Mycobacterial infections in cattle and pigs caused by *Mycobacterium avium* complex members and atypical mycobacteria in the Czech Republic during 2000–2004

I. Pavlik[1], L. Matlova[1], L. Dvorska[1], J.E. Shitaye[1], I. Parmova[1,2]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]State Veterinary Diagnostic Institute, Prague, Czech Republic

ABSTRACT: Bovine tuberculosis was last detected in cattle and pigs in the Czech Republic in 1995. Since March, 31, 2004 (Commission Decision No. 2004/320/EC) the Czech Republic has been included amongst states free from bovine tuberculosis within the European Union. The purpose of the present study was to evaluate results of intravital and post-mortem diagnosis of mycobacterial infections in slaughtered cattle and pigs from 2000 to 2004. When bovine tuberculosis in cattle was investigated, a tuberculin skin test with bovine tuberculin was performed every year and a skin test with avian tuberculin was simultaneously conducted in the animals with a positive response. A total of 2 419 889 animals were examined with a positive response being found in 123 (0.005%) of them. After slaughter, bovine tuberculosis was not detected in any of these animals by gross and/or laboratory examinations. With avian tuberculin, 40 349 animals were tested and positive responses were detected in 43 (0.1%) of them; the incidence was similar in all the years monitored. Tuberculous lesions were detected in 209 (0.01%) of 1 967 211 slaughtered cattle. Mycobacteria were present in 40 (21.3%) of 188 animals examined by laboratory methods: 26 isolates of *Mycobacterium avium* subsp. *avium* (18 isolates of serotype 2 and 8 isolates not typeable), 11 isolates of *M. a. hominissuis* (1 isolate of serotype 8 and 10 isolates not typeable), and 3 isolates of atypical mycobacteria. Tuberculous lesions were detected in 49 312 (0.22%) of 22 312 580 slaughtered pigs by veterinary-meat inspection. During the 5-year-period monitored, the incidence of tuberculous lesions decreased from 0.37% in 2000 to 0.10% in 2004. The following mycobacteria were isolated from 757 (33.5%) of 2 261 animals whose organs were examined by culture: 203 isolates of *M. a. avium* (180 isolates of serotype 2, 3 isolates of serotype 2/8, and 20 isolates not typeable), 442 isolates of *M. a*. *hominissuis* (1 isolate of serotype 1, 262 isolates of serotype 8, 35 isolates of serotype 9, 1 isolates of mixed serotypes 8/9, and 143 isolates not typeable), and atypical mycobacteria (*n* = 112). In both animal species, *M. avium* complex members prevailed: *M. a. avium* was the most prevalent subspecies in cattle, *M. a. hominissuis* in pigs. The main sources of *M. a. avium* and *M. a. hominissuis* are free living birds and contaminated external and stable environments (i.e. drinking water, feeds, and feed supplements), respectively. During the entire period monitored, miliary or generalized tuberculosis was not detected in any of the animals. The decreased incidence of tuberculous lesions in pigs was particularly a result of preventive measures adopted to control the occurrence of atypical mycobacteria.

Keywords: *Mycobacterium bovis*; IS*901*; IS*1245*; zoonosis

In the Czech Republic, bovine tuberculosis in cattle was brought under control in 1968. The incidence of bovine tuberculosis was gradually decreased in the subsequent post-elimination period. The last outbreak was found in 1995 (Pavlik et al., 1998, 2002a,b,c). The incidence of bovine tuberculosis was reduced not only in cattle, but also in other domestic animals. Bovine tuberculosis was

last detected in 1995 in pigs, and in 2002 it was diagnosed in two camels from the Prague zoological garden (Pavlik et al., 2002e). Among the isolated members of *Mycobacterium tuberculosis* complex *M. caprae* was identified in cattle in the last outbreak, in one farmed red deer (*Cervus elaphus*) in 1999 and in two camels in 2002 (Pavlik et al., 2002c,e; Erler et al., 2004).

