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ABSTRACT: The prevalence of Rhodococcus equi and atypical mycobacteria in the lymph nodes of pigs (n = 1 382) and cattle (n = 231) without clinical signs was studied in the Czech Republic over the period 1996–1998. R. equi alone was isolated from 7.4% of pigs, and in a mixed infection with atypical mycobacteria in another 2.3% of pigs and 1.7% of cattle. The frequency of R. equi was higher (p = 0.01) in pigs than in cattle. Atypical mycobacteria alone were more frequently isolated from pigs – 37.2% positive findings than from cattle – 28.2% positive findings (p = 0.01). Of the total of 546 mycobacterial strains isolated from pigs, 96.2% belonged to the Mycobacterium avium complex, and 3.8% (21 strains) belonged to other species (5 strains M. chelonae, 6 strains M. terrae, 2 strains M. phlei, 6 strains M. fortuitum and 2 strains not identified). A total of 65 mycobacterial strains belonging only to M. avium complex were identified in cattle. Of 492 pigs, R. equi alone was isolated from submaxillary lymph nodes at frequency of 19.5%, atypical mycobacteria were isolated at 30.1% and mixed infection at 4.9%. On the other hand, of the total of 639 pigs, R. equi alone was isolated from mesenteric lymph nodes in 0.5% of animals; atypical mycobacteria in 42.6% and mixed infection in 0.8% of pigs. The isolation of R. equi from submaxillary lymph nodes was more frequent (p = 0.01) than isolation from mesenteric lymph nodes. Examinations of lymph nodes from 218 pigs without tuberculous nodules (group S1) resulted in isolation of R. equi in 2.8% of animals compared with significantly higher (p = 0.01) isolation from 703 pigs with caseated tuberculous nodules (group S2) which was 13.7%. No R. equi was isolated in the 461 animals with calcified tuberculous nodules (group S3). The detection rate of atypical mycobacteria increased in these groups S1, S2 and S3, the values being 19.3, 37.3 and 45.6%, respectively, and those of R. equi and/or atypical bacteria from pigs were 23.4, 45.8 and 54.9%, respectively. The difference between the groups was highly significant (p = 0.01). The differences between the groups were highly significant (p = 0.01). Of a total of 765 pig and cattle farms R. equi was isolated from 93 farms. Contact of swine on these farms with horses or their faeces was confirmed in 19 (20.4%) cases.

tuberculosis; Rhodococcus equi; Mycobacterium avium complex; caseification and calcification of tuberculous nodules; submaxillary and mesenteric lymph nodes

ABSTRACT: Byla studována prevalence Rhodococcus equi a atypických mykobakterií v mízních uzlinách klinicky zdravých prasat (n = 1 382) a skotu (n = 231) v České republice v průběhu let 1996 až 1998. R. equi byl samostatně izolován od 7,4 % prasat, ve smíšené infekci s atypickými mykobakteriemi u dalších 2,3 % prasat a od 1,7 % kusu skotu. Frekvence výskytu R. equi u prasat byla statisticky výsločno významně vyšší (p = 0.01) než u skotu. Atypické mykobakterie byly samostatně izolovány od 37,2 % prasat a 28,2 % skotu, což byl statisticky výsloč významný rozdíl (p = 0.01). Z celkem izolovaných 546 mykobakteriálních kmenů od prasat jich 96,2 % patřilo ke komplexu Mycobacterium avium a 21 (3,8 %) k ostatním druhy (pět kmenů M. chelonae, šest M. terrae, dva kmeny M. phlei, šest kmenů M. fortuitum a dva kmeny nebyly určeny). Od skotu bylo izolováno 65 mykobakteriálních kmenů patřících pouze ke komplexu M. avium. R. equi byl samostatně izolován z podčetinových mízních uzlin 492 prasat u 19,5 %, atypické mykobakterie u 30,1 % a smíšená infekce u 4,9 % zvířat. Naproti

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INTRODUCTION

Bovine tuberculosis in farm animals including pigs and cattle was eradicated in the Czech Republic in 1968 (Pavlas, 1999). In the past decade, the detection rate of Mycobacterium bovis in pigs and cattle was very low with an almost zero occurrence in the last few years (Pavlik et al., 1998). However, tuberculosis changes in lymph nodes of cattle and pigs are still found during routine veterinary hygiene inspections in slaughterhouses. Data of the State Veterinary Administration of the Czech Republic indicates that 4 224 106 fattened pigs were examined in slaughterhouses in 1995. Tuberculosis changes, predominantly in mesenteric and head lymph nodes, were detected in 11 805 (0.27%) pigs (Pavlas, 1998). The cause of these changes must be investigated for epidemiological and veterinary public health reasons, as the disease causes great economic losses to farmers.

