# Tuberculous lesions in pigs in the Czech Republic during 1990–1999: occurrence, causal factors and economic losses

I. Pavlik[1], L. Matlova[1], L. Dvorska[1], J. Bartl[1], L., Oktabcova[2], J. Docekal[3], I. Parmova[4]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]District Veterinary Administration, Tachov, Czech Republic
[3]District Veterinary Administration, Znojmo, Czech Republic
[4]State Veterinary Institute, Praha, Czech Republic

**ABSTRACT**: In the decade monitored a total of 45 873 318 pigs were slaughtered and examined according to veterinary hygiene standards. Apart from 1991, when results of tuberculous findings were not obtained, tuberculous lesions were found in 134 088 (0.32%) of the 41 458 565 pigs examined in the remaining nine years. During a detailed analysis of the pathological anatomical examination of 190 940 pigs slaughtered in one district, tuberculous lesions in lymph nodes were found in 4 107 (2.2%) pigs: mesenteric (65.3% pigs), submandibular (18.6% pigs), inguinal (0.1% pigs) and simultaneously intestinal and head lymph nodes (15.9% pigs). Miliary tuberculosis was found only in the parenchymatous organs of four (0.1%) pigs. The following financial losses resulted: 6% for confiscating the head, intestines and stomach, and from 22 to 24% for assessing meat as conditionally edible after processing, i.e. intended only for heat-processed products. Mycobacteria were isolated from 7 246 (41.8%) pigs through the cultivation of tissue samples from 17 326 pigs. *Mycobacterium bovis* was detected in only five (0.07%) animals which originated from the last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995. *M. avium* complex (*MAC*) isolates came from 6 870 (94.8%) animals: 55.7% *M. a. avium* isolates were mainly of serotypes 2 and 3 and genotype IS*901*+ and IS*1245*+ and 39.2% *M. a. hominissuis* isolates were mainly of serotypes 4, 8 and 9 and genotype IS*901*– and IS*1245*+. Conditionally pathogenic mycobacteria (*M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum*) were isolated from 371 (5.1%) pigs. In the whole period monitored, two marked increases in the findings of tuberculous lesions were recorded: In the mid-1990s as a result of using deep bedding with wood shavings and at the end of the 1990s as a result of supplementing the pigs' feed with peat. The predominant occurrence of *M. a. avium* isolates of genotype IS*901*+ and IS*1245*+ in the first half of the 1990s was replaced above all by *M. a. hominissuis* isolates of genotype IS*901*- and IS*1245*+. The reason for this was probably a change in the sources of infection for pigs. While at the beginning of the 1990s the most frequent source of infection were wild and domestic birds, various parts of the external environment became the source of the infection for pigs from the mid-1990s. In the years 1996 to 1999, *Rhodococcus equi* was isolated from 203 (11.6%) of the 1 745 animals examined. It was solely isolated from 154 (8.8%) animals and from 49 (2.8%) animals together with mycobacteria.

**Keywords**: mycobacteriosis; PCR; risk assessment

In the Czech Republic, bovine tuberculosis was controlled in domestic animals including cattle and pigs in 1968 (Polak, 1969; Krucky, 1973; Kouba, 1999). Subsequently, a constant decline could be observed in the incidence of bovine tuberculosis in animals, which was last identified in cattle and domestic pigs in 1995 (Pavlik *et al.*, 1998, 2002a,d,e). During the veterinary meat inspection of pigs slaughtered in slaughterhouses, however, tuberculous lesions were still being found above all in the head and intesti-

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nal lymph nodes. Parts of the adjacent organs (or on occasion the whole body of the pig) were then assessed according to valid veterinary regulations to ensure that the consumer was protected against mycobacterial infection. For this reason, some organs were condemned and the whole bodies of animals were adjudged to be conditionally edible after processing, i.e. only for heat-treated products. Pig breeders of animals affected in this way, however, suffered major economic losses.

At the same time, it emerged from the results of laboratory investigations in the Czech Republic before 1989, that the most common isolates obtained from pig lymph nodes with tuberculous lesions were of the *Mycobacterium avium* (*MAC*) complex and, less commonly, also *Rhodococcus equi* (Krucky, 1981; Pavlas, 1989, Stika, 1989).

*MAC* is divided, as far as virulence for birds is concerned, into three different groups. **The first group** consisted of *M. a. avium* isolates entirely virulent for birds, which cause generalised avian tuberculosis. These isolates are mainly of serotypes 1, 2 and 3 and if they are isolated from field samples, more than 95% contain IS*901* (Pavlik *et al.*, 2000a; Mijs *et al.*, 2002). **The second group** consisted of *M. a. hominissuis* (also called intermediate group of isolates), which is only partially virulent for birds and which during intramuscular application cause lesions only at the site of the inoculation of pullets (Piening *et al.*, 1972; Mijs *et al.*, 2002). Serotypes 4 to 6, 8 to 11 and 21 belong to this group, not including IS*901*, but including IS*1245* in a greater number than 3 copies (Ritacco *et al.*, 1998). They occur above all in the external environment, where they are capable of multiplying at temperatures above 18 to 20°C (Horvathova *et al.*, 1997; Kazda, 2000). **The third group** consisted of *M. intracellulare* isolates of the remaining serotypes 7, 12 to 20 and 22 to 28, which do not include any of the above mentioned IS elements and are in practice not virulent for birds (Mijs *et al.*, 2002).

