = not tested

Table 4. Mycobacteria isolation from environmental samples during 1998 to 2003 (at least 20 samples were examined each year)

Examined samples		Period A		Period B		Total			Mycobacterial isolates[c]					
Material	Origin	No.	+ve	No.	+ve	No.	+ve	%	*MAH*	*MF*	*MN*	*MA*	*MS*	sp.[d]
Straw	the stack	7	2	6	0	13	2	15.4	2	0	0	0	0	0
	the stack with soil and moss	4	0	2	0	6	0	0	0	0	0	0	0	0
	stable loft	4	0	2	0	6	0	0	0	0	0	0	0	0
	pig pens	13	5	6	1	19	6	31.6	4	0	1	0	0	1
Group 1	subtotal	28	7	16	1	44	8	18.2	6	0	1	0	0	1
	%	100	25.0	100	6.3									
Water	stable	7	0	2	0	9	0	0	0	0	0	0	0	0
Feed	stable (feed concentrates)	10	0	4	0	14	0	0	0	0	0	0	0	0
Mouse[a]	stable (feed stock)	1	1	0	0	1	1	100	0	1	0	0	0	0
Group 2	subtotal	18	1	6	0	24	1	4.2	0	1	0	0	0	0
	%	100	5.6	100	0									
Dust	stable	6	1	4	0	10	1	5.3	1	0	0	0	0	0
Spider nets	stable	7	0	2	0	9	0	0	0	0	0	0	0	0
Scrapings	stable equipment	20	10	8	1	28	11	39.3	3	2	0	0	0	6
Group 3	subtotal	33	11	14	1	47	12	25.5	4	2	0	0	0	6
	%	100	33.3	100	7.1									
Invertebrates	earthworms near the straw stack	9	2	4	0	13	2	15.4	0	0	0	0	1	1
	diptera from the stable	20	6	10	0	30	6	20.0	2	4	0	0	0	0
	diptera from the farm surrounding	20	2	8	0	28	2	7.1	1	0	0	1	0	0
Group 4	subtotal	49	10	22	0	71	10	14.1	3	4	0	1	1	1
	%	100	20.4	100	0									
Faeces	pigs	8	0	4	0	12	0	0	0	0	0	0	0	0
	swallows and swallow nests	4	1	2	0	6	1	16.7	0	1	0	0	0	0
	marten[b] from the stable loft	0	0	1	1	1	1	100	0	0	0	0	0	1
Group 5	subtotal	12	1	7	1	19	2	10.5						
	%	100	8.3	100	14.3									
Total		140	30	65	3	205	33	16.1	13	8	1	1	1	9
	%	100	21.4	100	4.6		100		39.4	24.2	3.0	3.0	3.0	27.4

Period A: 1997–2001; Period B: 2002–2003; [a]mice *(Mus musculus)*; [b]stone marten (*Martes foina*); [c]*MAH = Mycobacterium avium* subsp. *hominissuis:* serotypes 4 (n = 1), 8 (n = 6), and 9 (n = 1), genotype *dnaJ*+, IS*901*–, IS*1245* +; *MF = Mycobacterium fortuitum; MN = Mycobacterium nonchromogenicum; MA = Mycobacterium abscessus; MS = Mycobacterium scrofulaceum;* [d]conditionally pathogenic mycobacteria not belonging to the *Mycobacterium avium* complex after the PCR examination and non-identified biochemically

initiated with the aim to investigate the source of mycobacteria (Table 1).

Despite the fact that all animals giving a reaction to avian tuberculin with subsequent detection of antibodies to *MAC* members have been consistently culled, reactions to skin tuberculin testing could be still found and even repeatedly in some pigs (Table 2). The original assumption that the pig farm environment was highly contaminated with *MAC* and CPM members was confirmed by culture examination of samples from the surrounding (Table 4). *MAH* and CPM likely participated as strong allergens in allergological and also serological reactions. Similar complications during avian tuberculosis diagnosing with avian tuberculin were recorded previously (Pearson et al., 1977; Monaghan et al., 1994; Kazda, 2000).

