ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

VETERINÁRNÍ MEDICÍNA

Veterinary Medicine – Czech

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

11

VOLUME 44
PRAHA
NOVEMBER 1999
ISSN 0375-8427

VETERINÁRNÍ MEDICÍNA

Mezinárodní vědecký časopis vydávaný z pověření Ministerstva zemědělství České republiky a pod gescí České akademie zemědělských věd

VETERINARY MEDICINE – CZECH

An international journal published under the authorization by the Ministry of Agriculture and under the direction of the Czech Academy of Agricultural Sciences

World Wide Web (URL): http://www.clark.cz/vri/casopis.htm

Cíl a odborná náplň: Časopis Veterinární medicína uveřejňuje původní vědecké práce a studie typu review ze všech oblastí veterinární medicíny v češtině, slovenštině a angličtině.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences, a abstrakty z časopisu jsou zahrnuty v těchto databázích: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicita: Časopis vychází měsíčně (12x ročně), ročník 44 vychází v roce 1999.

Přijímání rukopisů: Rukopisy ve třech vyhotoveních je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, vedoucí redaktorka, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Česká republika. Tel.: +420 2 24 25 79 39, fax: +420 2 24 25 39 38, e-mail: editor@login.cz. Podrobné pokyny pro autory jsou v redakci a na URL adrese http://www.clark.cz/vri/Pokyny.htm.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a zasílají se na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 1999 je 696 Kč.

Aims and scope: The journal Veterinární medicína original publishes papers and reviews from all fields of veterinary medicine written in Czech, Slovak or English.
The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences, abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicity: The journal is published monthly (12 issues per year), Volume 44 appearing in 1999.

Acceptance of manuscripts: Three copies of manuscript should be addressed to: Ing. Zdeňka Radošová, editor-in-chief, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Tel.: +420 2 24 25 79 39, fax: +420 2 24 25 39 38, e-mail: editor@login.cz. Detailed instructions for authors are available in the editorial office and at URL address http://www.clark.cz/vri/Pokynya.htm.

Subscription information: Subscription orders can be entered only by calendar year (January-December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2. Subscription price for 1999 is 159 USD (Europe), 167 USD (overseas).

F 4884

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 OF 1996–1998*

IZOLACE *RHODOCOCCUS EQUI* A ATYPICKÝCH MYKOBAKTERIÍ Z MÍZNÍCH UZLIN PRASAT A SKOTU Z CHOVŮ S VÝSKYTEM TUBERKULOIDNÍCH NÁLEZŮ V ČESKÉ REPUBLICE V LETECH 1996–1998

L. Dvorská,[1] I. Parmová[2], M. Lávičková[2], J. Bartl[1], V. Vrbas[1], I. Pavlík[1]

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

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 tuberculoid 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 tuberculoid nodules (group S2) which was 13.7%. No *R. equi* was isolated in the 461 animals with calcified tuberculoid 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 only in 19 (20.4%) cases.

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

ABSTRAKT: 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 % kusů skotu. Frekvence výskytu *R. equi* u prasat byla statisticky vysoce významně vyšší (p = 0,01) než u skotu. Atypické mykobakterie byly samostatně izolovány od 37,2 % prasat a 28,2 % skotu, což byl statisticky vysoce 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 druhům (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 podčelistních mízních uzlin 492 prasat u 19,5 %, atypické mykobakterie u 30,1 % a směsná infekce u 4,9 % zvířat. Naproti

* Partially supported by the Ministry of Agriculture of the Czech Republic (Grant No. EP 7172).

tomu z mízních uzlin mezenteriálních 639 prasat byl samostatně *R. equi* izolován pouze u 0,5 %, atypické mykobakterie u 42,6 % a směsná infekce u 0,8 % prasat. Izolace *R. equi* z *ln. submandibularis* byla statisticky vysoce významně vyšší (p = 0,01) než z *ln. mesenterialis*. Při vyšetření mízních uzlin 218 prasat bez tuberkulóních uzlíků (skupina S1) byl samostatně *R. equi* izolován pouze od 2,8 % zvířat oproti statisticky vysoce významně vyšší izolaci (p = 0,01) od 703 prasat s kazeifikovanými tuberkulózními uzlíky (skupina S2), která činila 13,7 % prasat. Od 461 prasat s kalcifikovanými tuberkulózními uzlíky (skupina S3) nebyl *R. equi* izolován ani u jednoho zvířete. Při izolaci atypických mykobakterií tato záchytnost v jednotlivých skupinách stoupala od 19,3 % (S1), přes 37,3 % (S2) po 45,6 % (S3). Rozdíly mezi skupinami byly statisticky vysoce významné (p = 0,01). Záchytnost *R. equi* a atypických mykobakterií od prasat stoupala v jednotlivých skupinách 23,4 % (S1), 45,8 % (S2) a 54,9 % (S3). Rozdíly mezi skupinami byly statisticky vysoce významné (p = 0,01). Ze 765 farem prasat a skotu byl *R. equi* izolován na 93 farmách; kontakt prasat s koňmi či jejich trusem byl prokázán jen na 19 (20,4 %) z nich.

tuberkulóza; *Rhodococcus equi*; *Mycobacterium avium* komplex; kazeifikace a kalcifikace tuberkuloidních uzlíků; *lymphonodus submandibularis*; *lymphonodus mesenterialis*

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 (Pavlík et al., 1998). However, tuberculoid changes in lymph nodes of cattle and pigs are still found during routine veterinary hygiene inspections in slaughter houses. Data of the State Veterinary Administration of the Czech Republic indicates that 4 224 106 fattened pigs were examined in slaughter houses in 1995. Tuberculoid 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 tuberculoid 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; Rao 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 micro-organism. 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 tuberculoid 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 anamnesis 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 1 382 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 Bioveta, 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 (*Ln. mesenterialis*) from 639 pigs and inguinal lymph nodes (*Ln. 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: *Ln. submandibularis* ($n = 5$), *Ln. mesenterialis* ($n = 185$), *Ln. inguinalis* ($n = 1$), *Ln. 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 sea 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 (Goodfelow, 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 χ^2-test (Stat Plus) was used for the statistical evaluation (Matoušková et al., 1992).