With respect to this favourable situation, the Czech Republic was included among states of the European Union that are officially free from bovine tuberculosis (Anonymous, 2004) on March, 31, 2004. For the first time in recent history general skin testing for bovine tuberculosis was cancelled; nowadays it is only performed in animals prior to transport. However, despite the favourable epizootiological situation in the Czech Republic, tuberculous lesions caused by other pathogens were detected in the lymph nodes from cattle and pigs during the entire post-elimination period. The results of the assessment of incidence of tuberculous lesions in animals between 1990 and 1999, show that *M. a. avium* prevailed among the isolates from cattle (Pavlik et al., 2002d; Dvorska et al., 2004). The main source was predominantly free living birds (Pavlik et al., 2000) and less frequently small terrestrial mammals (Fischer et al., 2000). Standardized IS*901* RFLP analysis (Dvorska et al., 2003) of mycobacterial isolates revealed wide heterogeneity of *M. a. avium*. This provides evidence that a wide spectrum of sources exist in the Czech Republic. The causative agent of paratuberculosis *M. a. paratuberculosis* was isolated from one mesenterial lymph node from one animal (Dvorska et al., 2004).

Some of the primo-isolates showing combined serotypes of both subspecies *M. a. avium* and *M. a. hominissuis* were cloned to independent colonies. IS*901* RFLP analysis of *M. a. avium* and IS*1245* standardized RFLP analysis (Van Soolingen et al., 1998) of *M. a. hominissuis* isolates showed that some of the animals were simultaneously infected with both these subspecies (Dvorska et al., 2004).

In contrast, *M. a. hominissuis* isolated from external environment in particular (Matlova et al., 2003), was most frequently isolated from pigs (Pavlik et al., 2003). Between the years 1990 and 1999, incidence of tuberculous lesions in lymph nodes from pigs increased in three periods. In the early 1990s, it was mainly avian tuberculosis that was recorded in herds, which was likely to be in association with transformed agriculture. The following increase

in 1995 was principally caused by the use of deep bedding containing sawdust and other wood waste. Various enzymes (such as Envistim) were applied for the biological degradation of wooden material; however, those were not able to devitalise or prevent propagation of atypical mycobacteria, which were subsequently isolated from samples of saw dust, deep litter, and lymph nodes of pigs with tuberculous lesions (Matlova et al., 2003, 2004a,b).

The last increase in the incidence of tuberculous lesions was recorded in pigs, between the years 1998 and 1999, when piglets started to be fed diets containing peat as a supplement. Peat was contaminated with atypical mycobacteria (particularly *M. a. hominissuis*) and reached up to 70 to 80% as detected in our previous study (Matlova et al., 2003). IS*1245* RFLP analysis revealed that the isolates of identical RFLP type were detected both in peat fed as a supplement and lymph nodes from pigs (Matlova et al., 2005).

From an epizootiological aspect, it is necessary to constantly monitor the occurrence of tuberculous lesions in cattle. Therefore, our attention was concentrated on the evaluation of statistical data obtained by intravital and post-mortem examinations of animals between 2000 and 2004. We likewise focused on the identification of the causative agents of tuberculous lesions. Last, but not least, it was also necessary to evaluate the results of skin tests with bovine and avian tuberculins performed on cattle because general tuberculin skin tests with bovine tuberculin in cattle have been discontinued since January, 1, 2005 due to the favourable epidemiologic situation.

The purpose of the present study was to analyse the results of intravital and post-mortem diagnosis of bovine tuberculosis in cattle and pigs between 2000 and 2004.