*M. a. avium* serotypes 1, 2 and 3 is a much more serious pathogen for pigs than *M. a. hominissuis* and *M. intracellulare* of remaining serotypes 4 to 28 which are most frequently isolated from the external environment (Pavlas and Patlokova, 1976; Pavlas, 1989; Hejlicek and Treml, 1996; Horvathova *et al.*, 1997). The higher virulence of *M. a. avium* isolates of these serotypes is also indicated by their detection not only in mesenteric, submandibular and inguinal lymph nodes but also in the muscle tissue (Pavlas and Patlokova, 1976, 1977).

The objectives of this work may be divided into two parts. In the first part the results of the assessment of all pigs slaughtered in the Czech Republic in the last decade (1990–1999) were analysed. The available results of tissue samples from several pigs slaughtered in slaughterhouses in this period which had been further examined in a laboratory were also evaluated, and the causes of these tuberculous lesions and the sources of infection caused by mycobacteria and *Rhodococcus equi* were monitored. In the second part, for reasons of increased occurrence of tuberculous lesions in the lymph nodes of pigs, one district was chosen, where the results of veterinary hygiene inspection at slaughterhouses between 1993 and 1999 were analysed.

## MATERIAL AND METHODS

### Part 1. Analysis of the results of veterinary hygiene inspection at slaughterhouses in the years 1990 to 1999

**Rules for the veterinary hygiene examination of pigs in slaughterhouses**. Throughout the whole period monitored, the examinations of all 41 458 565 pigs slaughtered in slaughterhouses between 1990 and 1999 (with the exception of 1991, when the results of tuberculous lesions in pigs in slaughterhouses were not statistically processed) were governed by Decree No. 121/87 Sb. Immediately after slaughter, the following lymph nodes were assessed in all pigs in slaughterhouses through adspection and incision: *Lymphonodi mandibulares*, *Lnn. tracheobronchales sin., med.* et *dex.*, *Lnn. tracheobronchales dextri*, *Lnn. hepatici*, *Lnn. gastrici*, *Lnn. jejunales* et *Lnn. inguinales superficiales*. Caseated or even calcified nodules of various size and shape (most commonly the size of a poppy) were adjudged as tuberculous lesions. If the preceding examinations were not sufficient, a deeper examination was carried out. In this, incisions were made in suspect parts and lymph nodes in their vicinity and in regional lymph nodes.

**The assessment of meat and parenchymatous organs from January 1st 1990 to September 15th 1996**. In this period, veterinary meat inspection following the discovery of tuberculous lesions in pigs was conducted according to Directive State Veterinary Administration (SVA) of the Czech Republic (CR) No. 1 of September 21st 1989, article 4, appendix No. 1 "Principles for making decisions about the

meat and organs of slaughterhouse animals". All tuberculous lesions in pigs were a reason for adjudging pork meat and organs as conditionally edible after processing (i.e. intended in their raw state for all heat-treated products).

**The assessment of meat and parenchymatous organs since September 16th 1996**. On the basis of a recommendation in the *Codex alimentarius*, taking account of European Union regulations and in order to reduce economic losses, on the September 16th 1996 SVA CR Supplement No. 2 was published to augment the above mentioned directive. Its primary principle was the protection of people against mycobacterial infections and the evaluation of the infectious situation in an area of mycobacterial infections in people and animals in the Czech Republic. In accordance with this supplement, isolated tuberculous findings in intestinal or head lymph nodes were only recorded and led to the condemnation of the intestines or head without further restriction. It was thus possible in a case where tuberculous lesions were found in one group of lymph nodes, i.e. head, gastric or jejunal to adjudge meat as edible (Table 1).

**Processing statistical data**. The results of examinations for tuberculosis annually formed part of the Annual Report of the SVA CR concerning veterinary hygiene activity (apart from 1991). Until 1995, the hygiene year was considered to be the calendar year. In 1996, following the association of the Czech Republic to the European Union, the hygiene year began on January 1st and ended on October 31st (only 10 months). The subsequent hygiene years always began in November of the preceding year

