

## Tuberculous lesions in pig lymph nodes caused by kaolin fed as a supplement

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**ABSTRACT:** An increased incidence of tuberculous lesions in head and mesenteric lymph nodes from slaughtered pigs weighing about 115 kg was recorded in a herd of pigs kept in two farms A and B in the Czech Republic. Tuberculous lesions were more frequently ( $P < 0.01$ ) diagnosed in pigs from Farm A (10.4%) than from Farm B (1.1%). The follow-up investigation of potential sources of infection on Farm A revealed that the piglets were fed kaolin from a nearby mine as a supplement. Among 20 samples from the pigs' environment, atypical conditionally pathogenic mycobacteria (ACPM) were detected in four samples as follows: dust ( $n = 2$ ), pig faeces ( $n = 1$ ) and kaolin fed as a supplement ( $n = 1$ ). *Mycobacterium avium* subsp. *hominissuis* of genotype IS901– and IS1245+ and serotype 8 was isolated from kaolin and pig lymph nodes, *M. fortuitum* from stable dust and pig faeces and other ACPM from stable dust. When kaolin feeding to piglets ceased, the incidence of tuberculous lesions in these pigs at slaughter 5–6 months later decreased from 16.1% to 3.4%. No ACPM was detected on Farm B in 27 samples from the environment. An investigation of surface kaolin mines did not detect mycobacteria in non-extracted kaolin. However, surface water (three isolates among 13 samples) from the pond used for kaolin levigation and 23 batches of the final product of kaolin (10 samples from each batch, i.e. a total of 230 samples were examined) were contaminated with ACPM. Among the latter, ACPM were isolated from three samples originating from three different batches. ACPM were likely to survive during transport of the kaolin, as a suspension through the pipeline, and during its further processing to the final product (sedimentation, addition of colloid substances, drying and other procedures).

**Keywords:** economic losses; veterinary meat inspection; *Mycobacterium avium* complex; zoonosis; food safety

Detection of tuberculous lesions in the organs of slaughtered pigs results in the condemnation of the affected organs or whole pig carcasses. Therefore tuberculosis in pigs still causes serious economic losses in agriculturally developed countries (Dey and Parham, 1993; Margolis et al., 1994; Morita et al., 1994a,b; Sigurdardottir et al., 1994; Cvetnic et al., 1998). Although the conditionally edible and condemned pig carcasses due to the tuberculous lesions decreased in the Czech Republic in the period 1999–2002 by more than 60 percent in comparison with 1995–1998 period (Kozak et al., 2003), the number of affected pigs is still high. Between 1990 and 1999, economic losses per slaughter pig with detected tu-

berculous lesions reached up to 24% of the slaughter price in the Czech Republic (Pavlik et al., 2003).

The incidence of tuberculous lesions in pigs in 1990s in the Czech Republic remained fairly stable (Pavlik et al., 2003). That situation persisted, although bovine tuberculosis had been controlled in 1968 (Pavlik et al., 1998) and the last detection of *Mycobacterium bovis* was registered in one small infected farm with cattle and domestic pigs in the year 1995 (Pavlik et al., 2002a,b). In the mid-1990s, an increased incidence of tuberculous lesions was recorded in domestic pigs in situations where sawdust was used as deep bedding (Pavlik et al., 2003; Matlova et al., 2004b).

In the late 1990s, farmers began to use novel feed supplements, such as peat, with the aim of reducing economic losses caused by diarrhea in piglets after birth and after weaning. However, the peat was contaminated with atypical conditionally pathogenic mycobacteria (ACPM), which were also isolated from pig tuberculous lesions (Matlova et al., 2004a). Members of the *M. avium* complex (MAC), particularly *M. avium* subsp. *hominissuis*, were detected in the peat supplement and in drinking water and the external environment (Pavlik et al., 2003; Matlova et al., 2003, 2004a). ACPM were also detected in invertebrate animals to a lesser extent (Fischer et al., 2001, 2003a,b) and in small terrestrial mammals (Fischer et al., 2000) trapped on pig farms.

Due to its absorbent capability and non-toxic nature, kaolin is considered a simple and effective means of preventing the adverse effects of many toxic agents, not only in the environment but also in living organisms (Trckova et al., 2004). Kaolinite is a raw material consisting particularly of the clay mineral kaolin. The mined raw material contains 20 to 30% kaolin; the rest is siliceous sand.