I. Isolation of *Rhodococcus equi* and atypical mycobacteria from swine lymph nodes from 1996–1998

Tissue samples	Number of examined animals	Isolation of the agent									
		R. equi only		Mycobacteria only				*R. equi* and mycobacteria			
		number	%	number	%	MAC	others	number	%	MAC	others
Lymphonodus submandibularis	492	96	19.5	148	30.1	143	5	24	4.9	20	4
Lymphonodus mesenterialis	639	3	0.5	272	42.6	264	8	5	0.8	5	0
Unidentified*	237	3	1.3	93	39.2	89	4	1	0.4	1	0
Lymphonodus inguinalis	14	0	0	1	7.1	1	0	2	14.3	2	0
Total	1382	102	7.4	514	37.2	497	17[a]	32	2.3	28	4[b]

Explanations:
MAC = *Mycobacterium avium* complex strains
* = *Lymphonodus submaxilaris*, and/or *Ln. retropharingealis*, and/or *Ln. mediastinales*, and/or *Ln. tracheobronchalis*, and/or *Ln. mesenteriales*
a = *M. chelonae* (4), *M. terrae* (6), *M. phlei* (2), *M. fortuitum* (3), not identified (2)
b = *M. fortuitum* (3), *M. chelonae* (1)

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. chelonei* ($n = 1$); *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 tuberculoid nodules, group S3 – with calcified tuberculoid nodules. In the changed lymph nodes tuberculoid 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

Patho-anatomical changes	Swine (%)					Cattle (%)			
	Number of animals	*R. equi* only	Mycobacteria only	*R. equi* and Mycobacteria	Total positive	Number of animals	*R. equi* only	Mycobacteria only	Total positive
Group S1									
Without changes	218 (15.8)	6 (2.8)	42 (19.3)	3 (1.4)	51 (23.4)	47 (20.3)	0 (0)	7 (14.9)	7 (14.9)
Group S2									
Lymph node caseification	703 (50.9)	96 (13.7)	262 (37.3)	28 (4.0)	386 (54.9)	13 (5.6)	0 (0)	0 (0)	0 (0)
Group S3									
Lymph node calcification	461 (33.3)	0 (0)	210 (45.6)	1 (0.2)	211 (45.8)	171 (74.1)	4 (2.3)	58 (33.9)	62 (36.3)
Total	1382	102	514	32	648	231	4	65	69
%		(7.4)	(37.2)	(2.3)	(46.9)		(1.7)	(28.1)	(29.9)

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

Tissue samples	Number of animals	Isolation of			
		R. equi only		MAC strains only	
		number	%	number	%
Lymphonodus submandibularis	5	0	0	1	20.0
Lymphonodus mesenterialis	185	3	1.6	57	30.8
Unidentified*	25	0	0	5	20.0
Lymphonodus inguinalis	1	1	100	0	0
Lymphonodus mediastinalis	15	0	0	2	13.3
Total	231	4	1.7	65	28.2

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

Tissue samples	Numbers	Isolated strains			
		R. equi		Mycobacteria	
		number	%	number	%
Lymphonodus submandibularis	497	120[a]	24.1	173	34.8
Lymphonodus mesenterialis	824	11	1.3	334[b]	40.5

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.

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 with 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 tuberculoid 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 devitalisation 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 tuberculoid 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 tuberculoid 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.

Cattle

McKenzie and Donald (1979) mentioned for the first time a possible participation of *R. equi* in the formation of tuberculoid lesions in bovine lymph nodes. Our study revealed that the involvement of *R. equi* in the formation of pathoanatomical changes in cattle, in contrast to pigs, is not important since the agent was isolated in only four (1.7%) of 231 examined animals. The strains were isolated from mesenteric lymph nodes (three animals) and inguinal lymph nodes (one animal). Similar results have also been reported by other authors (Mutimer and Woolcock, 1980; Takai and Tsubaki, 1985).

R. equi was isolated in 50% of faeces of the examined animals compared with negative culture results from retropharyngeal and submaxillary lymph nodes (Mutimer and Woolcock, 1980). Results show that the lymphatic tissue of the intestinal tract is permanently exposed to *R. equi*. A relatively sporadic occurrence of *R. equi* in these lymph nodes suggests its low importance in the pathological processes of the lymph nodes (McKenzie and Donald, 1979; Woolcock et al., 1980; Mutimer and Woolcock, 1980). *R. equi*, being an obligate aerobe, cannot multiply in the intestine of adult ruminants, thus cattle play a passive role in spreading the agent within the environment. Therefore, bovine faeces may contain only *R. equi* strains ingested from contaminated pasture (Prescott, 1991).

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 Tsubaki, 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 practised 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 tuberculoid 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 feedstuff 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 foodstuffs 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 Tsubaki (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 (Takai 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.

Acknowledgements

We are grateful to P. Švástová for her technical assistance. The authors wish to thank Prof. B. Skalka, DrSc., Dr. Wuhib Aayo Ayele (VRI Brno), Rebecca Norton (University of Wales, Aberystwyth, Wales, UK) and Ass. Prof. E. Baranyiová (Veterinary and Pharmaceutical University, Brno, Czech Republic) for their critical reading of the manuscript.

REFERENCES

Barton M. D., Hughes K. L. (1980): *Corynebacterium equi*: a review. Vet. Bull., *50*, 65–80.

Barton M. D., Hughes K. L. (1984): Ecology of *Rhodococcus equi*. Vet. Microbiol., *9*, 65–76.

Benson C. A., Ellner J. J. (1993): *Mycobacterium avium* complex infection and AIDS: advances in theory and practice. Clin. Infect. Dis., *17*, 7–20.

Berthelsen J. D. (1974): Economics of the avian TB problem in swine. J. Am. Vet. Med. Assoc., *164*, 307–308.

Cotchin E. (1943): *Corynebacterium equi* in the submaxillary lymph nodes of swine. J. Comp. Pathol., *53*, 298–309.

Dey B. P., Parham G. L. (1993): Incidence and economics of tuberculosis in swine slaughtered from 1976 to 1988. J. Am. Vet. Med. Assoc., *203*, 516–519.

Dvorská L., Parmová I., Bartl J., Švástová P., Pavlík I. (1999): Identification of *Mycobacterium avium* complex strains isolated from swine and cattle lymph nodes from the Czech Republic from 1996 till 1998. Vet. Med. – Czech, under preparation.

Feldman W. H., Moses H. E., Karlson A. G. (1940): *Corynebacterium equi* as a possible cause of tuberculosis-like lesions of swine. Cornell Vet., *30*, 465–481.

Golub B., Falk G., Spink, W. W. (1967): Lung abscess due to *Corynebacterium equi*. Report of first human infection. Ann. Int. Med., *66*, 1174–1177.

Goodfelow M. (1986): Genus *Rhodococcus* Zopf. 1891. In: Sneath P. H. A., Mair N. S., Sharpe M. E., Holt J. G. (eds.): Bergey's Manual of Systematic Bacteriology. Vol. 2. Baltimore, London, Los Angels, Sydney, Williams and Wilkins. 1472–1480.

Harvey R. L., Sunstrum J. C. (1991): *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. Rev. Infect. Dis., *13*, 139–145.

Hietala S. K., Ardans A. A. (1987): Neutrophil phagocytic and serum oppsonic response of the foal to *Corynebacterium equi*. Vet. Immunol. Immunopathol., *14*, 279–294.

Horsburg C. R. (1991): *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. N. Engl. J. Med., *324*, 1332–1338.

Horváthová A., Kazda J., Bartl J., Pavlík I. (1997): Occurrence of conditionally pathogenic mycobacteria in the environment and their impact on living organism (in Czech). Vet. Med. – Czech, *42*, 191–212.

Karlson A. G., Moses H. E., Feldman W. H. (1940): *Corynebacterium equi* (Magnuson, 1923) in the submaxillary lymph nodes of swine. J. Infect. Dis., *67*, 243–251.

Katsumi M., Kodama N., Miki Y., Hiramune T., Kikuchi R., Yanagawa R., Nakazawa M. (1991): Typing of *Rhodococcus equi* isolated from submaxillary lymph nodes of pigs in Japan. Zbl. Vet. Med. Reihe B, *38*, 299–302.