Of the changed lymph nodes of pigs, predominantly atypical, opportunistic mycobacteria were often isolated in the Czech Republic in the recent years. The strains of M. avium complex were most frequently isolated (Krucký, 1981; Pavlas, 1989; Dvorská et al., 1999), and they are the most important of the atypical mycobacteria. The strains of M. avium complex cause great financial losses mainly in swine and poultry farms (Berthelsen, 1974; Dey and Parham 1993). Recently, great attention has also been paid to tuberculosis caused by the strains of M. avium complex in human medicine, as these strains have been isolated in almost 55% of patients with AIDS (Yakrus and Good, 1990; Horsburg, 1991; Benson and Ellner, 1993). Different methods are being used at present for rapid diagnosis of the strains of M. avium complex, the most frequent being Accu-Probe (Inc., San Diego, California, USA).

In pigs with tuberculosis changes of lymph nodes, isolations of Rhodococcus equi are also frequent (Feldman et al., 1940; Karlson et al., 1940; Cotchin, 1943; Woodrofe, 1950; Barton and Hughes, 1980; Kao et al., 1982; Takai and Tsubaki, 1985; Takai et al., 1986; Katsumi et al., 1991). High prevalence of tuberculous infections in submaxillary lymph nodes in pigs caused by atypical mycobacteria together with R. equi has been described since the 1940’s (Karlson et al., 1940; Yachida and Shimizu, 1973). Isolation of R. equi from cattle is not frequent (McKenzie and Donald, 1979).

R. equi is a world-wide-spread micro-organism which is classified as an opportunistic organism. The first data concerning the isolation of R. equi, originally designated as Corynebacterium equi, were recorded in the early 1920’s (Magnusson, 1923). The author described the micro-organism as the causal agent of purulent bronchopneumonia of foals under 4 months of age. However, R. equi is considered to be primarily a soil microorganism. Its isolation from the intestinal mucosa of adult ruminants, and especially from their faeces, confirms transmission via contaminated feed. The highest counts of R. equi come from topsoil on horse breeding farms (Barton and Hughes, 1980; Takai and Tsubaki 1985; Prescott, 1991).

In cattle R. equi is found in tuberculous lesions of retropharyngeal and submaxillary lymph nodes (McKenzie and Donald, 1979). It can also be isolated from bovine small intestine and faeces (Mutimer and Woolcock, 1980). Sporadic findings of R. equi in cattle confirms its negligible role in spreading the micro-organism.

R. equi sporadically induces infections in other mammals as a result of its immunosuppressive effect. In man, R. equi was first isolated in the mid 1960’s from a patient with pulmonary abscesses (Golub et al., 1967). In the following years, R. equi was isolated in 12 other patients with pulmonary diseases. Infection with R. equi from a patient with acquired immunodeficiency syndrome (AIDS) was reported in 1986 (Samies et al., 1986), since then the number of cases of infection with R. equi in humans has risen to more than one hundred patients which corresponds with the spread of HIV infection (Votava et al., 1996, 1997). The most frequent entry point of R. equi infection in humans are the lungs and less frequently the intestinal tract or damaged skin. Contact with animals, manure or soil is reported in the amanessence of about one third of patients (Harvey and Sunstrum, 1991; Lasky et al., 1991).

Regarding the fact that R. equi and M. avium complex strains are causative agents of animal and human infections, it is necessary to control their spread in both human and animal populations. To assess the role of
individual pathogens in economic losses in fattening pigs and cattle, it is important to monitor their occurrence. Therefore, the objective of this study was to investigate the prevalence of R. equi strains and atypical mycobacteria in the lymph nodes of pigs and cattle from herds suspected of tuberculosis.