Kaolin-based medication is commonly used for the treatment of diarrhea and digestive problems in humans (Knezevich, 1998). A number of studies confirmed kaolin's ability to decontaminate aflatoxin (Abdel-Wahhab et al., 1999; Madden et al., 1999; Phillips, 1999), plant metabolites (alkaloids and tannins), enterotoxins (Dominy et al., 2004), pathogenic microorganisms, heavy metals (Hassen et al., 2003) and toxins (Knezevich and Tadic, 1994). *M. a. hominissuis* and other slowly growing non-specified mycobacteria have been isolated from kaolin mined in southern Moravia and used as a feed supplement for pigs in the Czech Republic (Matlova et al., 2003).

The purpose of the present study was to assess the significance of kaolin as a potential source of mycobacterial infections for pigs from Farm A, where a relatively high frequency of tuberculous lesions had been detected in the head and mesenteric lymph nodes from pigs. Kaolin was used on this farm as a feed supplement for the treatment of diarrhea in piglets after birth and after weaning. Farm B was managed similarly but did not have a high incidence of diarrhea and did not use kaolin. Other samples from the pigs' environment and samples from the kaolin mine were also examined in order to identify other potential sources of infection.

## MATERIAL AND METHODS

### Pig herds on Farms A and B

**Farm A.** Thirty-five boars and 800 sows, with an average monthly production of 1 800 weaned piglets, were kept on the farm. The animals were kept in four air-conditioned sheds from which birds as potential carriers of the causal agent of avian tuberculosis were excluded. Pigs were supplied with dried feed from a storage feed tank by means of a pipeline; contamination with bird faeces or dead birds was excluded. Tuberculin skin tests with avian tuberculin (AVITUBAL for simultaneous test produced by Bioveta Ivanovice na Hane, Czech Republic, 14 000 TU/ml, with the dose of 0.2 ml per animal) were performed in the breeding herd.

As the occurrence of diarrhea in piglets after birth and after weaning had increased on the farm, the farmer decided to use kaolin from nearby kaolin mine as a feed supplement. Kaolin was brought to the farm from the factory situated near the mine in sealed plastic bags (20 kg in each bag) and was stored in a dry storage room before use. Kaolin was not stored for longer than two months.

The piglets were fed kaolin after birth as follows: From Day 1 at about 0.5 kg/litter/day and from Day 5 or 6 at about 1 kg/litter/day combined with feed (prestarter), both in the piglets' trough for and on the pen. After the age of three weeks, provided the piglets were able to intake other feed, kaolin was gradually withdrawn. After weaning, kaolin was fed on the floor in the box.

**Farm B.** The same system of rearing and feeding piglets was used on Farm B. However, as the occurrence of diarrheal diseases in piglets was not very high, kaolin was not used. A tuberculin skin test with avian tuberculin (AVITUBAL for simultaneous skin test, 14 000 TU/ml with the dose of 0.2 ml) was carried out in 25% animals of the breeding herd, i.e. in 80 sows (among a total of 320 sows) and all 15 boars.

### Kaolin mining

Kaolin production from the extracted kaolinite or kaolinite-illitic gritstone or pudding-stone at the Moravian mine (U-ZN) was as follows: the superficial soil layer (about 50 cm) was removed and the raw material was floated to a suction pump by a

water cannon and transported to the factory in the form of dense suspension through a pipeline, about 150 m long and about 20 cm in diameter. Water originating from the small pond formed at the surface of the mine where the water cannon was used to wash off the raw material from the mine wall (Trckova et al., 2004). Samples for culture were collected from various stages of production, which consisted of rinsing and separating the kaolin according to the size of the particles, kaolin sedimentation and addition of colloid matter, and kaolin dehumidification in wire baskets.