Krucký J. (1981): Occurrence of mycobacteria in the examined samples during 1975–1979 (in Czech). Veterinářství, *31*, 362–365.

Lasky J. A., Pulkingham N., Powers M. A., Durack D. T. (1991): *Rhodococcus equi* causing human pulmonary infection: review of 29 cases. South. Med. J., *84*, 1217–1220.

Magnusson H. (1923): Spezifische Infektioese Pneumonie beim Fohlen. Ein neuer Eitererreger beim Pferd. Arch. Wiss. Prakt. Tierheilk., *50*, 22–238.

Matoušková O., Chalupa J., Cígler M., Hruška K. (1992): STAT Plus Manual (in Czech), 1st ed. Brno, Veterinary Research Institute. 168 pp.

McKenzie R. A., Donald B. A. (1979): Lymphadenitis in cattle associated with *Corynebacterium equi*: a problem in bovine tuberculosis diagnosis. J. Comp. Pathol., *89*, 31–38.

Mutimer M. D., Woolcock J. B. (1980): *Corynebacterium equi* in cattle and pigs. Vet. Quarterly, *2*, 25–27.

Pavlas M. (1989): Dynamics of the incidence of Mycobacteria in pigs in 1981–1985 (in Czech). Stud. Pneumol. Phtiseol. Czech., *49*, 152–157.

Pavlas M. (1998): Occurrence, diagnostics and epizootiological significance of porcine mycobacterial infections (in Czech). Veterinářství, *48*, 482–487.

Pavlas M. (1999): The 30th anniversary from eradication of bovine tuberculosis in cattle in Czechoslovakia. Acta Vet., Brno, *68*, 155–162.

Pavlík I., Bartl J., Parmová I., Havelková M., Kubín M., Bažant J. (1998): Occurrence of bovine tuberculosis in animals and humans in the Czech Republic in the years 1969 to 1996. Vet. Med. – Czech, *43*, 221–231.

Prescott J. F. (1991): *Rhodococcus equi* an animal and human pathogen. Clin. Microbiol. Rev., *4*, 20–34.

Prescott J. F., Coshan-Gauthier R., Barksade L. (1984): Antibody to *equi factor(s)* in the diagnosis of *Corynebacterium equi* pneumonia of foals. Can. J. Comp. Med., *48*, 370–373.

Rao M. S., Zaki S., Keshavamurthy B. S., Singh K. C. (1982): An outbreak of acute *Corynebacterium equi* infection in piglets. Indian Vet. J., *59*, 787–788.

Samies J. H., Hathaway B. N., Echols R. M., Veazey J. M., Pilon V. A. (1986): Lung abscess due to *Corynebacterium equi*: report of the first case in a patient with acquired immune deficiency syndrome. Am. J. Med., *80*, 685–688.

Skalka B. (1987): Dynamics of *equi-factor* antibodies in sera of foals kept on farms with differing histories of *Rhodococcus equi* pneumonia. Vet. Microbiol., *14*, 269–276.

Takai S. (1997): Epidemiology of *Rhodococcus equi* infections: a review. Vet. Microbiol., *56*, 167–176.

Takai S., Tsubaki S. (1985): The incidence of *Rhodococcus* (*Corynebacterium*) *equi* in domestic animal and soil. J. Vet. Med. Sci., *47*, 1291–1293.

Takai S., Takeuchi T., Tsubaki S. (1986): Isolation of *Rhodococcus* (*Corynebacterium*) *equi* and atypical mycobacteria from the lymph nodes of healthy pigs. J. Vet. Sci., *48*, 445–448.

Takai S., Ohbushi S., Koike K., Tsubaki S., Oishi H., Kamada M. (1991): Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. J. Clin. Microbiol., *29*, 2887–2889.

Takai S., Fukunaga N., Ochiai S., Imai Y., Sasaki Y., Tsubaki S., Sekizaki T. (1996): Identification of intermediately virulent *Rhodococcus equi* isolates from pigs. J. Clin. Microbiol., *34*, 1034–1037.

Votava M., Skalka B., Hrstková H., Tejkalová R., Dvorská L. (1996): Review of 105 cases of isolation of *Rhodococcus equi* in man (in Czech). Čas. Lék. Čes., *138*, 51–53.

Votava M., Skalka B., Tejkalová R. (1997): *Rhodococcus equi* – new discovered oportunistic human pathogen (in Czech). Epidemiol. Mikrobiol. Imunol., *46*, 58–66.

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. Vol. 2. Baltimore, London, Los Angels, Sydney, Williams and Wilkins. 1436–1457.

Woodrofe G. M. (1950): Studies on strains *Corynebacterium equi* isolated from pigs. Aust. J. Exp. Med. Sci., *28*, 399–409.

Woolcock J. B., Farmer M. T., Mutimer M. D. (1979): Selective medium for *Corynebacterium equi* isolation. J. Clin. Microbiol., *9*, 640–642.

Woolcock J. B., Mutimer M. D., Farmer M. T. (1980): Epidemiology of *Corynebacterium equi* in horses. Res. Vet. Sci., *28*, 87–90.

Yachida S., Shimizu K. (1973): Studies on atypical mycobacteria isolated from tuberculous lesions of the mesenteric lymph nodes of slaughtered pigs. Nippon. Juigaku. Zasshi., *35*, 459–471.

Yakrus M. A., Good R. (1990): Geographical distribution, frequency, and specimen source of *Mycobacterium avium* complex serotypes isolated from patients with acquired immunodeficiency syndrome. J. Clin. Microbiol., *28*, 926–929.

Received: 99–07–23
Accepted after corrections: 99–08–24

Contact Address:

MVDr. Ivo Pavlík, CSc., Výzkumný ústav veterinárního lékařství, Hudcova 70, 621 32 Brno, Česká republika
Tel.: +420 5 41 32 12 41, fax +420 5 41 21 12 29, e-mail: pavlik@vri.cz, http://www.vri.cz/wwwrflptext.htm