Despite the fact that the causative agent of avian tuberculosis was not detected in either the organs from pigs nor in samples from the external environment (Table 4), reactions to avian tuberculin and antibodies to *MAA* were repeatedly found in the examined pigs (Tables 1, 2 and 3). From the results obtained by culture examination of ln and organs of the breeding boar it was found that the *MAH* and CPM were likely transmitted to the boar organism through both oral (isolation from mesenteric ln) and aerogenic routes (isolation from pulmonary ln). Based on the above mentioned results, it is also evident that mycobacteria might have circulated in the blood (isolation from livers and spleen) and thus elicited positive responses, both allergological and serological (Tables 1 and 2).

MAH and CPM occurrence in the external environment of many European countries has been described (Kazda, 2000; Matlova et al., 2003). This was also confirmed by the results of our investigation; we detected CPM in various constituents of the external environment (straw, scrapings from the floors in pens, invertebrates, faeces from birds, mammals etc.). Proper mechanical cleaning with subsequent disinfection, disinfestation and extinction of small terrestrial mammals could reduce adverse effects of *MAH* and CPM. Those were not consistently performed before 2002–2003; however, in these years, occurrence of *MAH* and CPM and subsequent allergological reactions at avian tuberculin testing consequently decreased (Table 4).

Straw stored under moist conditions is usually contaminated with *MAC* members. CPM propagation mostly occurs during the summer months at ambient temperatures of above 18°C (Kazda, 2000). Matlova et al. (2003) detected that 40.0% of straw may be contaminated with *MAH* and CPM if stored under adverse conditions. The source of CPM may be soil and dust contaminating straw where *MAH*, *MI* and CPM are usually present (Windsor et al., 1984; Gardner and Hird, 1989; Horvathova et al., 1997; Pavlik et al., 2000; Kazda, 2000).

CPM sources for animals may also be insects and other invertebrates, as we found in Period A (Table 4) and as also described by some other authors (Fotedar and Banerjee, 1992; Matlova et al., 1998). Insect nets have been installed in the windows of stables in the investigated herd at the beginning of Period B in 2002. However, their efficiency was low because imagos were developed directly in the stables, i.e. in the environment containing pig faeces, feed leftovers and other biological materials (Fischer et al., 2001).

Water is the primary reservoir of *MAH* and most CPM species. It is above all surface water that plays a key role in the circulation of mycobacteria in the external environment (Engel et al., 1977; Kazda, 2000). Pelletier et al. (1988) noted that water from reservoirs usually used for watering pigs may be contaminated with low amounts of CPM. Matlova et al. (2003) investigated watering systems in pig farms. Study mostly focused on the expansion vessels in gravity flow systems. They isolated CPM from the sediment present in these vessels and from biofilm covering the surface of the conduit. Conditions favourable for mycobacteria propagation (increased water temperature) exist even in a conduit system made from synthetic material, placed in a stable loft (Pavlik et al., 2003). No expansion vessels were used on the investigated farm and water was piped to the stable through a metal pipeline from the own source situated above the hall. This system was convenient from an aspect of low risk of contamination of drinking water with mycobacteria, as shown by the fact that this type of contamination has never been detected there (Table 4).

According to Dalchow (1988) and Matlova et al. (2003) feed mixtures are not the primary sources of infection; this was confirmed on the farm investigated in the present study (Table 4). Matlova et al. (2003) noted that liquid feeds represent the main risk due to the fact that CPM contaminated water is used for their preparation. However, dried feeds were used on this farm and no CPM have been detected there during the entire study. However,

small terrestrial mammals also represent a risk of CPM spread because they may be CPM sources in the feed stocks. In the present study, *M. fortuitum* was isolated from one house mouse trapped in the mixed feed stock (Table 4).

Due to the fact, that diptera are present in stables during whole-year (our own non-published observation), regular desinsection was recommended in the stables, not only in the summer months (from May to September) when diptera are found outside the stables in the Czech Republic (Fischer et al., 2001). After adoption of preventive measures, not only numbers of animals that reacted to avian tuberculin decreased, but environmental contamination of the *MAH* and CPM was also reduced and CPM were no more isolated from invertebrates (Table 4).