Table 1. Tuberculous lesions found in slaughtered pigs during the years 1990 to 1999

| Year[1] | Total No. of slaughtered pigs | Tuberculous lesions and veterinary meat inspection | | | | | |
|---|---|---|---|---|---|---|---|
| | | without sequences[2] | | with sequences[3] | | total | |
| | | No. | % | No. | % | No. | % |
| 1990 | 5 159 137 | 0 | 0 | 22 979 | 0.45 | 22 979 | 0.45 |
| 1991 | 4 414 753 | nk | nk | nk | nk | nk | nk |
| 1992 | 4 741 464 | 0 | 0 | 15 366 | 0.32 | 15 366 | 0.32 |
| 1993 | 4 615 169 | 0 | 0 | 15 136 | 0.33 | 15 136 | 0.33 |
| 1994 | 4 431 560 | 0 | 0 | 11 659 | 0.26 | 11 659 | 0.26 |
| 1995 | 4 224 109 | 0 | 0 | 11 805 | 0.28 | 11 805 | 0.28 |
| 1996 | 4 369 965 | 0 | 0 | 18 864 | 0.43 | 18 864 | 0.43 |
| 1997 | 4 528 971 | 5 471 | 0.12 | 3 091 | 0.07 | 8 562 | 0.19 |
| 1998 | 4 720 939 | 11 219 | 0.24 | 2 795 | 0.06 | 14 014 | 0.30 |
| 1999 | 4 667 251 | 10 174 | 0.22 | 5 529 | 0.12 | 15 703 | 0.34 |
| Total[4] | 13 917 161 | 26 864 | 0.19 | 11 415 | 0.08 | 38 279 | 0.28 |
| Total[5] | 41 458 565 | | | 107 224 | 0.26 | 134 088 | 0.32 |

Explanations:

[1]in the hygiene year data was gathered from 1990 to 1995 from January to December, in 1996 from January to October and from 1997 to 1999 always 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

[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)

[4]total No. of animals slaughtered during the years 1997 to 1999

[5]total No. of animals slaughtered excluding 1991, when results of veterinary meat examination for tuberculosis were not available

nk = not known

and ended in October of the following year. From November 1st 1996 the administration, processing and transfer of data was conducted using common software. District Veterinary Administrations (DVAs) and City Veterinary Administrations (CVAs) had a central computer, which amassed the original data about slaughtered animals from computers located in slaughterhouses. Once a month, after checking by a senior inspector, data were sent by internal electronic mail to the SVA CR Information Centre in Liberec, Czech Republic. There, the data gathered were checked and processed for the use of SVA CR and returned for the use of all the DVAs and CVAs (Slanec and Valcl, 1996).

## The laboratory diagnosis of mycobacterial infections in pigs in the Czech Republic

**The collection of biological material in slaughterhouses**. Between 1990 and 1999, when a total of 45 873 318 pigs were slaughtered in slaughterhouses in the Czech Republic, biological material was collected from 17 326 (0.04%) pigs for laboratory examination. In sows and boars, tissue samples were collected from all animals with tuberculous lesions. From fattening pigs, biological material was collected for laboratory examination only if there were greater number of tuberculous lesions in the delivery from one herd, or after agreement with the relevant DVA or CVA during further following of sources of infection. Tissue samples were supplied to the laboratory immediately after collection, or were frozen to –20°C and delivered in this condition no more than one month later for laboratory examination.

**The system for laboratory diagnosis of mycobacterial infections in pigs**. In the period we monitored, three laboratories in the Czech Republic dealt with the cultivation of biological material from pigs for the presence of mycobacteria: **1.** the SVA CR Reference Laboratory for the Diagnosis of Paratuberculosis, Tuberculosis and Other Mycobacterial Infections in the State Veterinary Institute (SVI) Prague, **2.** the Mycobacteriological Laboratory at SVI Brno and **3.** the OIE Reference Laboratory for Paratuberculosis and the Methodological and Consultation Centre of SVA CR for Tuberculosis, Paratuberculosis and Other Mycobacterial Infections in Animals at the Veterinary Research Institute (VRI) Brno. The results of laboratory examination were always collected for the calendar year monitored in the Annual Report on Laboratory Diagnosis of the SVA CR.

**The culture examination of tissue for the presence of mycobacteria**. The method of isolating mycobacteria was described earlier (Kubin *et al.*, 1986; Fischer *et al.*, 2000). The sterilely collected tissue sample (approximately 1 g) was homogenised in a mortar and decontaminated with 1 N HCl for 15 min. The tissue was subsequently neutralised with 2 N NaOH until the colour changed to bromthymol blue as an indicator. After centrifuging (2 500 to 3 000 rpm/20 min) and re-suspending the sediment in a sterile physiological solution, 40 µl of the sediment was inoculated on egg media according to Stonebrink and Löwenstein-Jensen and into a liquid serous medium according to Sula (Sevac Praha, Czech Republic).