THE USE OF SORBENTS ON THE BASIS OF HUMIC ACIDS TO REDUCE AMMONIA LEVELS IN STABLE ENVIRONMENT*

VYUŽITÍ SORBENTŮ NA BÁZI HUMINOVÝCH KYSELIN KE SNÍŽENÍ HLADINY AMONIAKU VE STÁJOVÉM PROSTŘEDÍ

P. Suchý, I. Herzig, B. Písaříková

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Laboratory testing of sorption properties of humic substances, which were exposed to increased ammonia levels from 0 to 300 mg/g in a system, showed a highly significant ($P \leq 0.01$) increase of ammonia levels up to the value of 27.1 ± 1.32 mg/g of ground humic substances. The increase of ammonia level in the system to 400 and 500 mg/g had not any significant effect on its concentration in the humic substances (27.8 ± 2.08 and 28.7 ± 1.73 mg/g). The results of laboratory testing of ammonia binding to humic substances are shown in Fig. 1. In a series of experiments performed in experimental stables of the institute and aimed at testing the use of humic acids for the reduction of free ammonia level, 3% of sodium humate were administered in the ration for pigs in two subsequent periods (I and II). At the same time, nitrogen balance (the amount of nitrogen intake by feed and excretion by faeces and urine) was monitored. In another two periods (III and IV) 1 kg of ground humic substances was applied twice a day on the floor of experimental stable, i.e. 150 g/m^2/day. Eight pigs were included in the experiment, housed in balance cages, four in one cage in two identical separated stables. The pigs received feed mixture composed of 68.3% of wheat, 20.0% of barley, 10.0% of soya extracted meal and a mineral supplement. The average daily intake of feed mixture was 1.16, 1.27, 2.00 and 2.20 kg/animal/day in periods I, II, III and IV, respectively. The levels of free ammonia were recorded daily at 7 a.m. and 2.30 p.m. in both stables throughout the whole experimental period. Air temperature and relative humidity were measured in the stables as well as outdoor temperature and atmospheric pressure (Tab. I). Significantly lower ($P \leq 0.05$) average levels of free ammonia (6.9 ± 1.58 ppm) were found in period I in the stable where sodium humate was administered, compared with the stable without humate administration (10.4 ± 3.35 ppm). However, this result was not confirmed in period II. Nonsignificantly lower levels of free ammonia (6.4 ± 1.28 ppm) were found in the stable without humate administration compared with the stable with sodium humate administration (7.6 ± 2.00 ppm). Variations in average levels of free ammonia in both stables during period I and II are shown in Fig. 2 and 3. Recording of nitrogen balance (period I and II) revealed that pigs of the control group had daily nitrogen intake 34.9 ± 3.51 g and those of the experimental group 33.8 ± 4.84 g. Supplementation of feed mixture with 3% of sodium humate resulted in the experimental group in a decrease of daily intake of nitrogen by 3.2% (Tab. II). Daily output of nitrogen by faeces was significantly higher ($P \leq 0.01$) in the experimental group of pigs with administration of 5.35 ± 1.86 g/animal/day of sodium humate (15.8% of N) compared with 3.69 ± 0.68 g/animal/day (10.6% of N administered) in the control group. Daily excretion of nitrogen by urine was significantly lower ($P \leq 0.01$) in the experimental group reaching 11.2 ± 2.92 g/animal/day (33.1% of N administered) than in the control group reaching 14.6 ± 1.53 g/animal/day (41.9% of N administered) (Tab. II). In period III significantly lower ($P \leq 0.05$) levels of free ammonia (6.5 ± 1.12 ppm) were found in the stable where ground humic substances were applied on the floor compared with the control stable (11.0 ± 4.43 ppm). Like in period IV, significantly lower ($P \leq 0.01$) average levels of free ammonia (7.2 ± 2.44 ppm) were recorded in the experimental stable compared with the control one (12.0 ± 2.89 ppm). Variations of the average levels of free ammonia in both stables during periods III and IV are shown in Figs. 4 and 5.

pigs; sodium humate; humic substances; ammonia; nitrogen balance; emission; microclimate

ABSTRAKT: Při laboratorním ověření sorpčních vlastností oxihumolitu, který byl vystaven stoupajícím hladinám amoniaku 0 až 300 mg/g v systému, bylo prokázáno statisticky vysoce významné ($P \leq 0,01$) zvyšování hladin amoniaku do hodnoty 27,1 ± 1,32 mg/g mletého oxihumolitu. Zvýšení hladiny amoniaku v systému na 400 a 500 mg/g statisticky významně jeho koncentrace v oxihumolitu neovlivnilo (27,8 ± 2,08 a 28,7 ± 1,73 mg/g). V sérii pokusů uskutečněných v experimentálních stájích a zaměřených na ověření použití huminových látek ke snížení hladiny volného amoniaku, byla ve dvou po sobě jdoucích periodách (I a II) podávána 3 % humátu sodného v krmné dávce prasat. V dalších dvou periodách (III a IV) byl v pokusné stáji dvakrát denně aplikován 1 kg mletého oxihumolitu na podlahu, tj. 150 g/m^2/den. Do pokusů bylo zařazeno

* Supported by the National Agency for Agricultural Research in Prague (Grant No. EP 9402) and the Ministry of Industry of the Czech Republic (Grant No. 3310/51/97/02).

osm vepřů umístněných v bilančních klecích a rozdělených po čtyřech do dvou identických, stavebně oddělených stájí. V průběhu I. a II. periody byla zjištěna bilance dusíku. V I. periodě, ve stáji s podáváním humátu sodného v krmné dávce, byly zjištěny statisticky průkazně nižší ($P \leq 0,05$) průměrné hladiny amoniaku v ovzduší (6,9 ± 1,58 ppm) oproti stáji bez podávání humátu sodného (10,4 ± 3,35 ppm). Tento výsledek nebyl ve II. periodě potvrzen. Ve stáji bez podávání humátu sodného byly zjištěny nevýznamně nižší průměrné hladiny amoniaku ve stájovém ovzduší 6,4 ± 1,28 ppm oproti stáji s podáváním humátu sodného 7,6 ± 2,00 ppm. Při perorálním podávání humátu sodného byla v I. periodě zjištěna nižší hladina amoniaku ve stáji s podáváním humátu sodného o 3,5 ppm (33,6 %) oproti kontrole. Ve II. periodě byla naopak hladina amoniaku v pokusné stáji oproti kontrole vyšší o 1,2 ppm (15,8 %). Denní výdej dusíku výkaly byl v I. a II. periodě statisticky vysoce významně vyšší ($P \leq 0,01$) u pokusné skupiny prasat přijímajících humát sodný, a to 5,35 ± 1,86 g/kus/den (15,8 % přijatého N) oproti 3,69 ± 0,68 g/kus/den (10,6 % přijatého N) u kontrolní skupiny. Denní vylučování dusíku močí bylo statisticky vysoce významně nižší ($P \leq 0,01$) u skupiny přijímající humát sodný oproti kontrolní skupině, a to 11,2 ± 2,92 g/kus/den (33,1 % přijatého N) oproti 14,6 ± 1,53 g/kus/den (41,9 % přijatého N). Ve III., resp. IV. periodě byly ve stáji s aplikací mletého oxihumolitu na podlahu zjištěny statisticky průkazně nižší ($P \leq 0,05$) průměrné hladiny volného amoniaku, a to 6,5 ± 1,12 ppm oproti 11,0 ± 4,43 ppm (40,9 %), resp. 7,2 ± 2,44 ppm ($P \leq 0,01$) oproti 12,0 ± 2,89 ppm (40,0 %).

prasata; humát sodný; oxihumolit; amoniak; dusíková bilance; emise; mikroklima

ÚVOD

Intenzivní živočišná výroba ovlivňuje nejen prostředí ve kterém jsou zvířata ustájena, ale i kvalitu venkovního ovzduší. Pachy vznikající v důsledku koncentrace škodlivých a jedovatých plynů představují zásadní problém ustájení zvířat. Celosvětové emise amoniaku činí asi 54 Mt dusíku. Více než 60 % těchto emisí souvisí s lidskou činností, především s chovem zvířat a používáním průmyslových hnojiv (Asman aj., 1998). Vyšší koncentrace amoniaku v ekosystému způsobují eutrofizaci vod, snižování druhové diverzity rostlin, poškozování rostlinných porostů v blízkosti stájí, zvýšenou korozi kovů a poškozování fasád domů (Konopásek, 1995).