MATERIAL AND METHODS

Sources of statistical data

Statistical data on the results of tuberculin tests and examinations performed in veterinary laboratories were periodically evaluated by the State Veterinary Administration towards December, 31st of respective years. Tuberculin tests were performed yearly in 50% of cattle older than 24 months. In the monitored period before December 31st, 2001, tuberculin was produced from a strain of *M. bovis*

(AN 5) designated BOVITUBAL (14 000 TU/ml) and avian tuberculin was produced from a strain *M. a. avium* (D 4 ER) designated AVITUBAL for simultaneous skin tests (14 000 TU/ml) in a dose of 0.2 ml. From January 1st, 2002 to December 31st, 2004 the same tuberculin types were used, however the concentration was increased 28 000 TU/ml and the dose was reduced to 0.1 ml. The results obtained after a simple tuberculin skin test with bovine tuberculin were described previously (Pavlik et al., 2002d). To summarise: under single intradermal tuberculin testing with bovine tuberculin, a reaction number (RN) higher than or equal to 4.0 mm was considered a positive reaction, an RN between 2.0 and 3.9 mm as doubtful and a reaction number less than 1.9 mm as negative.

When a positive or dubious response to bovine tuberculin was investigated, bovine and avian tuberculin were concurrently used in simultaneous tuberculin skin tests performed at least 42 days apart. The results were described and explained in our previous study (Pavlik et al., 2002d).

Summary: Positive: reaction to bovine tuberculin exceeded by more than 4 mm the reaction to avian tuberculin and clinical symptoms did not appear at the point of application of the bovine tuberculin (diffuse or widespread swelling, exudation, necrosis, soreness or an infectious reaction of corresponding lymph vessels or lymph nodes).

Doubtful: reaction to bovine tuberculin was positive or doubtful and reaction was not more than 4 mm greater than the reaction to avian tuberculin and none of the above-mentioned clinical symptoms appeared.

Negative: reaction to bovine tuberculin was positive, doubtful or negative, but the reaction was the same or less than to avian tuberculin and in both cases none of the above-mentioned clinical symptoms were found.

Histopathology. A total of 62 samples of the lymph nodes (23 from cattle and 39 from pigs) with tuberculous lesions were formalin fixed, embedded in paraffin blocks, and stained by Ziehl-Neelsen (ZN) technique. Histological samples were observed by light microscopy (microscope Olympus B17, Japan).

Veterinary-meat inspection of cattle

Cattle and pigs slaughtered in abattoirs were examined for the presence of tuberculous lesions according to the previously described procedure (Pavlik et al., 2002d). Previous examinations that had been carried out were insufficient and so a detailed veterinary-meat inspection was performed. Suspect organs, adjacent lymph nodes, and regional lymph nodes were incised. Caseated or calcified nodules of various sizes and shapes (most commonly form the size of a poppy up to a pinhead) were noted as tuberculous lesions. All the adjacent organs or parts of the lymph nodes with tuberculous lesions were condemned: for example the intestine was condemned if tuberculous lesions were present in mesenteric lymph nodes and the head was condemned if tuberculous lesions were present in head lymph nodes. When the tuberculous lesions were found in parenchymatous organs or in the head and mesenteric lymph nodes concurrently the whole bodies of animals were adjudged to be conditionally edible after processing, i.e. only as raw material for heat treated products (Pavlik et al., 2002d).

Culture examination for mycobacteria

Collected biological material was either cooled to 4°C and immediately shipped to the laboratory for examination, or samples were frozen to −20°C and delivered to the laboratory within 2 to 3 weeks after collection. Culture examination for the presence of mycobacteria was conducted in the following two laboratories: the Reference diagnostic laboratory for animal tuberculosis of the State Veterinary Institute in Prague, and the Laboratory for Mycobacteriology of the State Veterinary Institute in Brno. The procedure of laboratory examination for mycobacteria has been described previously (Fischer et al., 2000). Only one sample from each animal was examined (respective lymph nodes, parenchymatous organs or their mixed samples).