### Material examined for the presence of mycobacteria

**Pig lymph nodes.** Fattened pigs from both the farms were slaughtered at an average weight of 115 kg. The veterinary inspection of slaughtered pigs from Farms A and B was conducted in accordance with the currently accepted standards of the Czech Republic, as described previously (Pavlik et al., 2003). Subsequently, pigs were divided into three groups according to localization of tuberculous lesions in lymph nodes: group 1 (tuberculous lesions found only in head lymph nodes), group 2 (tuberculous lesions found only in mesenteric lymph nodes) and group 3 (tuberculous lesions were detected in both head and mesenteric lymph nodes from one animal). Lymph nodes from 20 pigs with tuberculous lesions and seven pigs without tuberculous lesions were examined by culture for the presence of mycobacteria.

**Environmental samples from stables.** The samples for investigation of ACPM in the pig shed and external environment comprised 20 samples from Farm A (spider's webs, dust, feed concentrates with kaolin, faeces from boxes, and non-used kaolin) and 27 samples from Farm B stable environment (drinking water, dust, feed, and faeces from boxes).

**Kaolin. Farm A.** Seven samples of kaolin used as a feed supplement and three samples of feed combined with kaolin were examined.

**Mine U-ZN.** Non-extracted kaolin (3 samples) from a surface mine, kaolin from different steps of raw material processing after levigation and extraction from the mine (10 samples), water from the pond (13 samples) used for kaolin levigation and 10 samples from each of 23 batches of the finished product (a total of 230 kaolin samples) were examined for the presence of mycobacteria.

### Methods

**Culture examination for mycobacteria.** The methods of collection of biological materials in the slaughterhouse, the system of laboratory diagnosis of mycobacterial infections in the animals and the culture examinations of the organs for the presence of mycobacteria have been described previously (Pavlik et al., 2003). Firstly, samples from external environment and kaolin (approximately 5 g) were shaken in 30 ml of sterile distilled water and then left standing undisturbed at room temperature for 30 minutes. Heavy particles of the samples sedimented and 5 ml of suspension was collected from the upper part of the mixture. After centrifuging for 10 min at 2 500 to 3 000 rpm, samples were processed in the same way as tissues are routinely incubated at 25°C and 37°C (Matlova et al., 2003).

**Mycobacteria identification.** After microscopic examination for acid-fast rods (AFR) by Ziehl-Neelsen staining, all of the mycobacterial isolates were examined by PCR for the detection of: (i) *dnaJ* gene specific for genus *Mycobacterium* (Nagai et al., 1990), (ii) insertion sequence IS901 (Kunze et al., 1992) specific for *M. avium* subsp. *avium* of serotypes 1, 2, and 3, which are fully virulent for birds (Pavlik et al., 2000) and (iii) insertion sequence IS1245 (Guerrero et al., 1995) for the detection of *M. a. hominissuis* of genotype IS901– and IS1245+ and serotypes 4 to 6, 8 to 11, and 21 (Pavlik et al., 2000; Mijs et al., 2002; Pavlik et al., 2003). All MAC isolates were examined by serotyping (Wolinsky and Schaefer, 1973) modified by Sussland and Hrdinova (1976). The other mycobacterial isolates were identified by biochemical methods (Wayne and Kubica, 1986).

### Statistical analysis

The chi<sup>2</sup>-test (Stat Plus) was applied for the statistical evaluation of the results (Matouskova et al., 1992).

### RESULTS

The tuberculin skin tests carried out in the sows and boars with avian tuberculin on Farms A and B were negative in all the animals.

**Tuberculous lesions in the lymph nodes from pigs on Farms A and B.** Among 25 799 slaughtered

Table 1. Tuberculous lesions in lymph nodes of slaughtered pigs (Farm A) fed kaolin as a supplement

Kaolin fed	Month	Animals slaughtered <sup>b</sup>			Group 1 <sup>c</sup>		Group 2 <sup>d</sup>		Group 3 <sup>e</sup>	
		No.	with TB	%	No.	%	No.	%	No.	%
No	I	1 314	5	0.4	4	0.3	1	0.1	0	0
	II	1 456	25	1.7	1	0.1	24	1.6	0	0
Yes <sup>a</sup>	III <sup>f,g</sup>	2 246	465	20.7	127	5.7	256	11.4	82	3.7
	IV	1 450	112	7.7	39	2.7	62	4.3	11	0.8
	V	1 566	180	11.5	45	2.9	124	7.9	11	0.7
	VI	1 500	71	4.7	10	0.7	57	3.8	4	0.3
	VII	2 060	283	13.7	75	3.6	160	7.8	48	2.3
	VIII	1 851	612	33.1	36	1.9	473	25.6	103	5.6
	IX	1 375	94	6.8	9	0.7	54	3.9	31	2.3
No	X	1 690	38	2.3	4	0.2	34	2.0	0	0
	XI	1 840	20	1.1	0	0	20	1.1	0	0
Overall	I–XI	18 348	1 905	10.4	350	1.9	1 265	6.9	290	1.6
No	I, II, IX–XI	7 675	182	2.4	18	0.2	133	1.7	31	0.4
Yes <sup>a</sup>	III–VIII	10 673	1 723	16.1	332	3.1	1 132	10.6	259	2.4