Zvýšené koncentrace amoniaku a sirovodíku patří mezi predispoziční činitele ovlivňující zdraví zvířat i lidí. Bylo prokázáno, že zvýšené hladiny amoniaku se podílely na vývoji atrofické rhinitidy prasat (Robertson aj., 1990). Dále se ukázalo, že amoniak má negativní vliv na řadu biologických systémů zahrnujících metabolismus hormonů a reprodukci (Visek, 1984). Malayer aj. (1988) zkoumali vliv plynů unikajících z chlévské mrvy na pohlavní dospívání. Jejich zjištění ukázala, že amoniak snižuje stimulující vliv kanců na začátek pohlavního dospívání u prasniček. Nepříznivé působení vysokých koncentrací amoniaku je dobře zdokumentováno u selat, u kterých byl růst redukován o 12 % (Noyes aj., 1986).

Všeobecně lze říci, že amoniak má negativní účinky na užitkovost zvířat, zvyšuje jejich dispozici k řadě onemocnění a představuje riziko i pro zdraví lidí pracujících v provozech intenzivního chovu prasat a drůbeže (Drummont aj., 1980; Donham aj., 1995).

Amoniak může negativně ovlivňovat zdravotní stav již při nízkých hladinách (6 ppm); nejvyšší přípustná hladina amoniaku ve stájovém ovzduší je 25 ppm (Zeman, 1990). Donham (1991) doporučuje maximální koncentraci amoniaku v chovech prasnic 7 ppm.

Přístupy k ovlivnění hladin amoniaku ve stájích s omezeným pohybem zvířat, představují úpravy větracích systémů, změny v manipulaci s odpady, zvýšení kvality bílkovin a snížení obsahu dusíkatých látek v krmné dávce. Ke snížení emisí amoniaku může vést i zvýšení obsahu neškrobových polysacharidů v dietě výkrmových prasat (Canh aj., 1998).

Alternativní přístupy zahrnují použití sorbentů, například zeolitu v krmivu pro zvířata, použití přípravků obsahujících výtažky rostliny *Yucca schidigera*, esenciální oleje, rostlinné extrakty a další komerčně dostupné či ověřované přípravky, které jsou s úspěchem používány jako doplňky krmné dávky prasat, drůbeže a telat a přidávají se do lagun a nádrží s kejdou, nebo jsou rozprašovány na hnůj.

Cílem práce bylo ověřit, zda podáním huminových látek prasatům v krmivu, respektive aplikací do stájového prostředí, lze dosáhnout snížení koncentrace volného amoniaku ve stájovém ovzduší.

MATERIÁL A METODY

K pokusům byl použit humát sodný technický o sušině 90,9 %, s deklarovaným obsahem 61,9 % huminových kyselin a 32,1 % popela v sušině, a mletý oxihumolit o sušině 62,1 %, s deklarovaným obsahem 78,2 % huminových kyselin a 19,8 % popela v sušině. Dopočet do absolutní sušiny představují organické látky. Analýzy uvedených látek provedl VÚAnCh a.s., Ústí n. Labem.

1. Laboratorní ověření vazby plynného amoniaku na mletý oxihumolit v uzavřeném systému

Z chloridu amonného, předsušeného při teplotě 105 °C, byla připravena řada roztoků o koncentracích 5 až 500 mg amoniaku v 1 ml destilované vody. Do střední části misky dle Conwaye byl převeden 1 g mletého oxihumolitu. Do mezikruží misek byl napipetován 1 ml příslušného roztoku chloridu amonného a přidán 1 ml nasyceného roztoku uhličitanu draselného. Miska byla okamžitě hermeticky uzavřena a po promíchání roztoků kroužením ponechána při laboratorní teplotě 24 hodin. K utěsnění víčka byl použit glycerin.

Ve vzorcích mletého oxihumolitu, vystavených různým koncentracím amoniaku uvolněného z roztoků chloridu amonného, byl amoniak stanoven mikrodifuzí postupem uvedeným ve vyhlášce č. 222/1996 Sb. (Příloha č. 9 „Stanovení obsahu těkavých dusíkatých látek").

2. Experimentální použití huminových látek ve stájových podmínkách

Do sledování bylo zařazeno osm vepřů plemene BUxLa o počáteční průměrné hmotnosti v pokusu A 30 kg, v pokusu B 56 kg. Pokusná zvířata byla rozdělena na dvě skupiny (1 až 4 a 5 až 8) ustájené ve dvou identických, stavebně oddělených, uzavřených bezstelivových stájích (A, B), s hydromechanickým odklizem výkalů. Rozměry ustájovacích prostor jsou 3,0 x 4,5 x 4,5 m; každá z nich je propojena s vlastní přípravnou o rozměrech 2,2 x 3,0 x 2 m.

A. Podávání humátu sodného v krmné dávce

Uspořádání pokusu:

Číslo prasete	1	2	3	4	5	6	7	8
Stáj		A				B		
I. perioda (10 dnů)	Hu	Hu	Hu	Hu	0	0	0	0
Meziperioda (5 dnů)	–	–	–	–	–	–	–	–
II. perioda (10 dnů)	0	0	0	0	Hu	Hu	Hu	Hu

0 – kontrola, Hu – humát sodný

Prasata byla umístěna v bilančních klecích, umožňujících přesně evidovaný příjem krmiva a kvantitativní sběr výkalů a moče. Krmení probíhalo dvakrát denně v 7.00 a 16.00 hodin. Kontrolní skupina přijímala krmnou směs vlastní receptury (pšenice 68,3 %, ječmen 20,0 %, sojový extrahovaný šrot 10 % a minerální krmný doplněk), pokusná skupina stejnou krmnou směs s 3% podílem humátu sodného. Před zahájením pokusu bylo krmivo ověřeno analyticky (ČSN 46 7092).

Obsah hrubých živin v použitých krmivech:

		Základní krmná směs	Humát sodný
Sušina	(g/kg)	877,0	900,4
N-látky (N x 6,25)	**(g/kg)**	**183,2**	**63,9**
Tuk	(g/kg)	15,7	3,4
Vláknina	(g/kg)	25,7	6,2
Popel	(g/kg)	29,9	338,5
BNLV	(g/kg)	622,5	488,4
Organická hmota	(g/kg)	847,1	561,9

Průměrný příjem základní krmné směsi činil (kg/ks/den): v I. periodě 1,16, ve II. periodě 1,27, ve III. periodě 2,00 a ve IV. periodě 2,20.

V průběhu I. a II. periody pokusu byly 6. až 10. den kvantitativně sbírány výkaly a moč a uchovávány v tmavých zábrusových lahvích při teplotě 12 °C. Současně byl sledován příjem krmiva. Dusík v krmivu, moči a výkalech byl stanoven dle ČSN 46 7092 (1986). Na základě množství dusíku přijatého a vyloučeného byla vypočtena dusíková bilance.