**The identification of mycobacterial isolates**. Acid fast rods (AFR) positive isolates detected using direct microscopy after staining according to Ziehl-Neelsen (Z-N) were subcultured and subsequently identified using biochemical methods (Wayne and Kubica, 1986). From 1993 onwards, the Accu-Probe Probe (Accu-Probe Inc., San Diego, California) system was used: probes for *M. tuberculosis* complex (*MTC*), *MAC*, *M. avium* (including *M. a. avium* and *M. a. hominissuis*) and *M. intracellulare* isolates. The primers 5′-GCA ACG GTT GTT GCT TGA AA-3′ and 5′-TGA TAC GGC CGG AAT CGC GT-3′ (Kunze *et al.*, 1992) were used for the detection of IS*901* and the primers 5′-GCC GCC GAA ACG ATC TAC-3′ and 5′-AGG TGG CGT CGA GGA AGA-3′ (Guerrero *et al.*, 1995) were used for the detection of IS*1245*. *MAC* isolates were also identified by serotyping according to Wolinsky and Schaefer (1973) modified by Süssland and Hrdinova (1976). Apart from biochemical methods, a biological experiment on a guinea pig was also used for the identification of *M. bovis* isolates. A biological experiment on pullets was used in Laboratory 2 to identify *MAC* isolates of serotypes 1, 2 and 3 (Pavlas and Patlokova, 1977). From 1996 onwards, biological experiments on pullets for *MAC* isolates were replaced by the PCR method for detecting the insertion sequence IS*901*, which occurs only in *M. a. avium* field isolates of serotypes 1, 2 and 3 entirely virulent for birds (Dvorska, 1999a; Pavlik *et al.*, 1999a, 2000a). To compare whether the causal agent of tuberculous lesions in pigs changed in the period monitored, the proportion of for birds virulent *M. a. avium* (genotype IS*901*+ and IS*1245*+) and for birds non-virulent *M. a. hominissuis* (geno-

type IS*901*– and IS*1245*+) isolates was calculated for each year.

**The isolation of *R. equi*.** In Laboratory 1, all biological material from pigs also underwent culture examination for the presence of *R. equi*. Part of the sterile collected tissue was cultured on blood agar at 37°C for 48 hours prior to decontamination. *R. equi* isolates were identified according to SVA CR current methodology (Dvorska *et al*., 1999b). Only results of examinations from 1996 to 1999 were analysed (Table 2).

### Statistical assessment

The $\chi^2$-test (Stat Plus) was applied for statistical evaluation (Matouskova *et al*., 1992).

### Part 2. Analysis of the results of pig examinations for tuberculosis in one district T

In one selectively chosen district T, the results of the veterinary meat inspection of 190 940 pigs slaughtered from January 1st 1993 to October 31st 1999 were analysed in detail (Table 3). The animals were examined as in Part 1 and the results were processed according to the same criteria as those given in the preceding part.

## RESULTS

### Part 1. Tuberculous findings in pigs slaughtered in the Czech Republic in the years 1990 to 1999

During veterinary hygiene inspection, tuberculous lesions were found in 134 088 (0.32%) of the 41 458 565 pigs slaughtered (Table 1). Given that the numbers of pigs examined each year were generally in the order of millions, which may be considered representative selections, in evaluating the results, we may compare their relatively high number directly. The highest incidence of tuberculous lesions identified in pigs was found in 1990 (0.45%) and 1996 (0.43%). These values were more than twice than the value from 1997 (0.19%), which was the year with the lowest incidence of tuberculous lesions in pigs. In 1998 and 1999 a renewed growth of tuberculous lesions in pigs was recorded, to 0.30% and 0.34%. From a total of 17 326 pigs examined, mycobacteria were isolated from 7 246 (41.8%) animals. The culture yield in samples examined fluctuated from 29.5% in 1997 to 53.7% in 1994 (Table 4).

***MTC* isolates**. *M. bovis* was isolated in 1995 only from the tissues of two pigs with tuberculous lesions in the liver, mesenteric and head lymph nodes supplied for culture examination. In the other three pigs reared together and originating from the last

Table 2. Isolation of *Rhodococcus equi* and mycobacteria from pig's lymph nodes during the years 1996 to 1999

| Examined lymph Nodes | No. of examined pigs | Isolation of the causal agent | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *R. equi* only | | mycobacteria only | | | | *R. equi* and mycobacteria | | | |
| | | No. | % | No. | % | *MAC* | other spp. | No. | % | *MAC* | other spp. |
| Around the head[1] | 666 | 140 | 21.0 | 232 | 34.8 | 227 | 5 | 36 | 5.4 | 27 | 9 |
| Mesenteric | 759 | 7 | 0.9 | 369 | 48.6 | 361 | 8 | 7 | 0.9 | 7 | 0 |
| Undetermined[2] | 297 | 7 | 2.4 | 103 | 34.7 | 99 | 4 | 4 | 1.3 | 3 | 1 |
| Inguinal | 23 | 0 | 0 | 1 | 4.3 | 1 | 0 | 2 | 8.7 | 2 | 0 |
| Total | 1 745 | 154 | 8.8 | 705 | 40.4 | 688 | 17[2] | 49 | 2.8 | 39 | 10[3] |

Explanations:

MAC - *Mycobacterium avium* complex isolates

[1]lymph nodes around the head and/or retropharyngeal lymph nodes and/or mediastinal lymph nodes and/or tracheobronchial lymph nodes and/or mesenteric lymph nodes

[2]*M. chelonae* (*n* = 4), *M. terrae* (*n* = 6), *M. phlei* (*n* = 2), *M. fortuitum* (*n* = 3), not identified atypical mycobacteria (n = 2)

[3]*M. fortuitum* (*n* = 3), *M. chelonae* (*n* = 7)