CONCLUSIONS

Based on the results obtained in Period A (1997–2001), animal hygiene conditions in the herd have gradually improved since the beginning of Period B in 2002 due to a consistent adoption of preventive measures. Reduced exposure of pigs to *MAH* and CPM and decreased numbers of animals with reactions to avian tuberculin was a result. High frequency of non-specific skin reactions to avian tuberculin without a subsequent finding of tuberculous lesions in animals after slaughter was likely caused by *MAH* and CPM, repeatedly detected by culture of samples from the farm environment.

MAH and CPM were present in some constituents of the environment despite the adopted measures; these may complicate diagnosis of tuberculosis by skin testing with avian tuberculin in the future. Accordingly, the risk of CPM occurrence in the external environment should be kept in mind when complex preventive measures are proposed.

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REFERENCES

Alfredsen S., Skjerve D.E. (1993): An abattoir-based case control study of risk-factors for mycobacteriosis in Norwegian swine. Preventive Veterinary Medicine, 15, 253–259.

Balian S.C., Ribeiro P., Vasconcellos S.A., Pinheiro S.R., Ferreira Neto J.S., Guerra J.L., Xavier J.G., Morais Z. M., Telles M.A. (1997): Tuberculosis lymphadenitis in slaughtered swine from the State of Sao Paulo, Brazil: microscopic histopathology and demonstration of mycobacteria (in Portuguese). Revista de Saude Publica, 31, 391–397.

Bartos M., Hlozek P., Svastova P., Dvorska L., Bull T., Matlova L., Parmova I., Kuhn I., Stubbs J., Moravkova M., Kintr J., Beran V., Melicharek I., Ocepek M., Pavlik I. (2006). Identification of members of *Mycobacterium avium* species by AccuProbes, serotyping, and single IS*900*, IS*901,* IS*1245* and IS*901*-flanking region PCR with internal standards. Journal of Microbiological Methods, 64, 333–345.

Cvetnic Z., Kovacic H., Ocepek M. (1998): Mycobacteria in the environment and in the feed of swine in Croatia (in German). Wiener Tierarztliche Monatsschrift, 85, 18–21.

Dalchow W. (1988): Mycobacteriosis in pigs fed cereal waters (in German). Tierarztliche Umschau, 43, 62–74.

Dey B.P., Parham G.L. (1993): Incidence and economics of tuberculosis in swine slaughtered from 1976 to 1988, food animal economics. Journal of American Veterinary Medical Association, 203, 516–519.

Dvorska L., Parmova I., Lavickova M., Bartl J., Vrbas V., Pavlik I. (1999): Isolation of *Rhodococcus equi* and atypical mycobacteria from lymph nodes of pigs and cattle in herds with the occurrence of tuberculoid gross changes in the Czech Republic over the period 1996–1998. Veterinarni Medicina, 44, 321–330.

Dvorska L., Bull T.J., Bartos M., Matlova L., Svastova P., Weston R.T., Kintr J., Parmova I., Van Soolingen D., Pavlik I. (2003): A standardised restriction fragment length polymorphism (RFLP) method for typing *Mycobacterium avium* isolates links IS*901* with virulence for birds. Journal of Microbiological Methods, 55, 11–27.

Dvorska L., Matlova L., Ayele W.Y., Fischer O.A., Amemori T., Weston R.T., Alvarez J., Beran V., Moravkova M., Pavlik I. (2007): Avian tuberculosis in naturally infected captive water birds of the Ardeidae and Threskiornithidae families studied by serotyping, IS*901* RFLP typing and virulence for poultry. Veterinary Microbiology, 119, 366–374.

Engel H.W.B., Groothuis D.G., Wouda W., Konig C.D.W., Lendfers L.H.M. (1977): Pig-compost as a source of *Mycobacterium avium* infection in swine (in German). Zentralblatt fur Veterinarmedizin, Reihe B, Berlin, 25, 373–382.