B. Aplikace oxihumolitu do stájového prostředí

Uspořádání pokusu:

Stáj	A	B
III. perioda (9 dnů)	Hu	0
Meziperioda (5 dnů)	–	–
IV. perioda (11 dnů)	0	Hu

0 – kontrola, Hu – oxihumolit

Vlastní pokus probíhal ve dvou periodách během nichž byl na podlahu jedné ze stájí (A či B) dvakrát denně, po odklizu výkalů, aplikován 1 kg oxihumolitu, tj. 150 g/m^2/den. Mezi periody byla zařazena pětidenní meziperioda, bez aplikace oxihumolitu.

V průběhu I. až IV. periody byly v prostředí obou stájí dvakrát denně, v 7.00 a 14.30 hodin, sledovány hladiny amoniaku titrační metodou (Bena aj., 1955). Současně byla zjišťována teplota vzduchu a relativní vlhkost ve stájích, venkovní teplota a tlak vzduchu (tab. I). Z naměřených hodnot byl vypočten průměr pro každý den pokusu.

Získaná data byla vyhodnocena *t*-testem, pomocí statistického a grafického systému Stat-Plus (Matoušková aj., 1992).

VÝSLEDKY

1. Laboratorní ověření vazby plynného amoniaku na mletý oxihumolit v uzavřeném systému

Ve vzorcích mletého oxihumolitu, vystavených působení plynného amoniaku v množství od 5 do 300 mg/g, bylo zjištěno statisticky vysoce významné zvyšování ($P \leq 0,01$) průměrné koncentrace vyvázaného amoniaku od 1,2 ± 0,10 mg/g při hladině amoniaku v systému 5 mg, do 27,1 ± 1,32 mg/g při hladině amoniaku v systému 300 mg. Další zvyšování hladiny amoniaku v systému na 400 a 500 mg nepřineslo statisticky významné zvýšení průměrné koncentrace vyvázaného amoniaku (27,8 ± 2,08 a 28,7 ± 1,73 mg/g; obr. 1).

2. Experimentální použití huminových látek ve stájových podmínkách

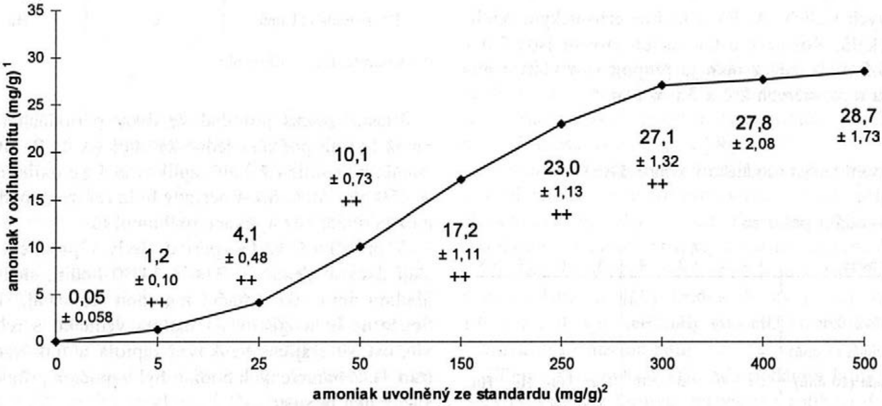
A. Podávání humátu sodného v krmné dávce

V I. periodě ve stáji A, kde byl podáván humát sodný *per os*, byly zjištěny statisticky průkazně nižší ($P \leq 0,05$)

I. Makro- a mikroklimatické ukazatele v průběhu pokusu – Macro- and micro-climate parameters during the experiment

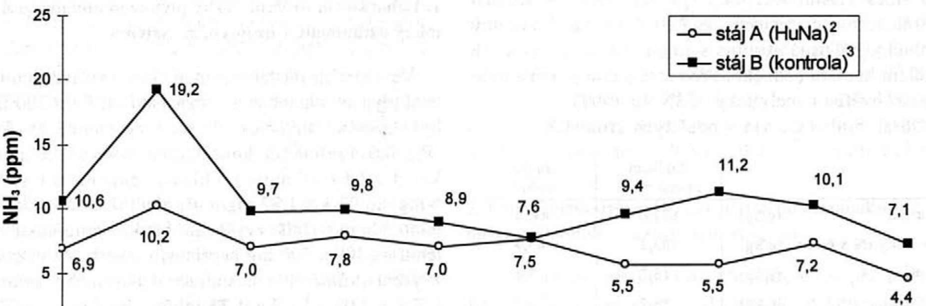
Perioda[1]		Teplota ve stáji[2] (°C) A	Teplota ve stáji[2] (°C) B	Relativní vlhkost[3] (%) A	Relativní vlhkost[3] (%) B	Průměrná denní venkovní teplota[4] (°C)	Atmosferický tlak[5] (hPa)
I.	$\bar{x}$	23,6	23,1	69	70	17,8	1 011,6
	± s	1,23	0,92	4,8	2,3	2,41	4,99
II.	$\bar{x}$	24,5	23,5	67	72	19,2	1 016,3
	± s	1,64	0,85	9,9	6,3	2,20	2,13
III.	$\bar{x}$	22,8	24,3	66	70	13,3	1 006,6
	± s	1,02	0,82	4,1	5,6	2,06	4,96
IV.	$\bar{x}$	19,9	20,1	60	65	3,4	1 015,5
	± s	0,52	0,52	4,8	3,3	1,74	6,35

[1]period, [2]stable temperature, [3]relative humidity, [4]average daily outside temperature, [5]atmospheric pressure



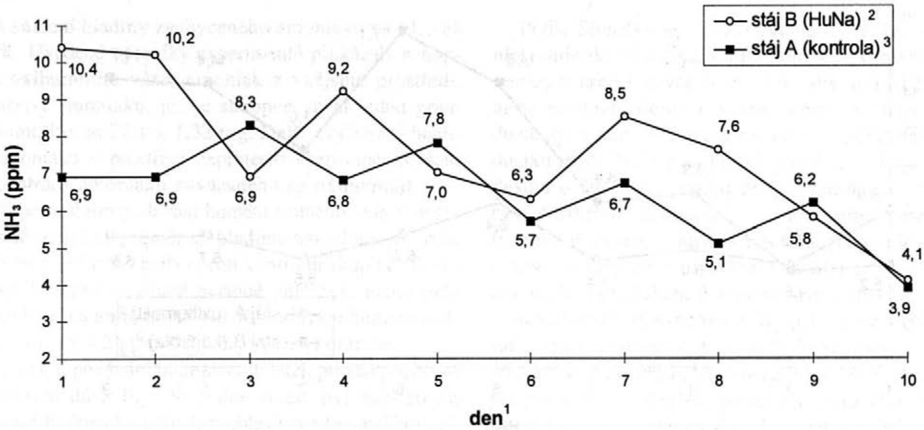
1. Změny koncentrací amoniaku v mletém oxihumolitu po působení plynného amoniaku (ze standardního roztoku NH_4Cl o přepočteném obsahu amoniaku 5 až 500 mg) – Variations of ammonia concentrations in ground oxyhumolith following the effect of gaseous ammonia (evolving from standard solution of NH_4Cl with converted content of ammonia 5–500 mg)