MATERIAL AND METHODS

1. Origin of pigs and cattle

Pigs

Animals were from herds with tuberculoid findings in lymph nodes or with allergic reactions to avian tuberculin after simultaneous tuberculin testing at a dose of 1 600 TU/dosi (Bioveta, Ivanovice na Hané, Czech Republic). Lymph nodes were examined from 1382 clinically healthy pigs originating from 575 herds of 45 districts of the Czech Republic in the period of 1996–1998.

Cattle

Samples were collected from animals with allergic reactions to bovine tuberculin Bovitubal (16 000 TU/dosi) made in Biroveta, Ivanovice na Hané in the Czech Republic or from animals with tuberculoid changes in lymph nodes. Lymph nodes from 231 animals originating from 190 herds of 30 districts were examined.

Relationship between examined animals and horse farms

Considering a possible transmission of R. equi from horse herds to swine and cattle herds, the following history data was collected in all the districts with R. equi isolation: herd origin and direct or indirect contact of animals with horses during the past 15 years.

2. Sampling of the necropsy materials

Lymph nodes were collected immediately after slaughter and transferred to the laboratory either fresh or deep-frozen (–20 °C). One culture examination was performed from each animal.

Pigs

Head lymph nodes (Lymphonodus submaxillaris) were examined from 492 pigs; mesenteric lymph nodes (L. mesenterialis) from 639 pigs and inguinal lymph nodes (L. inguinalis) from 14 pigs. Lymph nodes from 237 pigs belonging to one of the three categories that could not be identified were also cultured.

Cattle

Different lymph nodes were examined from 231 cattle: L. submandibularis (n = 5), L. mesenterialis (n = 185), L. inguinalis (n = 1), L. mediastinalis (n = 15), and a mixture of the above lymph nodes (n = 25).

3. Patho-anatomical assessment of lymph nodes

During tissue sampling from swine and bovine lymph nodes for culture examinations, visual assessment of pathoanatomical changes was performed. Based on this assessment, animals were divided into the following three groups:

Group S1 – with no patho-anatomical changes (n = 265),
Group S2 – with caseous tuberculoid changes (n = 716),
Group S3 – with calcified tuberculoid changes (n = 632).

4. Culture examinations of tissues from swine and bovine lymph nodes

Lymph node samples were aseptically collected and cultured on blood agar supplemented with 5% ram’s blood at 37 °C for 48 h. For culture examination of mycobacteria, 1 g of tissue was homogenised with sand in a mortar and incubated with 1N HCl for 15 min. Consequently, by adding bromthymol blue as an indicator, it was neutralised with 1N NaOH until the colour turned blue. After centrifugation (3 000 rpm/20 min) and resuspension in sterile saline, the sediment was inoculated at a dose of 0.2 ml onto three media: egg medium by Stonebrink (one tube), egg medium by Löwenstein-Jensen (two tubes), and a liquid serum medium by Šula for isolation of mycobacteria (two tubes).

5. Identification of the isolated bacterial strains

R. equi strains

The strains of R. equi were isolated on blood agar or solid egg media for mycobacterial isolation and identified by biochemical tests (Goodfellow, 1986).

Mycobacterial strains. Fast-growing mycobacteria at 37 °C (growing to 7 days) were identified by biochemical tests (Wayne and Kubica, 1986). Slowly growing mycobacteria were identified by Accu-Probe test (Accu Probe Inc., San Diego, California); probes for M. avium complex (MAC), M. avium (MA) and M. intracellulare (MI) strains.

Tests were carried out according to the manufacturer’s instructions.