Identification of mycobacterial isolates

Isolated mycobacterial isolates were examined by Accu Probe (Gen-Probe Incorporated, San Diego, California, USA) with probes for *M. tuberculosis* complex, *M. avium* and *M. intracellulare* isolates. The isolates of *M. avium* complex were further identified by serotyping (Wolinsky and Schaefer, 1973) modified by Süssland and Hrdinova (1976),

or by the PCR method for IS*901* detection (Kunze et al., 1992; Pavlik et al., 2000). Isolates that could not be determined by the above described methods were assessed using biochemical methods (Wayne and Kubica, 1986). Only one isolate from each sample was examined. Virulence of 10 *M. a. avium* and 10 *M. a. hominissuis* isolates in the year 2000 was studied after the intramuscular infection of pullets with 1 to 5 mg of bacterial wet substance per kg of life weight (Pavlik et al., 2000).

RESULTS

Tuberculin test in cattle

Between 2000 and 2004, a total of 2 419 889 skin tests with bovine tuberculin were performed. A positive response was detected in 123 (0.005%) animals. The most commonly performed tests were simultaneous skin tests (bovine and avian tuberculins) with animals giving a positive response to bovine tuberculin being slaughtered. Simultaneous skin tests were performed in 40 349 animals; 43 (0.1%) of them gave positive response (Table 1).

Tuberculous lesions in cattle

From a total of 1 967 211 slaughtered cattle, tuberculous lesions were detected by veterinary-meat meat inspection in 209 (0.011%) of them; incidence of tuberculous lesions detected during the whole period ranged between 0.009% and 0.016% (Table 2).

Laboratory examination of samples from cattle

In all 23 lymph nodes with tuberculous lesions granulomatous inflammation was diagnosed by histopathology examination. Tissue samples from 188 animals were examined; culture examination for mycobacteria was positive in 38 (20.2%) of them. The detection rate of mycobacteria in respective years varied from 17.1% to 33.3%. No member of *M. tuberculosis* complex was isolated from any of the animals. Members of *M. avium* complex were isolated most frequently (92.1% of positive culture examinations); among them, *M. a. avium* and *M. a. hominissuis* were isolated in 63.2% and 28.9%, respectively. The *M. a. avium* to *M. a. hominissuis* ratio showed a decreasing trend from 4.0 in 2001 to 0.4 in 2004. From the other mycobacterial species, one strain of each of *M. terrae*, *M. chelonae*, and *M. phlei* was isolated (Table 2).

Tuberculous lesions in pigs

From a total of 22 312 580 slaughtered pigs, tuberculous lesions were detected in 49 312 (0.22%) of animals; prevalence of tuberculous lesions detected during the whole period ranged between 0.37% and 0.10% in 2000 and 2004, respectively (Table 3).

Laboratory examination of samples from pigs

In all 39 lymph nodes with visible tuberculous lesions granulomatous inflammation was diagnosed

Table 1. Skin testing of cattle with bovine and avian tuberculin (No. of animals)

Year*	Bovine tuberculin			Avian tuberculin		
	tested	positive	%	tested	positive	%
2000	668 850	52	0.0078	13 559	20	0.1475
2001	662 646	30	0.0045	11 586	13	0.1122
2002	391 274	11	0.0028	1 350	10	0.7407
2003	374 625	1	0.0003	1 730	0	0.0000
2004	322 494	29	0.0090	12 124	0	0.0000
Total	2 419 889	123	0.0051	40 349	43	0.1066

*in the hygiene year data was gathered from November of the previous year to October of the year of evaluation

Table 2. Results of the examination of slaughtered cattle for tuberculosis and culture results for mycobacteria

| Year | No. of animals[1] | | | Culture examination[2] | | | Mycobacterial isolates identification[3] | | | | | | Ratio of M. a. avium to M. a. hominissuis |
	slaughtered	TB lesions[4] (histopathology)	%	total	positive	%	total	M. a. avium[5] 2	M. a. avium[5] nt	M. a. hominissuis[5] 8	M. a. hominissuis[5] nt	other spp.	
2000	429 267	69 (5)	0.016	34	7	20.6	7	6	1	0	0	0	0
2001	384 712	39 (3)	0.010	33	11	33.3	11	6	2	0	2	1	4.0
2002	406 878	31 (5)	0.008	35 (4)	7	20.0	7	4	1	0	2	0	2.5
2003	392 686	36 (6)	0.009	49 (13)	7	14.3	7	2	2	1	1	1	2.0
2004	353 668	34 (4)	0.010	37 (3)	8	21.6	8	0	2	0	5	1	0.4
Total	1 967 211	209 (23)	0.011	188 (20)	40	21.3	40	18	8	1	10	3	2.4
%					100		100	45.0	20.0	2.5	25.0	7.5	