<sup>a</sup>slaughtered pigs fed kaolin as a supplement before weaning (28 days) and 14 to 21 days after weaning

<sup>b</sup>veterinary-meat inspection was carried out in all pigs after slaughter according to the currently accepted regulations (Pavlik et al., 2003)

<sup>c</sup>tuberculous lesions were detected only in head lymph nodes

<sup>d</sup>tuberculous lesions were detected only in mesenteric lymph nodes

<sup>e</sup>tuberculous lesions were detected in both head and mesenteric lymph nodes originating from one animal

<sup>f</sup>investigation of the farm and collection of samples from the environment

<sup>g</sup>collection of samples from lymph nodes of slaughtered pigs in the slaughterhouse

TB = tuberculous lesions

pigs from Farms A and B (Tables 1 and 2) during the 11 months of investigation, no tuberculous lesions were detected either in the lungs or in parenchymatous organs (liver, spleen, kidneys, etc.).

Tuberculosis was detected in the lymph nodes of pigs from Farm A (Table 1, Figure 1) more frequently (10.4% of 18 348 slaughter pigs) than on Farm B (1.1% of 7 451 slaughter pigs;  $P < 0.01$ ; Table 2, Figure 1). The occurrence of tuberculous lesions in pigs from Farm A slaughtered over five months (I, II, IX to XI) which had not been fed kaolin as a supplement was compared with a six month period (III to VIII) during which pigs had been fed kaolin as a supplement (Table 1). Tuberculous lesions were detected more frequently ( $P < 0.01$ ) in the lymph nodes from pigs fed kaolin (16.1% of 10 673 slaughter pigs) than in pigs which were not fed kaolin as a supplement (2.4% of 7 675 slaughter pigs).

Tuberculous lesions were consistently found more commonly in intestinal lymph nodes than in head lymph nodes on Farm A (Table 1). On Farm A, the highest frequency of tuberculous lesions was recorded in the intestinal lymph nodes in months III and VIII. On Farm B, the highest frequency of tuberculous lesions was detected in month III.

**Mycobacterial isolates from pig lymph nodes.** From Farm A, *M. a. hominissuis* of genotype *dnaJ*+, IS901– and IS1245+ and serotype 8 was isolated from two of 20 pigs with detected tuberculous lesions. In contrast, no mycobacteria were isolated from the lymph nodes that did not have tuberculous lesions from seven pigs (Table 3).

**Isolation of mycobacteria from the environment of the stables.** Farm A. Among 20 samples from the environment of the pig shed on Farm A, four isolates were detected: *M. a. hominissuis* of genotype *dnaJ*+, IS901– and IS1245+ and of serotype 8 only in

Table 2. Tuberculous lesions in lymph nodes from slaughtered pigs from Farm B

Kaolin Fed	Month	Animals slaughtered <sup>b</sup>			Group 1 <sup>c</sup>		Group 2 <sup>d</sup>		Group 3 <sup>e</sup>	
		No.	with TB	%	No.	%	No.	%	No.	%
	I	613	0	0	0	0	0	0	0	0
	II	532	8	1.5	5	0.9	3	0.6	0	0
	III	690	31	4.5	0	0	31	4.5	0	0
	IV	580	2	0.4	1	0.2	1	0.2	0	0
	V	940	13	1.4	2	0.2	10	1.1	1	0.1
No <sup>a</sup>	VI	467	2	0.4	1	0.2	1	0.2	0	0
	VII	955	23	2.4	4	0.4	18	1.9	1	0.1
	VIII	700	4	0.6	1	0.1	2	0.3	1	0.1
	IX	618	0	0	0	0	0	0	0	0
	X	706	0	0	0	0	0	0	0	0
	XI	650	0	0	0	0	0	0	0	0
Overall	I–XI	7 451	83	1.1	14	0.2	66	0.9	3	0 <sup>f</sup>