6. Statistical assessment

The χ²-test (Stat Plus) was used for the statistical evaluation (Matoušková et al., 1992).
## RESULTS

### 1. Isolation of *R. equi* and atypical mycobacteria from swine (Tab. I)

*R. equi* strains were isolated from 134 pigs originating from 89 herds in 41 districts of the Czech Republic during the period 1996–1998. From 17 districts only one herd was examined, and in 24 districts 72 herds were examined. Repeated discovery of *R. equi* during a three-year-period was confirmed in 8 herds. Of the 134 examined pigs, *R. equi* alone was identified in 102 (76.1%) animals, and in the remaining 32 (23.9%) animals this isolation also yielded atypical mycobacteria. Identification of 28 mycobacterial strains revealed that 87.5% belonged to the *M. avium* complex and 4 (12.5%) to other mycobacterial species: *M. chelonae* (*n* = 1); *M. fortuitum* (*n* = 3). From 1,382 examined pigs atypical mycobacteria were solely confirmed in 514 (37.2%) animals.

Of 546 isolated mycobacterial strains 525 (96.2%) belonged to the *M. avium* complex. All these strains reacted positively with MAC and MA probes and negatively with MI probe of the Accu-Probe test. Of the remaining 21 strains, 5 strains (0.1%) were identified as *M. chelonae*, 6 (1.1%) as *M. terrae*, 2 (0.4%) as *M. phlei*, 6 (1.1%) as *M. fortuitum*, and 2 strains (0.4%) were not identified.

### Isolation of *R. equi* and atypical mycobacteria in pigs from different lymph nodes (Tab. I)

**Infection with *R. equi* only.** *R. equi* was isolated from mesenteric lymph nodes only in three (0.5%) of 639 animals whereas from submaxillary lymph nodes it was isolated in 96 (19.5%) of 492 animals. The difference was highly significant (*p* = 0.01). In 237 cases where the type of collected lymph nodes could not be identified, *R. equi* alone was isolated in three animals. In 14 animals *R. equi* was not isolated from inguinal lymph nodes.

### Infection with atypical mycobacteria only.** The highest detection rate of atypical mycobacteria was found in mesenteric lymph node cultures (42.6%). A highly significant low rate (*p* = 0.01) of isolation was found in submaxillary and inguinal lymph nodes with 30.1% and 7.1%, respectively.

### Mixed infection with *R. equi* and atypical mycobacteria. The lowest detection rate of both infectious agents (0.8%) was found in the mesenteric lymph nodes. However, their isolation from submaxillary (4.9%) and inguinal lymph nodes (7.1%) was significantly higher (*p* = 0.01).

### Isolation of *R. equi* and atypical mycobacteria from pigs with patho-anatomical lesions in lymph nodes (Tab. II)

Isolation of *R. equi* and atypical mycobacteria was different in each group of animals which were characterised on the basis of gross lesions in lymph nodes: group S1 – without gross lesions, group S2 – with caseous tuberculous nodules, group S3 – with calcified tuberculous nodules. In the changed lymph nodes tuberculous nodules of different sizes from poppy seed to bean were demonstrated.

### Isolation of *R. equi* only.** An examination of lymph nodes in 218 pigs from group S1 revealed *R. equi* in 6 (2.8%) pigs; in group S2, 703 pigs *R. equi* were examined and isolated in 96 (13.7%) animals. The difference between the two groups was highly significant (*p* = 0.01). However, in group S3, comprising 461 animals, no *R. equi* was isolated.

### Isolation of atypical mycobacteria only.** In the isolation of atypical mycobacteria the rate of detection increased in all groups: 19.3% in group S1, 37.3% in group S2 and 45.6% in group S3. Differences among all three groups were highly significant (*p* = 0.01).

### Isolation of *R. equi* and atypical mycobacteria. In mixed infection of pigs with both agents, the rate of detection was only 0.2% in group S3 and 4% in group S2; the difference was highly significant (*p* = 0.01). However, there was no significant difference observed...
II. Relationship between the isolation of *R. equi* and mycobacteria and patho-anatomical changes in swine lymph nodes

<table>
<thead>
<tr>
<th>Patho-anatomical changes</th>
<th>Swine (%)</th>
<th>Cattle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of animals</td>
<td><em>R. equi</em> only</td>
</tr>
<tr>
<td>Group S1</td>
<td>218 (15.8)</td>
<td>6 (2.8)</td>
</tr>
<tr>
<td>Without changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group S2</td>
<td>703 (50.9)</td>
<td>96 (13.7)</td>
</tr>
<tr>
<td>Lymph node caseification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group S3</td>
<td>461 (33.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lymph node caseification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1382 (7.4)</td>
<td>102 (7.4)</td>
</tr>
</tbody>
</table>