[1] in the hygiene year data was gathered from November of the previous year to October of the year of evaluation

[2] culture for mycobacteria was always evaluated from January to December of the relevant calendar year; (4) in parenthesis is given No. of laboratory examined animals with positive skin test to bovine tuberculin but with the absence of tuberculous lesions in lymph nodes or laboratory examined animals with diagnosed suspected adenopathy (without the presence of tuberculous lesions in lymph nodes)

[3] *M. a. avium* (*M. avium* subsp. *avium* of genotype IS901+ and IS1245+); *M. a. hominissuis* (*M. avium* subsp. *hominissuis* isolates of genotype IS901– and IS1245+)

[4] tuberculous lesions in lymph nodes and/or in parenchymatous organs were condemned; (5) No. of tissue samples with granulomatous lesions confirmed by histopathology examination

[5] isolates were identified by serotyping according to Wolinsky and Schaefer (1973) modified by Süssland and Hrdinova (1976)

other spp. = *M. terrae*, *M. chelonae*, and *M. phlei* were isolated

nt = not tested due to auto-agglutination or the growth in R form

Table 3. Tuberculous lesions found in slaughtered pigs during 2000–2004

Year[1]	Total No. of slaughtered pigs	Tuberculous lesions and meat-inspection evaluation					
		without sequences [2]		with sequences [3]		total	
		No.	%	No.	%	No.	%
2000	4 513 596	12 918	0.29	3 798	0.08	16 716	0.37
2001	4 465 822	10 269	0.23	3 226	0.07	13 495	0.30
2002	4 625 445	8 467	0.18	1 614	0.03	10 081	0.22
2003	4 478 756	4 285	0.10	517	0.01	4 802	0.11
2004	4 228 961	1 735	0.04	2 483	0.06	4 218	0.10
Total	22 312 580	37 674	0.17	11 638	0.05	49 312	0.22

[1]in the hygiene year data was gathered from November of the previous year to October of the following year

[2]the result of the examination of the whole bodies of slaughtered pigs was assessed without restrictions (only adjacent organs or parts of the lymph nodes with tuberculous lesions were condemned)

[3]the whole bodies of slaughtered pigs were adjudged according to Supplement No. 2 from September 16th, 1996 of SVA CR Directive No. 1 from September 21st, 1989 as consumable with restrictions (conditionally edible as processed, i.e. intended in the raw state for use in all heat-treated products)

by histopathology examination. From a total of 2 261 pigs examined by laboratory methods, mycobacteria were isolated from 757 (33.5%) animals. Culture positivity increased from 28.8% in 2000 to 36.3% in 2004. No *M. tuberculosis* complex member was isolated either from pig or cattle samples. Members of *M. avium* complex were most commonly isolated in respective monitored years. All of 10 *M. a. avium* isolates were fully virulent for pullets after the intramuscular infection and caused miliary tuberculosis in parenchymatous organs during the 4 to 8 weeks period. All of 10 *M. a. hominissuis* isolates were partially virulent for pullets and caused tuberculous lesions only at the point of inoculation after 8 weeks period. During whole investigated period, members of subspecies *M. a. avium* and *M. a. hominissuis* isolates represented 85.2%. The *M. a. avium* to *M. a. hominissuis* ratio was shown to range between 0.2 and 0.6 (Table 4).