<sup>a</sup>slaughtered pigs were not fed kaolin as a supplement

<sup>b</sup>veterinary-meat inspection was carried out in all pigs after slaughter according to the currently accepted legislation (Pavlik et al., 2003)

<sup>c</sup>tuberculous lesions were detected only in head lymph nodes

<sup>d</sup>tuberculous lesions were detected only in mesenteric lymph nodes

<sup>e</sup>tuberculous lesions were detected in both head and mesenteric lymph nodes originating from one animal

<sup>f</sup>0.04%

TB = tuberculous lesions

kaolin supplemented feed, *M. fortuitum* in the dust from the stables and pig faeces from the boxes and a slowly growing isolate which was not a member of the MAC and was not further identified, in the dust from the stable (Table 3).

*Farm B*. Among 27 environmental samples from Farm B, no mycobacteria were isolated (Table 3).

**Mycobacteria isolation from kaolin from the mine.** Among 256 kaolin samples from the mines, the processing plant and the finished products,

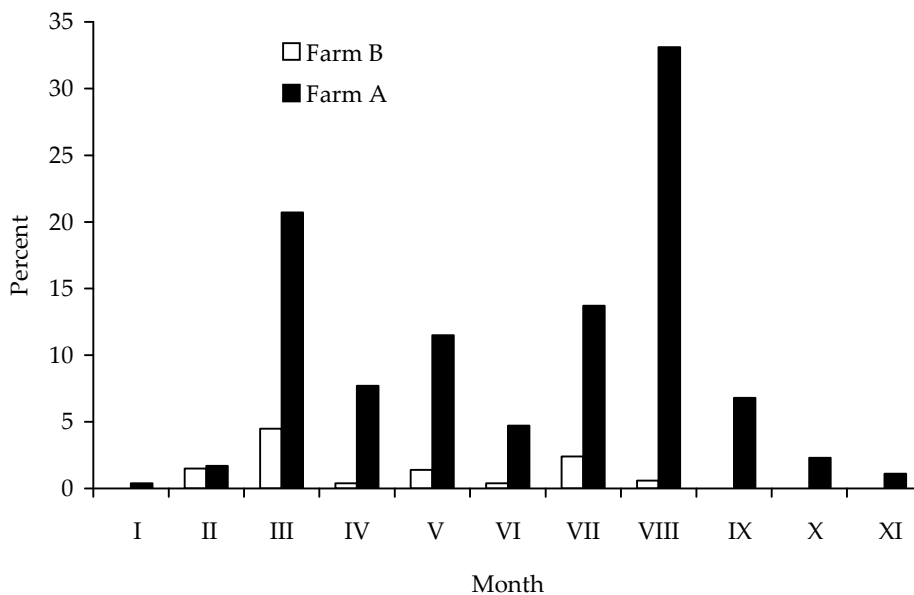


Figure 1. Occurrence of tuberculous lesions in slaughtered pigs from farms A and B

Table 3. Isolation and identification of mycobacterial isolates from pig lymph nodes, kaolin and environment from the Farms A and B

Locality	Samples examined			No. of mycobacterial isolates		
	Sample	No.	Positive	<i>M. a. hominissuis</i> <sup>a</sup>	<i>M. fortuitum</i>	Others <sup>b</sup>
Farm A	Spider webs	1	0	0	0	0
	Dust	6	2	0	1	1
	Feed with kaolin	3	1	1 <sup>e</sup>	0	0
	Faeces in boxes	3	1	0	1	0
	Non-used kaolin	7	0	0	0	0
	Subtotal	20	4	1 <sup>e</sup>	2	1
	%	100	20.0			
	Lymph nodes (+) <sup>c</sup>	20	2	2 <sup>e</sup>	0	0
	Lymph nodes (-) <sup>d</sup>	7	0	0	0	0
	Subtotal	27	2	2	0	0
%	100	7.4				
Farm B	Drinking water	16	0	0	0	0
	Dust	3	0	0	0	0
	Feed	3	0	0	0	0
	Faeces in boxes	5	0	0	0	0
	Subtotal	27	0	0	0	0
	%	100	0	0	0	0
Kaolin Mine U-ZN	Mined	3	0	0	0	0
	Sediment in factory	10	0	0	0	0
	Water pond	13	3	0	0	3
	Final product <sup>f</sup>	230	3	1 <sup>e</sup>	0	2
	Subtotal	256	6	1 <sup>e</sup>	0	5
%	100	2.3				