III. Isolation of *Rhodococcus equi* and *Mycobacterium avium* complex strains from cattle lymph nodes from 1996–1998

<table>
<thead>
<tr>
<th>Tissue samples</th>
<th>Number of animals</th>
<th>Isolation of <em>R. equi</em> only</th>
<th>MAC strains only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>number</td>
<td>%</td>
</tr>
<tr>
<td>Lyphomononas submandibularis</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lyphomononas mesenterioli</td>
<td>185</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Unidentified</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lyphomononas inguinalis</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Lyphomononas mediastinalis</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>231</td>
<td>4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Explanations:
MAC = *Mycobacterium avium* complex

between the detection rate of group S1 (1.4%) and the other groups.

**Isolation of *R. equi* and/or atypical mycobacteria.**
The degree of gross lesions on lymph nodes and a subsequent identification of at least one of the agents was evaluated. The rate of detection was in group S1 (23.4%), in group S3 (45.8%) and in group S2 (54.9%), the lowest and the highest being group S1 and S2, respectively. Statistical analysis of the three groups revealed that the difference between them was highly significant (p = 0.01).

2. Isolation of *R. equi* and atypical mycobacteria from cattle (Tab. III)

Of 231 examined animals *R. equi* strains were isolated from 4 (1.7%) and atypical mycobacteria from 65 (28.2%) animals. Not a single case of mixed infection in animals by both agents was detected.

**Isolation of *R. equi* and atypical mycobacteria from different lymph nodes in cattle** (Tab. III)

Because of a limited number of examined animals we found no significant differences in isolation of both agents from different lymph nodes.

Isolation of *R. equi* and atypical mycobacteria from lymph nodes with patho-anatomical lesions in cattle (Tab. II)

*R. equi* was isolated from 4 animals in group S3 only. The rate of detection of atypical mycobacteria in group S3 was (33.9%) highly significant (p = 0.05) compared with group S1 (14.9%). No atypical mycobacteria were isolated from group S2.

3. Comparison of *R. equi* and atypical mycobacteria isolation from cattle and pigs (Tab. I, III, IV)

**Occurrence of *R. equi* and atypical mycobacteria (Tab. I, III)**

The frequency of both *R. equi* and atypical mycobacteria was significantly higher (p = 0.01) in pigs than in cattle.

**Isolation of *R. equi* and atypical mycobacteria from submaxillary and mesenteric lymph nodes (Tab. IV)**

In the following analysis all isolations of *R. equi* and atypical mycobacteria from submaxillary and mesenteric lymph nodes of pigs and cattle were calculated.
IV. Comparison of isolation of *R. equi* and/or mycobacteria from swine and cattle submandibular and mesenteric lymph nodes

<table>
<thead>
<tr>
<th>Tissue samples</th>
<th>Numbers</th>
<th>Isolated strains</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>R. equi</em></td>
<td>Mycobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>number</td>
<td>%</td>
<td>number</td>
<td>%</td>
</tr>
<tr>
<td>Lymphonodus submandibularis</td>
<td>497</td>
<td>120a</td>
<td>24.1</td>
<td>173</td>
<td>34.8</td>
</tr>
<tr>
<td>Lymphonodus mesenterialis</td>
<td>824</td>
<td>11</td>
<td>1.3</td>
<td>334b</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Explanations:

a = p = 0.01: compared with mesenteric lymph nodes

b = p = 0.01: compared with submandibular lymph nodes

The number of *R. equi* isolates from submaxillary lymph nodes (24.1% of animals) was significantly higher (p = 0.01) than the isolates from mesenteric lymph nodes (1.3% of animals). On the other hand, the number of isolated mycobacterial strains from mesenteric lymph nodes (40.5% of animals) was significantly higher (p = 0.05) than from submaxillary lymph nodes (34.8% of animals).

In the remaining animals with isolation of *R. equi* from inguinal and pulmonary lymph nodes, it is difficult to draw a valid conclusion, due to a limited number of samples.