Falkinham J.O. (2003): Factors influencing the chlorine susceptibility of *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*. Applied and Environmental Microbiology, 69, 5685–5689.

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., Du Maine 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 causal 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. (2004a): 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., Peral D.L., Weston R.T., Bartos M., Pavlik I. (2004b): Beetles as possible vectors of infections caused by *Mycobacterium avium* species. Veterinary Microbiology, 102, 247–255.

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* subsp. *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.

Fotedar R., Banerjee U. (1992): Nosocomial fungal infections – study of the possible role of cockroaches (*Blattella germanica*) as vectors. Acta Tropica, 50, 339–343.

Gardner I.A., Hird D.W. (1989): Environmental source of mycobacteriosis in a California swine herd. Canadian Journal of Veterinary Research, 53, 33–37.

Guerrero C., Bernasconi J., Burki D., Bodmer T., Telenti A. (1995): A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. Journal of Clinical Microbiology, 33, 304–307.

Horvathova A., Kazda J., Bartl J., Pavlik I. (1997): Occurrence of conditionally pathogenic mycobacteria in life environment and their influence on living organism (in Slovak). Veterinarni Medicina, 42, 191–212.

Kazda J. (2000): The Ecology of the Mycobacteria. 1st ed. Kluwer Academic Publishers, Dordrecht, Boston, London. 72 pp.

Komijn R.E., De Haas P.E., Schneider M.M., Eger T., Nieuwenhu S.J.H., Van Den Hoek R.J., Bakker D., Van Zijderveld F.G., Van Soolingen D. (1999): Prevalence of *Mycobacterium avium* in slaughter pigs in The Netherlands and comparison of IS*1245* restriction fragment length polymorphism patterns of porcine and human isolates. Journal of Clinical Microbiology, 37, 1245–1249.

Kozak A., Vecerek V., Chloupek P., Tremlova B., Malena M. (2003): Veterinary meat inspection of pig carcasses in the Czech Republic during the period of 1995–2002. Veterinarni Medicina, 48, 207–213. http://www.vri.cz/docs/vetmed/48-8-207.pdf

Kunze Z.M., Wall S., Appelberg R., Silva M.T., Portaels F., McFadden J.J. (1991): IS*901*, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. Molecular Microbiology, 5, 2265–2272.

Kunze Z.M., Portaels F., McFadden J.J. (1992): Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS*901*. Journal of Clinical Microbiology, 30, 2366–2372.

Leinemann H., Berner H., Gerlach H., Kalau H., Kosters J. (1993): Mycobacteriosis on pigs due to *Mycobacterium avium-intracellulare*. Tierarztliche Umschau, 48, 713–718.

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.

Masaki S., Shimizu K., Cho N., Hirose T. (1982): Isolation of mycobacteria from lymph nodes of pigs and their environment. Nippon Juigaku Zasshi, 44, 213–221.

Matlova L., Fischer O., Kazda J., Kaustova J., Bartl J., Horvathova A., Pavlik I. (1998): Occurrence of mycobacteria in invertebrates and poikilothermic animals and their role in man and other animals (in Czech). Veterinarni Medicina, 43, 115–132.

Matlova L., Dvorska L., Bartl J., Bartos M., Ayele W.Y., Alexa M., Pavlik I. (2003): Mycobacteria isolated from

the environment of pig farms in the Czech Republic during the years 1996 to 2002. Veterinarni Medicina, 48, 343–357. http://www.vri.cz/docs/vetmed/48-12-343.pdf

Matlova L., Dvorska L., Bartos M., Docekal J., Trckova M., Pavlik I. (2004a): Tuberculous lesions in pig lymph nodes caused by kaolin fed as supplement. Veterinarni Medicina, 49, 379–388. http://www.vri.cz/docs/vetmed/49-10-379.pdf

Matlova L., Dvorska L., Palecek K., Maurenc L., Bartos M., Pavlik I. (2004b): Impact of sawdust and wood shavings in bedding on pig tuberculous lesions in lymph nodes, and, IS*1245* RFLP analysis of *Mycobacterium avium* subsp. *hominissuis* of serotypes 6 and 8 isolated from pigs and environment. Veterinary Microbiology, 102, 227–236.