[1]ammonia in oxyhumolith (mg/g), [2]ammonia evolved from standard solution (mg/g)



2. Hladina amoniaku v experimentálních stájích v průběhu pokusu s perorálním podáváním humátu sodného (I. perioda) – Ammonia levels in experimental stables during the experiment with peroral administration of sodium humate (period I)

[1]day, [2]stable A (HuNa), [3]stable B (control)



3. Hladina amoniaku v experimentálních stájích v průběhu pokusu s perorálním podáváním humátu sodného (II. perioda) – Ammonia levels in experimental stables during the experiment with peroral administration of sodium humate (period II)

[1]day, [2]stable A (HuNa), [3]stable B (control)

II. Výsledky bilancí dusíku (n = 8) – Results of nitrogen balance (n = 8)

Skupina[1]	Kontrola[2]	Humát sodný[3]
Celkový příjem (g/ks/den)[4]		
$\bar{x}$	34,9	33,8
± s	3,51	4,84
V (%)	10,1	14,3
Vyloučeno výkaly (g/ks/den)[5]		
$\bar{x}$	3,69	5,35^{++}
± s	0,68	1,06
V (%)	18,6	19,9
Bilanční stravitelnost (%)[6]		
$\bar{x}$	89,4	84,2^{++}
± s	2,05	1,92
V (%)	2,30	2,28
Vyloučeno močí (g/ks/den)[7]		
$\bar{x}$	14,6	11,2^{++}
± s	1,53	2,92
V (%)	10,5	26,0
Uloženo ze stráveného (%)[8]		
$\bar{x}$	53,3	61,0^{++}
± s	3,02	5,89
V (%)	5,67	9,65

++ – $P \leq 0{,}01$

[1]group, [2]control, [3]sodium humate, [4]total intake (g/animal/day), [5]N output by faeces (g/animal/day), [6]apparent digestibility (%), [7]N output by urine (g/animal/day), [8]N deposition from N digested (%)

průměrné hladiny amoniaku v ovzduší 6,9 ± 1,58 ppm, oproti 10,4 ± 3,35 ppm ve stáji B bez podávání humátu sodného. Kolísání průměrných hladin amoniaku v obou stájích je uvedeno na obr. 2. Průměrná teplota a relativní vlhkost byly ve stáji A 23,6 ± 1,23 °C a 69 ± 4,8 %, ve stáji B 23,1 ± 0,92 °C a 70 ± 2,3 % (tab. I).

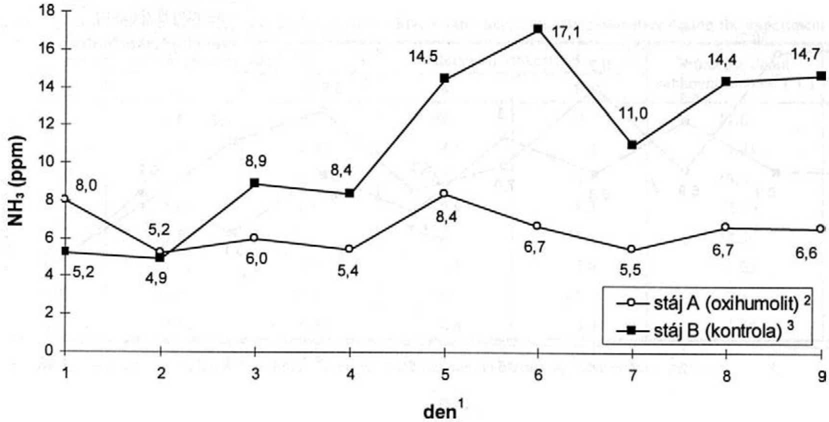
Ve II. periodě ve stáji A, bez podávání humátu sodného, byly zjištěny průměrné hladiny amoniaku ve stájovém ovzduší 6,4 ± 1,28 ppm, ve stáji B, kde byl podáván humát sodný *per os*, pak 7,6 ± 2,00 ppm. Rozdíly mezi průměrnými hladinami amoniaku nebyly v tomto období statisticky průkazné. Kolísání průměrných hladin amoniaku v obou stájích je uvedeno na obr. 3. Průměrná teplota a relativní vlhkost ve stáji A byly 24,5 ± 1,64 °C a 67 ± 9,9 %, ve stáji B 23,5 ± 0,85 °C a 72 ± 6,3 % (tab. I).

Souhrnné výsledky dusíkových bilancí (perioda I a II) jsou uvedeny v tab. II. Prasata v kontrolní skupině přijímala denně 34,9 ± 3,51 g dusíku, v pokusné skupině 33,8 ± 4,84 g dusíku. Zařazením 3% podílu humátu sodného do krmné směsi se u pokusné skupiny denní příjem dusíku snížil o 3,2 %.

U prasat v pokusné skupině, přijímajících humát sodný, bylo zjištěno statisticky vysoce průkazně vyšší ($P \leq 0{,}01$) denní vylučování dusíku výkaly, a to 5,35 ± 1,06 g dusíku (15,8 % přijatého N) oproti 3,69 ± 0,68 g dusíku (10,6 % přijatého N) u kontrolní skupiny. Dále byla u pokusné skupiny zjištěna statisticky vysoce významně nižší ($P \leq 0{,}01$) bilanční stravitelnost dusíku (84,2 ± 1,92 % oproti 89,4 ± 2,05 %). Denní vylučování dusíku močí bylo u skupiny přijímající humát sodný oproti kontrolní skupině vysoce statisticky významně nižší ($P \leq 0{,}01$), a to 11,2 ± 2,92 g dusíku (33,1 % přijatého) oproti 14,6 ± 1,53 g dusíku (41,9 % přijatého) u kontrolní skupiny. Statisticky vysoce významně vyšší ($P \leq 0{,}01$) bylo ukládání stráveného dusíku prasaty pokusné skupiny oproti kontrole (61,0 ± 5,89 % oproti 53,3 ± 3,02 %) (tab. II).

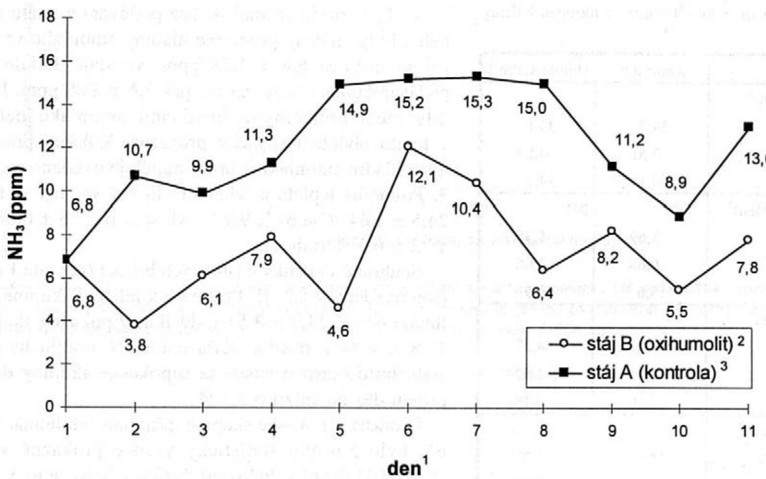
B. Aplikace oxihumolitu do stájového prostředí