The number of *R. equi* isolates from submaxillary lymph nodes (24.1% of animals) was significantly higher (p = 0.01) than the isolates from mesenteric lymph nodes (1.3% of animals). On the other hand, the number of isolated mycobacterial strains from mesenteric lymph nodes (40.5% of animals) was significantly higher (p = 0.05) than from submaxillary lymph nodes (34.8% of animals).

In the remaining animals with isolation of *R. equi* from inguinal and pulmonary lymph nodes, it is difficult to draw a valid conclusion, due to a limited number of samples.

4. Contacts between *R. equi* infected cattle and swine herds with horse herds

**Infected swine herds**

Of a total of 575 examined swine herds, *R. equi* was isolated from 134 pigs originating from 89 (15.5%) herds. Anamnestic data concerning all the infected herds revealed the following facts: in 10 (11.2%) farms horses were kept in the past 10–15 years; 7 swine herds (7.9%) were in the vicinity (within 5 km) of horse farms; and in 72 fully-automated large-scale swine farms there was no contact either with horses or their faeces.

**Infected cattle herds**

From a total of 190 examined herds, *R. equi* was isolated in 4 of them. In the first farm horses were kept on the premises previously; the second farm was adjacent of a horse farm and the remaining two herds had no contact either with horses or their faeces.

**DISCUSSION**

Since 1968 bovine tuberculosis has been eradicated from the Czech Republic and the epidemiological situation has changed markedly. In the early 1970's the strains of *M. avium* complex began to predominate over *M. bovis* isolates. In the years 1975–1979 only 340 *M. bovis* strains were isolated from 21 748 samples as compared to 5 331 strains of *M. avium* complex in the same period (Krucký, 1981). As the rate of detection of mycobacteria in the lymph nodes with gross lesions was relatively low (under 60%), the search was directed towards other intracellular agents. These intracellular bacterial species were also isolated from lymph nodes with tuberculoïd nodules. During allergenodiagnostic tests in pigs, in some cases, non-specific reactors to avian tuberculosis remained unexplained. Therefore in the early 1990's the State Veterinary Diagnostic Institute in Prague started to culture samples not only on media for mycobacteria but also on blood agar where *R. equi* strains were predominantly isolated from the lymph nodes of pigs (Štika, 1992 – personal information).

1. **Clinical symptoms of *R. equi* infection**

Clinical symptoms of *R. equi* infection are manifested particularly in foals up to 6 months of age (Woolcock et al., 1980; Hietala and Ardans, 1987; Prescott, 1991). Foals with low level of antibodies are more susceptible to spontaneous infection (Skalka, 1987).

In man, disease caused by *R. equi* infection is rare. It is predominantly characterised by pneumonia, and rare extrapulmonary abscesses. In young animals and AIDS patients the disease has the same course manifested by abscessing pneumonia. In HIV-negative individuals the disease is most frequently extrapulmonary, resembling the infection in pigs (Votava et al., 1996, 1997).

As the purpose of our work was to assess intradermal test reactor animals and to monitor gross lesions in abattoirs, all examined animals were without clinical signs of the infection. However, the gross changes caused by *R. equi* found in lymph nodes could not be distinguished from lymphadenitis caused by mycobacteria.

Because at present no rapid and reliable diagnostic method (direct detection of DNA from tissue by PCR) is available in the laboratories in the Czech Republic, additional examinations by culture methods appears to be necessary.

2. **Patho-anatomical findings**

*R. equi* and atypical mycobacteria (especially *M. avium* complex strains) are facultative intracellular pathogens surviving inside macrophages and inducing granulomatous inflammation. At the advanced stage of
the disease the macrophages become destroyed and a caseous necrosis of the lymph nodes develop. As the disease progresses, these necrotic lymph nodes may calcify.

In animals, lungs are often the organ most affected by *R. equi* (especially in horses), and so a heavy oral infection can also cause lymphadenitis of the gastrointestinal tract accompanied by intestinal ulceration (Prescott, 1991). During meat inspection at the slaughter house, it is difficult to differentiate whether the gross lesions of the lymph nodes are caused either by *R. equi* or by mycobacteria.