Matlova L., Dvorska L., Ayele W.Y., Bartos M., Amemori T., Pavlik I. (2005): Distribution of *Mycobacterium avium* complex isolates in tissue samples of pigs fed peat naturally contaminated with mycobacteria a supplement. Journal of Clinical Microbiology, 43, 1261–1268.

Matouskova O., Chalupa j., Cigler M., Hruska K. (1992): Stat Plus – Manual (in Czech). 1st ed. Veterinary Research Institute, Brno, Czech Republic. 168 pp.

Mijs W., De Haas P., Rossau R., Van Der Laan T., Rigouts L., Portaels F., Van Soolingen D. (2002): Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. *avium* to bird-type isolates and *M. avium* subsp. *hominissuis* for the human/porcine type of *M. avium*. International Journal of Systematic and Evolutionary Bacteriology, 52, 1505–1518.

Monaghan M.L., Doherty M.L., Collins J.D., Kazda J.F., Quinn P.J. (1994): The tuberculin test. Veterinary Microbiology, 40, 111–124.

Morita Y., Maruyama S., Katsube Y. (1994): Prevalence of atypical mycobacteriosis in slaughtered swine in Gunma prefecture and the serovars of the isolates. Journal of Veterinary Medicine Science, 56, 475–479.

Nagai R., Takewaki S., Wada A., Okuzumi K., Tobita A., Ohkubo A. (1990): Rapid detection and identification of mycobacterial DNA by PCR (in Japanese). Rinsho Byori 38, 1247–1252.

Nishimori K., Eguchi M., Nakaoka Y., Onodera Y., Ito T., Tanaka K. (1995): Distribution of IS*901* in strains of *Mycobacterium avium* complex from swine by using IS*901*-detecting primers that discriminate between *Mycobacterium avium* and *Mycobacterium intracellulare*. Journal of Clinical Microbiology, 33, 2102–2106.

Offermann U., Bodmer T., Audige L., Jemmi T. (1999): The prevalence of *Salmonella*, *Yersinia* and Mycobacteria in slaughtered pigs in Switzerland (in German). Schweizer Archiv fur Tierheilkunde, 141, 509–515.

Pavlas M., Michalska A., Hunady M. (1993): Diagnosis of avian tuberculosis-mycobacteriosis by rapid agglutination. Acta Veterinaria Brno, 62, 63–69.

Pavlik I. (2006): The experience of new European Union Member States concerning the control of bovine tuberculosis. Veterinary Microbiology, 112, 221–230.

Pavlik I., Svastova P., Bartl J., Dvorska L., Rychlik I. (2000): Relationship between IS*901* in the *Mycobacterium avium* complex strains isolated from birds, animals, humans and environment and virulence for poultry. Clinical and Diagnostic Laboratory Immunology, 7, 212–217.

Pavlik I., Ayele W.Y., Parmova I., Melicharek I., Hanzlikova M., Kormendy B., Nagy G., Cvetnic Z., Ocepek M., Fejzic N., Lipiec M. (2002): Incidence of bovine tuberculosis in cattle in seven Central European countries during the years 1990–1999. Veterinarni Medicina, 47, 45-51. http://www.vri.cz/docs/vetmed/47-3-45.pdf

Pavlik I., Matlova L., Dvorska L., Bartl J., Oktabcova L., Docekal J., Parmova I. (2003): Tuberculous lesions in pigs in the Czech Republic during 1990–1999: occurrence, causal factors and economic loses. Veterinarni Medicina, 48, 113–125. http://www.vri.cz/docs/vetmed/48-5-113.pdf

Pearson C.W., Conner L.A., Leper A.W.D. (1977): Tuberculin sensitivity of cattle inoculated with atypical mycobacteria isolated from cattle, feral pigs and trough water. Australian Veterinary Journal, 53, 67–71.