DISCUSSION

In the monitored period between 2000 and 2004 the number of animals with positive reactions was comparable with those detected by tuberculin skin tests between 1990 and 1999 (Pavlik et al., 2002d), with the exception of the year 2003, when only one (0.0003%) animal gave a positive response. The prevalence of tuberculous lesions in cattle in the monitored period since 2000 to 2004 is likewise comparable with the frequency of findings between 1990 and 1999 (Table 1; Pavlik et al., 2002d). However, from 1996 onwards, the number of animals examined by culture was lower than the number of animals with detected tuberculous lesions (Tables 2 and 3; Pavlik et al., 2002d, 2003). That situation can be viewed as non-sufficient from the aspect of potential detection of the pathogen causing tuberculous lesions in cattle.

It is evident from the results obtained by laboratory examinations of tissues shown in Table 2 that the number of animals with detected tuberculous lesions was higher than the number of animals examined in the laboratory. It is necessary to view this fact as highly risky, similarly as in the previously analysed period between 1990 and 1999 (Pavlik et al., 2002d). It is necessary to consider absence of any *M. tuberculosis* complex member in the examined samples as highly favourable from an epidemiological aspect (Table 2). It is well-known that *M. tuberculosis* was isolated e.g. from cattle and pigs in the other states of Central Europe (Pavlik et al., 2002a,e, 2005) and *M. africanum* was isolated from one antelope in Croatia (Pavlik et al., 2005).

The number of animals with tuberculous lesions did not exceed 0.37% (Table 3) in any of the monitored years; this can be viewed as highly favourable in comparison with the previous decade (Pavlik et al., 2002d, 2003). Decreasing trend of prevalence

Table 4. Laboratory examination for mycobacterial infections in pigs

Year	No. of examined pigs[1]			Isolated mycobacterial species[2]										Ratio of
	total	positive	%	total No.	*M. a. avium*[3]			*M. a. hominissuis*[3]					other spp.	*M. a. avium* to *M. a. hominissuis*
					2	2/8	nt	4	8	8/9	9	nt		
2000	459	132 (7)	28.8	132	47[4]	0	0	1	48[5]	0	11[6]	11	14	0.8
2001	426	142 (9)	33.3	142	30	3	1	0	52	0	7	37	12	0.4
2002	385	131 (9)	34.0	131	20	0	0	0	55	1	4	31	20	0.2
2003	437	151 (8)	34.6	151	33	0	6	0	43	0	6	32	31	0.5
2004	554	201 (6)	36.3	201	50	0	13	0	64	0	7	32	35	0.6
Total	2 261	757 (39)	33.5	757	180	3	20	1	262	1	35	143	112	0.5
%				100	23.8	0.4	2.7	0.1	34.6	0.1	4.6	18.9	14.8	

[1]examined tissue samples originated from animals with tuberculous lesions and/or from animals skin testing positive for bovine or avian tuberculosis; (7) No. of tissue samples with granulomatous lesions confirmed by histopathology examination

[2]*M. a. avium* (*M. avium* subsp. *avium* isolates of genotype IS901+ and IS1245+); *M. a. hominissuis* (*M. avium* subsp. *hominissuis* isolates of genotype IS901– and IS1245+)

[3]isolates identified by serotyping according to Wolinsky and Schaefer (1973) modified by Süssland and Hrdinova (1976); 2/8 and 8/9 (mixed serotypes were detected)

[4]virulence for pullets after the intramuscular infection was tested in 10 isolates, which were fully virulent and caused miliary tuberculosis in parenchymatous organs during the 4 to 8 weeks period

[5]virulence for pullets after the intramuscular infection was tested in 8 isolates, which were partially virulent for pullets and caused tuberculous lesions only at the point of inoculation after 8 weeks period

[6]virulence for pullets after the intramuscular infection was tested in 25 isolates, which were partially virulent for pullets and caused tuberculous lesions only at the point of inoculation after 8 weeks period

other spp. = *M. terrae*, *M. chelonae*, *M. phlei*, *M. fortuitum* etc. were isolated

nt = not tested due to auto-agglutination or the growth in R form

of tuberculous lesions in respective herds from 0.37% in 2000 to 0.10% in 2004 (Table 3) gives evidence of effective prevention against animal contamination with atypical mycobacteria from the environment in respective pig herds. More frequent findings of tuberculous lesions in pigs in comparison with cattle might have been caused by higher susceptibility of pigs to mycobacterial infections as observed in our previous studies (Pavlik et al., 2002d, 2003).