Table 3. Distribution of tuberculous lesions in slaughtered pigs in District Veterinary Administration T during 1993 to 1999

| Year | Slaughtered pigs | | | Lymph nodes | | | | | | | | Parenchymatous organs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | total No. | with lesions | % | around the head | | mesenteric | | inguinal | | mixed* | | | |
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % |
| 1993 | 32 936 | 33 | 0.1 | 0 | 0 | 33 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1994 | 25 289 | 1 572 | 6.2 | 200 | 12.7 | 881 | 56.0 | 1 | 0.1 | 487 | 31.0 | 3[1] | 0.2 |
| 1995 | 23 689 | 323 | 1.4 | 18 | 5.6 | 301 | 93.2 | 1 | 0.3 | 3 | 0.9 | 0 | 0 |
| 1996 | 21 585 | 1 367 | 6.3 | 373 | 27.3 | 887 | 64.9 | 0 | 0 | 107 | 7.8 | 0 | 0 |
| 1997 | 12 865 | 320 | 2.5 | 107 | 33.4 | 213 | 66.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1998 | 32 623 | 191 | 0.6 | 18 | 9.4 | 173 | 90.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1999 | 41 953 | 301 | 0.7 | 49 | 16.3 | 197 | 65.4 | 0 | 0 | 54 | 17.9 | 1[2] | 0.3 |
| Total | 190 940 | 4 107 | 2.2 | 765 | 18.6 | 2 685 | 65.3 | 2 | 0.1 | 651 | 15.9 | 4 | 0.1 |

Explanations:

*tuberculous lesions were found minimally in two groups (around the head and/or mesenteric and/or inguinal) of lymph nodes

[1]in three pigs miliar tuberculous lesions caused by *M. a. avium* isolates of serotype 2 were found in liver and lungs

[2]in one pig tuberculous lesions were found in liver

outbreak of bovine tuberculosis in cattle in the Czech Republic (Pavlik *et al.*, 1998, 2001, 2002a), tuberculous lesions were also found in the same organs, which were not, however, examined for the presence of mycobacteria. All of these five pigs (0.1%) were therefore considered as animals infected with bovine tuberculosis (Table 4).

***MAC* isolates.** A total of 6 870 (94.8%) *MAC* isolates were detected: 55.7% *M. a. avium* isolates were mainly of serotypes 2 and 3 (genotype IS*901*+ and IS*1245*+) and 39.2% *M. a. hominissuis* isolates were mainly of serotypes 4, 8 and 9 (genotype IS*901*– and IS*1245*+). The proportion of *M. a. avium* isolates and *M. a. hominissuis* isolates between 1990 and 1992 fell from 9.2 to 2.0. After 1993 it was always lower than 1.1 and in 1996 and 1998 this proportion was even as low as 0.4 (Table 4). Statistically highly significant *MAC* isolates ($P < 0.01$) were identified more frequently from mesenteric lymph nodes (48.6%) compared to their identification from head lymph nodes (34.8%). The identification of *MAC* isolates from inguinal lymph nodes (4.3%) was statistically highly significantly lower ($P < 0.01$) than the identification of *MAC* isolates from head or mesenteric lymph nodes (Table 2).

**Remaining mycobacterial species.** A total of 371 (5.1%) isolates were identified as conditionally pathogenic species (*M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum*). In individual years, their proportion in the total number of mycobacterial isolates never exceeded 9% (Table 4).

***R. equi*.** Over the years 1996 and 1999, from the lymph nodes of 1 745 pigs, *R. equi* was solely isolated from 154 (8.8%) pigs and in mixed infection with mycobacteria in a further 49 (2.8%) pigs (Table 2). Statistically highly significant *R. equi* ($P < 0.01$) was isolated more frequently from head lymph nodes (21.0%) compared to mesenteric lymph nodes (0.9%). Even in cases of concurrent infection with mycobacteria and *R. equi* both causal agents were statistically highly significantly identified more often ($P < 0.01$) in head lymph nodes as opposed to intestinal lymph nodes (0.9%).

## Part 2. The incidence of tuberculous lesions in pigs slaughtered in district T

Out of 190 940 pigs slaughtered, tuberculous lesions were found in 4 107 (2.2%) pigs, of which tuberculous lesions were found in the mesenteric lymph nodes of 65.3% pigs, head lymph nodes of 18.6% pigs and inguinal lymph nodes of 0.1% pigs. Tuberculous lesions were found simultaneously in