Pelletier P.A., Dumoulin G.C., Stottmeier K.D. (1988): Mycobacteria in public water supplies: comparative resistance to chlorine. Microbiological Science, 5, 147–148.

Ramasoota P., Chansiripornchai N., Kallenius G., Hoffner S.E., Svenson S.B. (2001): Comparison of *Mycobacterium avium* complex (*MAC*) strains from pigs and humans in Sweden by random amplified polymorphic DNA (RAPD) using standardized reagents. Veterinary Microbiology, 78, 251–259.

Ritacco V., Kremer K., Van Der Laan T., P.Nenburg J.E., de Haas P.E.W., Van Soolingen D. (1998): Use of IS*901* and IS*1245* in RFLP typing of *Mycobacterium avium* complex: relatedness among serovar reference strains, human and animal isolates. International Journal of Tuberculosis and Lung Diseases, 2, 242–251.

Shitaye J.E., Parmova I., Matlova L., Dvorska L., Horvathova A., Vrbas V., Pavlik I. (2006): Mycobacterial and *Rhodococcus equi* infections in pigs in the Czech Republic between the years 1996 and 2004: the causal factors and distribution of infections in the tissues. Veterinarni Medicina, 51, 497–511. http://www.vri.cz/docs/vetmed/51-11-497.pdf

Skoric M., Shitaye J.E., Halouzka R., Fictum P., Trcka I., Heroldova M., Tkadlec E., Pavlik I. (2007): Tuberculous and tuberculoid lesions in free living small terrestrial mammals and the risk of infection to humans and animals: a review. Veterinarni Medicina, 52, 144–161. http://www.vri.cz/docs/vetmed/52-4-144.pdf

Sussland Z., Hrdinova V. (1976): Use of rapid agglutination in the serotyping of the *Mycobacterium avium-intracellulare* complex (in Czech). Veterinarni Medicina, Praha, 21, 209–213.

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 boars (*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

Trckova M., Matlova L., Dvorska L., Pavlik I. (2004): Kaolin, bentonite, and zeolites as feed supplements for animals: health advantages and risks. Veterinarni Medicina, 49, 389–399. http://www.vri.cz/docs/vetmed/49-10-389.pdf

Trckova M., Matlova L., Hudcova H., Faldyna M., Zraly Z., Dvorska L., Beran V., Pavlik I. (2005): Peat as a feed supplement for animals: a literature review. Veterinarni Medicina, 50, 361–377. http://www.vri.cz/docs/vetmed/50-8-361.pdf

Trckova M., Zraly Z., Matlova L., Beran V., Moravkova M., Svobodova J., Pavlik I. (2006a): Effects of peat feeding on the performance and health status of fattening pigs and environmentally derived mycobacteria. Veterinarni Medicina, 51, 533–543. http://www.vri.cz/docs/vetmed/51-12-533.pdf

Trckova M., Zraly Z., Bejcek P. Matlova L., Beran V., Horvathova A., Faldyna M., Moravkova M., Shitaye J.E., Svobodova J., Pavlik I. (2006b): Effect of feeding treated peat as a supplement to newborn piglets on the growth, health status and occurrence of conditionally pathogenic mycobacteria. Veterinarni Medicina, 51, 544–554. http://www.vri.cz/docs/vetmed/51-12-544.pdf

Wayne L.G., Kubica G.P. (1986): Genus *Mycobacterium* Lehmann and Neumann 1896, 363AL. In: Sneath P.H.A., Mair N.S., Sharpe M.E., Holt J.G. (eds.): Bergey's Manual of Systematic Bacteriology, 2. The Williams & Wilkins Co., Baltimore. 1436–1457.

Windsor R.S., Durrant D.S., Burn K.J. (1984): Avian tuberculosis in pigs: *Mycobacterium intracellulare* infection in a breeding herd. Veterinary Record, 19, 497–500.

Wolinsky E., Schaefer W.B. (1973): Proposed numbering scheme for mycobacterial serotypes by agglutination. International Journal of Systematic Bacteriology, 23, 182–183.

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