Detection of *M. avium* complex members prevailed in both animal species: *M. a. avium* was most commonly isolated from cattle; the sources of this pathogen are particularly free living birds. *M. a. hominissuis* was mostly isolated from pigs; the source of this pathogen is particularly contaminated external and stable environment: drinking water, feeds, and feed supplements such as peat and kaolin (Engel et al., 1977; Songer et al., 1980; Windsor et al., 1984; Gardner and Hird, 1989; Horvathova et al., 1997; Dürrling et al., 1998; Matlova et al., 2003, 2004a, 2005; Trckova et al., 2004). Various small terrestrial mammals and invertebrate species are further reservoirs of *M. avium* complex associated with cattle and pig infections (Fischer et al., 2000, 2001, 2003a,b, 2004a,b). During the entire monitored period, miliary or generalized tuberculosis was not observed in any of the animals. Due to prevention of atypical mycobacteria occurrence in pig herds, the frequency of findings of tuberculous lesions in pigs was reduced.

Tuberculoid lymph node lesions both in pigs and cattle may be also caused by other bacterial species such as *Rhodococcus equi* (Dvorska et al., 1999). Prevalence of *R. equi* and atypical mycobacteria in the lymph nodes of pigs and cattle was studied in the Czech Republic during the period 1996–1999 (Dvorska et al., 1999; Pavlik et al. 2003). *R. equi* alone was isolated from 8.8% of pigs, and in another 2.8% of pigs and 1.7% of cattle in a mixed infection with atypical mycobacteria. The frequency of *R. equi* was higher in pigs than in cattle (Dvorska et al., 1999). Isolation of *R. equi* from mesenteric lymph nodes predominated in both animal species (Dvorska et al., 1999; Pavlik et al. 2003).

The use of bran as alternative bedding, instead of frequently contaminated sawdust, poses a risk of infection to piglets (Fischer et al., 2004b). Bran contaminated with atypical mycobacteria (where mostly *M. a. hominissuis* was isolated) was spread by beetles of the species *Tenebrio molitor* in pig herds (Fischer et al., 2004b).

CONCLUSIONS

From epizootiological, epidemiological, and veterinary-hygienic aspects, it is necessary to adhere to the following measures in the Czech Republic as well as in other countries:

1. Not to use hazardous feeds, feed supplements, and drinking water contaminated with atypical mycobacteria and to prevent cattle and pigs coming in to contact with free living animals (particularly with birds and small terrestrial mammals) as much as possible.

2. To perform thorough veterinary-meat inspection in the slaughterhouses in all slaughtered animals of all age categories.

3. To examine biological material from all tissues with tuberculous lesions (lymph nodes and tissues of parenchymatous organs) from cattle and pigs for presence of mycobacteria by culture, or lymph nodes from animals with positive response in tuberculin skin test, even if gross lesions are not observed.

4. To assume that *M. tuberculosis* complex members other than *M. bovis* and *M. caprae* can occur in animals.

REFERENCES

Anonymous (2004): Commission Decision of 31st March 2004 amending Decisions 93/52/EEC, 2001/618/EC and 2003/467/EC as regards the status of acceding countries with regard to brucellosis (*B. melitensis*), Aujeszky's disease, enzootic bovine leukosis, bovine brucellosis and tuberculosis and of France with regard to Aujeszky's disease (notified under document number C(2004) 1094) (text with EEA relevance, 2004/320/EC). Official Journal of the European Communities, 07.04.2004, L 102, 75.

Dürrling H., Ludewig F., Uhlemann J., Gericke R. (1998): Peat as a source of *Mycobacterium avium* infection for pigs (in Germany). Tierärztliche Umschau, 53, 259–261.