Table 4. Laboratory examination for mycobacterial infections in pigs during the years 1990 to 1999

| Year | Examined pigs | | | Isolated mycobacterial species | | | | | | | | Ratio of *M. a. avium* to *M. a. hominissuis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | total No. | positive | | *M. bovis* | | *M. a. avium* | | *M. a. hominissuis* | | atypical myc. | | |
| | | No. | % | No. | %[1] | No. | %[1] | No. | %[1] | No. | %[1] | |
| 1990 | 4 025 | 1 600 | 39.8 | 0 | 0 | 1 257 | 78.6 | 293 | 18.3 | 50 | 3.1 | 4.3 |
| 1991 | 2 405 | 905 | 37.6 | 0 | 0 | 807 | 89.2 | 88 | 9.7 | 10 | 1.1 | 9.2 |
| 1992 | 2 294 | 886 | 38.6 | 0 | 0 | 545 | 61.5 | 268 | 30.3 | 73 | 8.2 | 2.0 |
| 1993 | 2 192 | 875 | 39.9 | 0 | 0 | 319 | 36.5 | 493 | 56.3 | 63 | 7.2 | 0.6 |
| 1994 | 1 938 | 1 041 | 53.7 | 0 | 0 | 497 | 47.7 | 471 | 45.3 | 73 | 7.0 | 1.1 |
| 1995 | 1 327 | 568 | 42.8 | 5[2] | 0.9 | 261 | 45.9 | 274 | 48.2 | 28 | 4.9 | 1.0 |
| 1996 | 1 560 | 795 | 51.0 | 0 | 0 | 199 | 25.0 | 565 | 71.1 | 31 | 3.9 | 0.4 |
| 1997 | 569 | 168 | 29.5 | 0 | 0 | 65 | 38.7 | 91 | 54.2 | 12 | 7.1 | 0.7 |
| 1998 | 416 | 153 | 36.8 | 0 | 0 | 44 | 28.8 | 98 | 64.0 | 11 | 7.2 | 0.4 |
| 1999 | 600 | 255 | 42.5 | 0 | 0 | 39 | 15.3 | 196 | 76.9 | 20 | 7.8 | 0.2 |
| Total | 17 326 | 7 246 | 41.8 | 5 | 0.1 | 4 033 | 55.7 | 2 837 | 39.2 | 371 | 5.1 | 1.4 |

Explanations:

*M. a. avium* = *Mycobacterium avium* subsp. *avium* isolates of serotypes 1, 2 and 3 and genotype IS*901*+ and IS*1245*+, virulent for poultry (generalized avian tuberculosis in pullets)

*M. a. hominissuis* = *Mycobacterium avium* subsp. *hominissuis* isolates of serotypes from 4 to 6, 8 to 11 and 21 and genotype IS*901*– and IS*1245*+, partially virulent for poultry (tuberculous lesions only in the place of inoculation to the muscle of pullets)

atypical myc. = atypical mycobacteria (the most often isolated species: *M. chelonae, M. terrae, M. phlei* and *M. fortuitum*)

[1]of positive samples

[2]from the last outbreak of bovine tuberculosis infection in cattle herd in the Czech Republic five pigs commonly housed were slaughtered: pigs were fed with milk from 9 infected cows; in all pigs were found tuberculous lesions in liver, spleen and mesenteric lymph nodes; culture examination was done in two animals with positive result for *M. bovis* (Pavlik *et al*., 2001, 2002a)

the intestinal and head lymph nodes of 15.9% animals. Miliary tuberculosis in parenchymatous organs (liver, spleen and kidneys) was only diagnosed in four (0.1%) pigs. Generalised chronic tuberculosis was not found in any pig (Table 3).

## DISCUSSION

### Part 1. Tuberculous findings in pigs slaughtered in the Czech Republic in the years 1990 to 1999

In the time monitored a higher incidence of tuberculous lesions was recorded in the Czech Republic in two periods (Table 1). The first sharp increase occurred in the mid-1990s, when enzymatically (e.g. using Envistim) split sawdust began to be more commonly used in pig rearing as deep bedding (Nemec, 1995; Pecina, 1995; Jedlicka, 1996; Bartl *et al.*, 1997a,b). When sawdust, wood-shavings or other wood-based products were used, it generally proved impossible to prevent the multiplication of *M. a. hominissuis* of serotypes 4, 8 and 9, with which this raw material was very often contaminated (Horvathova *et al.*, 1997). Subsequently, therefore, because of the increased discovery of tuberculous lesions in pigs, most pig-breeders gradually gave up this technology with deep bedding using sawdust (Pavlik *et al.*, 2000b).

The second increase in incidence of tuberculous lesions in the lymph nodes of pigs occurred at the end of the 1990s (Table 1), when peat began to be used as an addition to feed for piglets for 2 up to 4 weeks after birth. The conditionally pathogenic mycobacteria isolated from the organs of pigs with tuberculous lesions were identical to the species isolated from peat (Pavlik *et al.*, 1999b).

## Direct economic losses through the occurrence of tuberculous lesions in slaughtered pigs

The level of direct economic losses in the period from January 1st 1990 to September 15th 1996 in slaughtered pigs with tuberculous lesions was as high as 24% of their final price. On the discovery of tuberculous lesions in parenchymatous organs, the organs and meat were adjudged unfit for consumption. After September 16th 1996, the average loss from the total price of one pig upon the discovery of tuberculous lesions in only the head lymph nodes (condemnation of the whole head) was 6.0%. Following the discovery of tuberculous lesions in only the mesenteric lymph nodes, the loss was 1.3% (condemnation of the intestines = 0.8% and condemnation of the stomach = 0.5%). However, the greatest losses, reaching 22 to 24% of the price of the slaughtered pig, arose upon the discovery of numerous tuberculous lesions simultaneously in the nodes and/or the gastric and/or the jejunal and/or inguinal head lymph which led to the meat being adjudged as conditionally edible.