**Pigs**

In our study we have found both agents, either independently or in combination, in 54.9% of animals with caseous lymph nodes and in 45.8% of animals with calcified lymph nodes. However, the detection rate of the two agents differed depending on the stage of gross lesions. In the caseous lymph nodes *R. equi* alone was detected in 13.7% of animals, and in a mixed infection with atypical mycobacteria in another 40%. On the other hand, no *R. equi* alone was isolated from 461 pigs with calcified nodules, and only in one animal (0.2%) was it isolated together with atypical mycobacteria (Tab. II). It seems that during the immune response of the host organism to *R. equi* infection the subsequent calcification of the inflammatory process results in a total deactivation of the agent. On the other hand, the rate of detection of atypical mycobacteria (especially the strains of *M. avium* complex) tends to increase (Tab. II), the highest detection rate being found in calcified lymph nodes (45.6%).

**Cattle**

In contrast to pigs the rate of detection of *R. equi* in 231 examined cattle was as low as 4 (1.7%). However, in all animals with isolation of *R. equi*, calcification of tuberculous nodules was observed (Tab. II, III). According to other authors, cattle are highly resistant to *R. equi* infection (Woolcock et al., 1980; Mutimer and Woolcock, 1980). Isolation of *R. equi* in these 4 animals suggests an impaired natural resistance resulting in the failure to eliminate the bacteria from the inflammatory lesions by calcification.

**3. Pathogenesis caused by *R. equi***

**Pigs**

The primary role of *R. equi* in the pathogenesis of granulomatous lymphadenitis remains unclear because *R. equi* was isolated from lymph nodes showing tuberculous lesions (Cotchin et al., 1943; Prescott 1991) as well as from normal lymph nodes (Woodrofe 1950; Takai et al., 1986, 1996). The detection rate of *R. equi* from the normal lymph nodes ranged between 2 and 8% (Cotchin, 1943; Takai et al., 1986). Our results show that *R. equi* strains alone were isolated from 2.8% and in mixed infections from 1.4% of pigs with no patho-anatomical changes of the lymph nodes (Tab. II). These animals were in most cases culled due to allergic reactions to avian tuberculin. Therefore intravital diagnosis of mycobacterial infection in pigs is complicated by *R. equi* strains as the surface antigenic structures of both bacterial species are evidently very similar (Prescott, 1991).

To elucidate pathogenesis of the disease, some authors attempted to induce, though unsuccessfully, patho-anatomical changes in swine lymph nodes by oral administration of *R. equi* (Feldman et al., 1940; Karlson et al., 1940; Cotchin, 1943; Takai and Tsubaki, 1985; Katsumi et al., 1991). Therefore it is likely that some hitherto undisclosed predisposing factors are responsible for the expression of lesions in infected pigs (Takai et al., 1996; Takai, 1997). These factors may include changes in nutrition, stress during transportation of piglets after weaning, their repeated infection, current state of cell-mediated immunity etc.

**4. Localisation of gross lesions in lymph nodes of pigs and cattle and isolation of the causative agents**

**Localisation of gross lesions**

The present study confirms the conclusions of many other authors (Karlson et al., 1940; Cotchin 1943; Takai
and Tsukuba. 1985; Takai et al., 1986; Katsumi et al., 1991; Takai, 1997) that submaxillary lymph nodes of pigs are favourable for the survival of R. equi and atypical mycobacteria. Most probably the mesenteric lymph nodes offer better conditions for atypical mycobacteria (especially M. avium complex) than for R. equi (Tab. I). However, the concurrent presence of both R. equi (19.5%) and atypical mycobacteria (30.1%) in the submaxillary lymph nodes is not the result of a dependant relationship.

Mixed infection of R. equi and atypical mycobacteria

The epidemiological problem of the occurrence of R. equi and atypical mycobacteria in the submaxillary and mesenteric lymph nodes of pigs, in which Takai et al. (1986) were engaged, has not yet been satisfactorily solved. According to their studies mesenteric lymph nodes create a more favourable environment for the survival of the following atypical mycobacteria: M. chelonei spp. chelonei, M. fortuitum and M. xenopi (19% positive out of 90 examined samples). However, the mesenteric lymph nodes do not provide suitable conditions for the survival of R. equi. It is most probable that R. equi and atypical mycobacteria can collaborate with each other in the formation of caseous lymphadenitis in swine following certain stress factors.