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., Bartos M., Parmova I., Bartl J., Svastova P., Bull T.J., Pavlik I. (2004): Study of *Mycobacterium avium* complex strains isolated from cattle in the Czech Republic between 1996 and 2000. Veterinary Microbiology, 99, 239–250.

Erler W., Martin G., Sachse K., Naumann L., Kahlau D., Beer J., Bartos M., Nagy G., Cvetnic Z., Zolnir-Dovc M., Pavlik I. (2004): Molecular fingerprinting of *Mycobacterium bovis* subsp. *caprae* isolates from Central Europe. Journal of Clinical Microbiology, 42, 2234–2238.

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

Fischer O., Matlova L., Bartl J., Dvorska L., Melicharek I., Pavlik I. (2000): Findings of mycobacteria in insectivores and small rodents. Folia Microbiologica, 45, 147–152.

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.

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

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

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.

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 as a supplement. Journal of Clinical Microbiology, 43, 1261–1268.

Pavlik I., Bartl J., Parmova I., Havelkova M., Kubin M., Bazant J. (1998): Occurrence of bovine tuberculosis in animals and humans in the Czech Republic in 1969 to 1996. Veterinarni Medicina, 43, 221–231.

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., Körmendy B., Nagy G., Cvetnic Z., Ocepek M., Fejzic N., Lipiec M. (2002a): 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., Bures F., Janovsky P., Pecinka P., Bartos M., Dvorska L., Matlova L., Kremer K., Van Soolingen D. (2002b): The last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995 was caused by *Mycobacterium bovis* subspecies *caprae*. Veterinarni Medicina, 47, 251–263. http://www.vri.cz/docs/vetmed/47-9-251.pdf.

Pavlik I., Dvorska L., Bartos M., Parmova I., Melicharek I., Jesenska A., Havelkova M., Slosarek M., Putova I., Martin G., Erler W., Kremer K., Van Soolingen D. (2002c): Molecular epidemiology of bovine tuberculosis in the Czech Republic and Slovakia in the period 1965–2001 studied by spoligotyping. Veterinarni Medicina, 47, 181–194. http://www.vri.cz/docs/vetmed/47-7-181.pdf.

Pavlik I., Dvorska L., Matlova L., Svastova P., Parmova I., Bazant J., Veleba J. (2002d): Mycobacterial infections in cattle in the Czech Republic during 1990–1999. Veterinarni Medicina, 47, 241–250.http://www.vri.cz/docs/vetmed/47-9-241.pdf.

Pavlik I., Machackova M., Ayele W.Y., Lamka J., Parmova I., Melicharek I., Hanzlikova M., Körmendy B., Nagy G., Cvetnic Z., Ocepek M., Lipiec M. (2002e): Incidence of bovine tuberculosis in domestic animals other than cattle and in wild animals in six Central European countries during 1990–1999. Veterinarni Medicina, 47, 122–131.http://www.vri.cz/docs/vetmed/47-5-122.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.

Pavlik I., Trcka I., Parmova I., Svobodova J., Melicharek I., Nagy G., Cvetnic Z., Ocepek M., Pate M., Lipiec M.

(2005): Incidence of bovine and human tuberculosis in cattle and other animals in seven Central European countries during the years 2000–2004. Veterinarni Medicina, 50, 291–299. http://www.vri.cz/docs/vetmed/50-7-291.pdf.

Songer J.G., Bicknell E.J., Thoen C.O. (1980): Epidemiological investigation of swine tuberculosis in Arizona. Canadian Journal of Comparative Medicine, 44, 115–120.

Süssland 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.

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.

Van Soolingen D., Bauer J., Leao S., Pavlik I., Vincent V., Rastogi N., Gori A., Bodmer T., Garzelli C., Garcia M.J. (1998): *IS1245* Restriction fragment length polymorphism typing of *Mycobacterium avium* isolates: proposal for standardization. Journal of Clinical Microbiology, 36, 3051–3054.

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.

Corresponding Author

Assoc. 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