<sup>a</sup>*Mycobacterium avium* subsp. *hominissuis* of serotypes 4–6, 8–11 and 21 and genotype IS901– and IS1245+ (Pavlik et al., 2000; Mijs et al., 2002)

<sup>b</sup>other mycobacterial species than *M. avium* complex including *M. a. hominissuis*, *M. a. avium* and *M. intracellulare* and *M. fortuitum*

<sup>c</sup>pig lymph nodes with tuberculous lesions (one lymph node examined from each pig)

<sup>d</sup>pig lymph nodes without tuberculous lesions (one lymph node examined from each pig)

<sup>e</sup>*Mycobacterium avium* subsp. *hominissuis* of serotype 8 and genotype *dnaJ*+, IS901– and IS1245+

<sup>f</sup>from each of 23 batches of final product, 10 samples were examined

*M.* = *Mycobacterium*

mycobacteria were detected in six (2.3%) samples. *M. a. hominissuis* (serotype 8; genotype *dnaJ*+, IS901– and IS1245+) was found in one sample from one kaolin batch. Five isolates of slow-growing

non-MAC mycobacteria were detected in pond water from the mine surface and in two samples of kaolin from two different batches of the product (Table 3).

## DISCUSSION

Kaolin was suspected of being a source of mycobacteria after the initial visit to Farm A in month III and to the kaolin mine. It was apparent that the potential existed risk for the surface water, used for levigation of the extracted raw material, to have been contaminated with ACPM (Kazda, 2000; Lescenko et al., 2003; Matlova et al., 2003). The farmer immediately ceased using kaolin as a feed supplement for pigs.

Nevertheless sharp differences in the incidence of tuberculous lesions in lymph nodes existed between months III to VIII, when the pigs were being fed kaolin and were slaughtered (Table 1). This incidence may indicate the irregular distribution of mycobacteria in the kaolin. This was shown during the examination of batches of kaolin, when mycobacteria were isolated from only one sample of kaolin within three sets examined out of 23. On the other hand, in the period when kaolin stopped being added to the pig feed, in month IX a relatively high incidence of tuberculous lesions in lymph nodes (6.8%) continued to be found. This incidence may indicate the feeding of remnants of the kaolin to the pigs or indeed infection from an environment contaminated by kaolin (troughs, equipment used to prepare feed). At the same time, the farmer did not undertake any other changes in farm management that may have affected mycobacterial incidence.

Like Farm A, Farm B also saw a weak occurrence of tuberculous lesions in slaughtered pigs in the period from month II to month VIII (Table 2). We were unfortunately unable to establish its cause, since the examination of samples from the external environment for mycobacteria proved negative. We may therefore assume that the cause was poor zoohygienic conditions on Farm B, or perhaps the use of kaolin in feed, about which we were not informed.

The piglets that had been fed the supplement were fattened for the next five months up to the slaughter weight of 115 kg. The much higher frequency of tuberculous lesions in the lymph nodes from pigs on Farm A compared with Farm B ( $P < 0.01$ ) and the higher occurrence of tuberculous lesions in pigs fed kaolin on Farm A ( $P < 0.01$ ) supported the suspicion that kaolin was the source of mycobacteria.

The risk of tuberculous lesions in pig lymph nodes caused by the ACPM occurring in kaolin is considerable, as detected on Farm A (Table 1). In contrast to peat, which is likewise used by farmers as a feed supplement (Matlova et al., 2004a), only

one *M. a. hominissuis* isolate (Table 3) was detected in kaolin on Farm A, investigated in our study. On this farm peat had not been used previously in feed. The low frequency of *M. a. hominissuis* and other ACPM findings in kaolin (2.3%) was reflected in the rather low frequency of tuberculous lesions in lymph nodes from the pigs (16.1%) in comparison with the frequency of *M. a. hominissuis* and other ACPM findings in peat (73.4%) where the frequency of tuberculous lesions in lymph nodes from the pigs was 41.0% (Matlova et al., 2004a).