5. Ecological background of the occurrence of infections caused by R. equi and atypical mycobacteria

The importance of horse keeping

After the preparation of selective media NANAT by Woolcock et al. (1979), it was possible to carry out extensive epidemiological and ecological studies of R. equi from soil, faeces and manure samples. It was confirmed that multiplication of R. equi can take place in manure and soil which has a high content of organic matter. Manure can become an important source of infection when left on pasture (Prescott et al., 1984). Takai et al. (1991) studied the ecology of R. equi in soils from horse breeding farms and concluded that massive propagation of R. equi from soil occurs in a chain existing between the horse and its soil environment. The highest R. equi counts were found on the surface of the soil, while a 30 cm deep layer contained no R. equi. The results confirm that horses, especially foals, are infected by inhaling R. equi germs, abundant in dusty and windy weather. Therefore we preferred to collect anamnestic data recording a possible direct contact of the animals (pigs-cattle) with horses or indirect infection through contaminated soil.

The importance of horse rearing and infection with R. equi in the Czech Republic

Our results show that in the last 15 years only 20.4% of 765 swine and cattle farms had come into contact with horses, manure or horse farms. It is necessary to remember that the number of horses involved in agricultural production has markedly decreased during the last decades. Specialisation of animal production on a large scale basis is more practiced and so the possible contact of horses with other farm animals has been reduced to a minimum.

Sources of R. equi infection

From the results of our study it can be inferred that in the Czech Republic, the most important intracellular parasites causing tuberculosis changes in lymph nodes are atypical mycobacterial strains of M. avium complex and R. equi. The sources of infection in M. avium complex are infected birds and other animals, whereas for R. equi horses and their environment are incriminated. These opportunistic microorganisms are also present in the environment. R. equi is a soil microorganism which occurs frequently in the manure of herbivores and the environment of grazing animals. The organism is often isolated from the faeces and intestinal tract of cattle, red deer, goats, horses, pigs, sheep and wild birds. Information concerning the isolation of R. equi from faeces is scarce (Prescott, 1991).

Atypical mycobacteria, mainly M. avium complex strains, are a natural inhabitant of the ecosystem in which they participate in the degradation of biological materials (Horváthová et al., 1997). Therefore, the presence of R. equi and atypical mycobacteria in the intestinal tract of pigs is a result of feed contamination. Consequently the possible distribution of the bacteria from the intestine to other predilection sites, submaxillary and mesenteric lymph nodes, is performed by macrophages (Takai et al., 1986). As a result of this massive contamination of feedstuffs and consequent infection of cattle and swine, gross lesions are likely to be detected in abattoirs.

Prevention of the infection in swine herds. From the above mentioned facts it is necessary to prevent the possible contact of pigs with arable land which might be contaminated with R. equi. Therefore it is equally important to reduce soil contamination of feedstuffs like potatoes, carrot and other roughage. Pig runs for all age categories should be kept clean, and faeces regularly removed.

6. The risk of infection of human population of the Czech Republic

The risk of R. equi transmission from farm animals to man obviously provokes the basic question as to whether people are infected directly from animals or primarily from the soil. To solve this problem Takai and Tsukuba (1985) examined samples from the soil and farm animals. A higher occurrence of R. equi was detected in samples from soil and feeding areas, the lower amount being from faecal samples.
Infection of pigs, or other atypical hosts, is similar to the R. equi infection in HIV-negative individuals, which is characterised by extrapulmonary lesions. In HIV-positive patients and in young foals the course of the disease is similar and is manifested by pneumonia (Votava et al., 1996, 1997). Another common feature of R. equi infection in man and pigs is that the R. equi isolates obtained from swine lymph nodes and from patients suffering from AIDS contain the same protein antigen 20-kDa on their surface (Takah et al., 1996). However, it has not yet been adequately explained whether pigs are the source of infection for HIV-positive individuals or whether both share the same source of infection.

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