The extent of these economic losses, however, also fluctuated sharply in connection with the demand for pork meat on the market. In a case of shortage, only losses for the condemnation of the head, intestines and stomach (i.e. up to 7.3%) were charged to the breeder. On the contrary, when there was a surfeit of pork meat on the market, breeders were faced in some cases with substantially higher losses, which reached up to 70% of the total price of one pig. In particular, the slaughter of animals in small slaughterhouses, which did not process the meat into meat products, led to a growth of costs connected with storage (refrigerating or freezing) and with the transfer of meat to far-away processing plants. When these problems arose, the discovery of tuberculous lesions could in exceptional cases lead to the condemnation of the whole animal.

As a result of the change in assessing meat upon the discovery of tuberculous lesions on September 16th 1996, meat between 1997 and 1999 was less often (statistically highly significant, $P < 0.01$) adjudged to be conditionally edible, i.e. intended in the raw state for use in all heat-treated products (Table 1).

Economic losses in pig herds as a result of tuberculosis will nevertheless continue to occur, because the new Decree of the Ministry of Agriculture of the Czech Republic No. 287/1999 "On veterinary requirements for animal products" has been effective since January 1st 2000. According to this, parts of the head and organs with the occurrence of tuberculous lesions should be condemned. As conditionally edible meat, intended in the raw state for processing in all heat-treated products, pigs with the occurrence of tuberculous lesions are assessed in two of three groups of lymph nodes (head, gastric and jejunal) and the whole body of the animal, which reacted positively to avian tuberculin and was without tuberculous lesions after the slaughter.

## Laboratory examination of the tissues samples of pigs

The limited yield of live mycobacteria through the culture examination of the tissues of pigs (Table 4) probably had two causes. The first of them is the experimentally proven finding that the gradual devitalisation of mycobacteria takes places during the course of granulomatous infection of the lymph nodes of pigs. This leads to results where a negative culture for mycobacteria is also found in microscopically positive tuberculous changed autopsy material (Pavlas *et al.*, 1984; Kovarik *et al.*, 1995). The second cause may be seen in the proportion of other bacterial agents (above all *R. equi*), which may also cause the formation of tuberculous lesions in pig's lymph nodes (Dvorska *et al.*, 1999b).

***MTC* isolates.** The infection of five (0.07%) pigs with *M. bovis* occurred when they were housed together with infected cattle and fed milk from these infected cows (Pavlik *et al.*, 2001, 2002a). Apart from these five pigs, over the whole period, no other mycobacterial *MTC* species, which in addition to *M. bovis* include *M. tuberculosis*, *M. bovis* BCG, *M. bovis* subsp. *caprae*, *M. africanum*, *M. microti* and *M. canetti*, was found in pigs (Kremer *et al.*,

1999; Aranaz *et al.*, 1999; Dvorska *et al.*, 2001). None of the above mentioned species was isolated in other animals in this period, with the exception of one isolation of *M. bovis* in 1999 from one farm-reared deer (Pavlik *et al.*, 2002b,c,d,e; Machackova *et al.*, 2000, 2003). Only in the preceding decade in 1984 *M. tuberculosis* was identified from caseated pleuritis in an old dog (a cross-breed), whose owner (an older man) was also infected with *M. tuberculosis* (Cada F., 2000, State Veterinary Institute Plzen, Czech Republic, personal communication). In Slovakia, however, *M. tuberculosis* was isolated from three pigs in this period in 1994, 1997 and 1998, in which the source of infection was an infected tender (Badalik *et al.*, 1997, 1999; Melicharek, 2000; Pavlik *et al.*, 2003).

In the Czech Republic, however, a risk factor is the employment of persons who, given the relatively unfavourable epizootiological situation in certain countries (O.I.E., 1999, 2000), may be infected with *M. bovis*. Domestic pigs are also receptive to infection in *M. tuberculosis* (Zorawski *et al.*, 1974; EuroTB: http://www.ceses.org/eruotb/erotb.htm).

***MAC* isolates.** After the elimination of bovine tuberculosis in the Czech Republic, from 1969 to 1989 the most important causal agents of tuberculous lesions in pigs became members of *MAC* (Pavlas and Patlokova, 1977; Krucky, 1981; Pavlas *et al.*, 1984, 1985; Stika, 1989; Pavlas, 1989, 1998). The same applies also in the last decade (1990 to 1999), when the capture of *MAC* also dominated in biological material taken from pigs, forming 94.9% of all mycobacterial isolates (Table 4). The situation was similar in cattle in the Czech Republic (Pavlik *et al.*, 2002c).