The same causal agent (*M. a. hominissuis* of genotype *dnaJ*+, IS901– and IS1245+ and serotype 8) was isolated from pig lymph nodes from kaolin samples and the feed supplemented with kaolin (Table 3). When kaolin feeding ceased being used as a supplement for pigs, there was a sharp decline in the subsequent occurrence of tuberculous lesions in the lymph nodes at slaughter (Table 1); indicating that the ACPM source was most likely the kaolin from mine U-ZN. Although mycobacteria were not detected in the mined raw material, it was most probably contaminated with ACPM in the surface water from the pond during kaolin levigation (Table 3). Surface water has been shown to be a major ACPM source (Beerwerth, 1973; Kazda, 2000; Matlova et al., 2003). Other sources of ACPM might also be considered, such as dust, soil layer above kaolin, bird faeces and other risk raw materials (Beerwerth and Schurmann, 1969; Dawson, 1971; Beerwerth and Kessel, 1976a,b).

In our study no animals were found on Farm A or B which reacted following skin testing with avian tuberculin because the pigs were not infected with *M. a. avium* (serotype 1–3, genotype *dnaJ*+, IS901+, IS1245+). Nevertheless in the case of a mass infection with ACPM, non-specific reactions after skin testing with avian tuberculin may be expected in female piglets, sows and other categories of pigs reared in this way, given the stimulation of cell immunity after ingesting them with feed or water.

Contamination of kaolin both with *M. a. hominissuis*, which was most frequently isolated from pigs (Pavlik et al., 2000), *M. fortuitum* species and other ACPM, may result in formation of tuberculous lesions in the lymph nodes (Matlova et al., 2003).

Since extracted kaolin is a mineral with a minimum content of nutrients and no nutrients are purposely added during its production, contamination with ACPM may be controlled during production by immediately packing it in airtight bags. The risk

may be reduced further during the long-term storage of kaolin below 18°C, as propagation of ACPM in the external environment does not occur under those conditions (Kazda, 2000). Other measures may include using ACPM-free water for kaolin levigation and heat-treatment during kaolin processing to destroy ACPM.

Although bovine tuberculosis has been eradicated in the Czech Republic more than 30 years ago, mycobacterioses are still important not only as a cause of paratuberculosis in cattle and other ruminants (Hruska, 2004), but also in pigs. In the Czech Republic in the period 1999–2002 there was found a rapid decrease of amount of tuberculous lesions in pig carcasses (Kozak et al., 2003), which could be affected by better knowledgeability of sources of mycobacteria (peat, kaolin, drinking water and others) in stable environment (Matlova et al., 2004a,b).

Molecular studies have found that humans, especially immunocompromised patients, and pigs have been infected from the same environmental MAC (Komijn et al. 1999; Pavlik et al., 2000). Although it is not known if pigs are a source of human MAC infection, care should be taken when using feed supplements, such as peat and clay minerals (kaolin, bentonite, and zeolite) that may increase the risk of pigs becoming infected with MAC. Human infections caused by the members of MAC are considered serious diseases very often resistant to and tituberculous treatment (Dvorska et al., 2002; Bartos et al., 2004).

## CONCLUSIONS

1. Our investigation confirmed that *M. a. hominis-suis* and ACPM caused the formation of tuberculous lesions in pig lymph nodes. It was found that a new source of these mycobacteria was kaolin floated in water contaminated with *M. a. hominis-suis* and ACPM.

2. The source of mycobacteria contaminating the kaolin was water used for floating, therefore it is necessary to warn the breeder that this raw material may be used only if, during the production of the kaolin, such temperatures are reached that ACPM is securely devitalised. In view of the possible additional contamination of kaolin from the environment during transport or storage in a warm and damp environment, it is more appropriate to disinfect it shortly before use in feed.

3. In view of the fact that after the ingestion of atypical mycobacteria, cell immunity is stimulated, non-specific reactions may be expected in female piglets, sows and other categories of pigs reared in this way after skin testing with avian tuberculin.

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