At the beginning of 1990s, property rights changed in agriculture in the Czech Republic, which also caused changes in the rearing of pigs. Not only pigs were reared by less experienced breeders, but also pig feed was not adequately separated from stocks of other domestic animals, in particular poultry. As a result, the risk of the transmission of *M. a. avium*, the causal agent of avian tuberculosis into herds of pigs rose (Pavlik *et al.*, 1997). A second risk factor was represented by wild birds: above all the house and tree sparrows (*Passer domesticus, Passer montanus*), Eurasian collared doves (*Streptopelia decaocto*), turtle doves (*Streptopelia turtur*) and feral pigeons (*Columba livia* f. *domestica*), which, especially in smaller herds using traditional feed technology, had free access to feed in both stores and in pig-troughs in barns (Bartl *et al.*, 1997a,b). The dominance of the yield of *M. a. avium* isolates over *M. a. hominissuis* isolates up until 1992 also bears testimony to these presumptions (Table 4). Because, however, tuberculosis of pigs caused by *MAC* isolates was diagnosed, we also sought other sources. While examining small vertebrates from places several kilometres away from human dwellings and barns (rodents and insectivores) we found that they too had been infected with *MAC* (Fischer *et al.*, 2000, 2001). In the autumn, at the time when these small vertebrates migrate into the barns of pigs, they evidently may become vectors for the transmission of pathogenic mycobacterial species for pigs.

The predominance of *M. a. avium* isolates over *M. a. hominissuis* isolates in the second half of the 1990s was probably caused by a change in the sources of mycobacteria especially in large herds of pigs. The sources of these *M. a. hominissuis* isolates were non-traditional raw materials (sawdust, peat and others), which were to a great extent contaminated with these mycobacteria (Bartl *et al.*, 1997a,b; Pavlik *et al.*, 1999b, 2000b). From the epidemiological perspective it is interesting that these *M. a. hominissuis* isolates are also most commonly those detected in people (Havelkova *et al.*, 1998; Ritacco *et al.*, 1998; Pavlik *et al.*, 2000a; Mijs *et al.*, 2002). This fact is probably connected with a reduction in the sources of *M. a. avium*, the most common source of which for livestock and the human population are birds. Avian tuberculosis caused by these types of *M. a. avium* does not occur in domestic birds kept using large-scale. In large herds of pigs, contact between pigs and wild or domestic birds is made impossible. However, contact between pigs and small vertebrates, which may also be infected with *MAC*, cannot always be ruled out (Fischer, 1999; Fischer *et al.*, 2001, 2003a,b).

**Isolates of other mycobacterial species.** The sources of other species of mycobacteria isolated from the tissues of pigs originated probably from the external environment, in which they are a natural component of the ecosystem and are capable of multiplying at temperatures higher than 18 to 20°C to concentrations high enough to initiate a pathological process in the lymph node of a pig (Horvathova *et al.*, 1997; Pavlik *et al.*, 1999a; Kazda, 2000). They occur in abundance in different samples of the external barn environment (bedding, feed, soil in the runs, drinking water etc.), where, in a case of massive incidence, they may sensitise the organism of pigs and provoke non-specific reactions during skin testing with bovine and/or avian tuberculins (Herzig *et al.*, 1982; Pavlas and

Patlokova, 1985; Bartl *et al.*, 1997a,b; Horvathova *et al.*, 1997; Urban and Kvapilik, 1995; Vanicek and Prasek, 1995). Amongst other things, these mycobacteria occur in a wide range of poiki-lothermic and homeothermic animals, which may serve as vectors of infection (Matlova *et al.*, 1998; Fischer, 1999; Fischer *et al.*, 2000, 2001).

***R. equi* isolates.** *R. equi* is considered a soil micro-organism and is most often found in the surface of the soil on farms of horses (Barton and Hughes, 1980; Takai and Tsubaki, 1985; Prescott, 1991). The isolation of *R. equi* from the lymph nodes of cattle is rare in contrast to its isolation from pigs (McKenzie and Donald, 1979; Dvorska *et al.*, 1999b). The reduction in the numbers of horses in the Czech Republic to 20 700 in 1998 (O.I.E., 1999) and a high specialisation of herds of pigs in which all direct or indirect contact with horses and their excrement was eliminated did not lead in pigs to a disappearance of infection caused by *R. equi* (Table 2). Infection with *R. equi* was identified in 93 out of 765 pig and cattle farms in the Czech Republic. Of these, contact (direct or indirect) with horses in the preceding 10 years before diagnosis of the *R. equi* infection was found in only 19 (20.4%) farms. This result probably indicates infection of *R. equi* from the soil or from other sources (Dvorska *et al.*, 1999b).

## Part 2. The incidence of tuberculous lesions in pigs slaughtered in district T

The discovery of an incidence of tuberculous lesions in pigs in slaughterhouses in this district that was five times higher (2.2%) than the average for the whole Czech Republic in the monitored period (0.43%) was significant. Together with the fact that the incidence of miliary tuberculosis was also found here, it may be assumed that pigs in certain herds in this district were massively infected with *M. a. avium* and *M. a. hominissuis* (Table 3).

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*Corresponding Author*

Assoc. Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 541 321 241, fax: +420 541 211 229, e-mail: pavlik@vri.cz
www: http://www.vri.cz/labs/tbc/default.htm