Ve III. periodě byly ve stáji A, při aplikaci oxihumolitu na podlahu, zjištěny statisticky průkazně nižší



4. Hladina amoniaku v experimentálních stájích v průběhu pokusu s aplikací oxidumolitu na podlahu (III. perioda) – Ammonia levels in experimental stables during the experiment with oxyhumolith application on the floor (period III)

[1]day, [2]stable A (oxyhumolith), stable B (control)



5. Hladina amoniaku v experimentálních stájích v průběhu pokusu s aplikací oxidumolitu na podlahu (IV. perioda) – Ammonia levels in experimental stables during the experiment with oxyhumolith application on the floor (period IV)

[1]day, [2]stable A (oxyhumolith), stable B (control)

($P \leq 0{,}05$) průměrné hladiny amoniaku ve stájovém ovzduší (6,5 ± 1,12 ppm oproti stáji B 11,0 ± 4,43 ppm), kde nebyl oxihumolit na podlahu aplikován. Kolísání průměrných hladin amoniaku v obou stájích je uvedeno na obr. 4. Průměrná teplota a relativní vlhkost byly ve stáji A 22,8 ± 1,02 °C a 66 ± 4,1 %, ve stáji B 24,3 ± 0,82 °C a 70 ± 5,6 % (tab. I).

Ve IV. periodě byly ve stáji A (oxihumolit nebyl aplikován) zjištěny vysoce statisticky průkazně vyšší ($P \leq 0{,}01$) průměrné hladiny amoniaku (12,0 ± 2,89 ppm oproti stáji B, kde byl aplikován oxihumolit, 7,2 ± 2,44 ppm). Kolísání průměrných hladin amoniaku v obou stájích je uvedeno na obr. 5. Průměrná teplota a relativní vlhkost byly ve stáji A 19,9 ± 0,52 °C a 60 ± 4,8 %, ve stáji B 20,1 ± 0,52 °C a 65 ± 3,3 % (tab. I).

DISKUSE

Porovnáním koncentrace amoniaku ve vzorcích oxihumolitu a hladin jimž byly vystaveny, byl zjištěn nejvyšší podíl navázaného amoniaku při hladinách 5 až 50 mg/g (24,0 až 20,2 %), další zvyšování hladiny amoniaku v systému (150 až 500 mg/g) přineslo relativní snížení hladiny zachyceného amoniaku na 11,5 až 5,7 %. Uvedené výsledky experimentů prokázaly schopnost oxihumolitu vázat amoniak z vnějšího prostředí. Množství amoniaku, jež je schopen vázat jeden gram oxihumolitu, je 27,1 ± 1,32 mg. Další zvyšování hladiny amoniaku v prostředí nepřineslo významné zvýšení koncentrace amoniaku navázaného na oxihumolit.

Při perorálním podávání humátu sodného byla v první periodě zjištěna průměrná hladina amoniaku ve stáji o 3,5 ppm (33,6 %) nižší oproti kontrolní skupině. Tento výsledek nebyl ve druhé periodě potvrzen, neboť průměrná hladina amoniaku byla při podávání humátu sodného vyšší o 1,2 ppm (15,8 %) oproti kontrole.

Perorální podávání huminových látek přináší pozitivní i negativní důsledky. Na jedné straně byl deklarován pozitivní biologický účinek v oblasti tvorby chelátových sloučenin s rizikovými chemickými prvky (Klöcking, 1994; Herzig aj., 1994) stimulační vlastností na antitoxickou a detoxikační činnost jater (Lotosch aj., 1988), zvýšením metabolické aktivity s vyšším přísunem živin do extracelulární tekutiny (Visser, 1988) apod. Naproti tomu bylo potvrzeno, že vysoký příjem huminových kyselin ve vodě ovlivnil výskyt poruch štítné žlázy v populaci (Huang aj., 1994). Vzhledem k ne zcela bezproblémovým výsledkům pokusů s perorálním podáváním huminových látek zvířatům, spojených například s purifikací huminových látek, bude třeba důsledky tohoto způsobu aplikace i nadále studovat.

Při aplikaci mletého oxihumolitu na podlahu experimentálních stájí byla ve třetí periodě zjištěna nižší průměrná hladina amoniaku ve stáji s aplikovaným oxihumolitem o 4,5 ppm (tj. 40,9 %). Tento výsledek byl potvrzen i ve čtvrté periodě, kdy byla průměrná hladina amoniaku ve stáji s aplikovaným oxihumolitem nižší o 4,8 ppm (tj. 40,0 %) oproti kontrole. Snížení emisí amoniaku o 40 % představuje, při produkci amoniaku u prasat 17,5 až 30,0 kg/DJ (Konopásek, 1995), pokles na 10,5 až 18,0 kg/DJ. Tento pokles je srovnatelný s výsledky pokusů prováděných například s přípravkem obsahujícím výtažky rostliny *Yucca schidigera*, kdy Kott aj. (1997) udávají průměrný obsah amoniaku v halách pro 23 750 ks nosnic bez podávání 9,10 mg/m^3 a s podáváním přípravku v krmné směsi 4,36 mg/m^3 , tj. nižší o 52 %.

V bilančním pokusu došlo při perorálním podávání humátu sodného ke zvýšení hladiny dusíku ve vyloučených výkalech o 45 % a snížení exkrece dusíku močí o 23,3 %. Bilanční (zdánlivá) stravitelnost dusíku se snížila, ale došlo k vyššímu využití stráveného dusíku prasaty v pokusné skupině. Denní snížení exkrece dusíku výkaly a močí celkem činilo 9,5 %.

Snížení exkrece dusíku do prostředí, a následně snížení množství volného amoniaku, je možné také opatřeními ve výživě prasat. Snížení vylučování dusíku močí a výkaly lze dosáhnout využíváním krmiv s vysokou stravitelností dusíkatých látek, zvýšením kvality proteinu a snížením obsahu dusíku v krmné dávce na minimum potřeby, při současném vybalancování esenciálních aminokyselin.

Podle Šimečka aj. (1996) vybalancováním esenciálních aminokyselin (lyzinu, metioninu, tryptofanu a treoninu) v krmné dávce prasat tak, aby se blížily ideálnímu proteinu, dojde k významnému poklesu obsahu dusíkatých látek v dietě a následně k poklesu exkrece dusíku močí. Valaja aj. (1993) uvádějí snížení exkrece dusíku o 29 % při použití diety s redukovaným obsahem dusíkatých látek a o 20 % zařazením syntetického lyzinu a treoninu. Z našich autorů například Prokop aj. (1994) uvádějí snížení exkrece dusíku o 14 až 16 %, bez snížení produkční účinnosti krmných směsí.

Kombinací doporučovaných opatření ve výživě prasat, s aplikací huminových látek do krmiva, respektive do stájového prostředí, lze omezit emise amoniaku, a tím přispět nejen ke zlepšení podmínek stájového prostředí s příznivým dopadem na zdraví ustájených zvířat a ošetřujícího personálu, ale i ke zmírnění negativních vlivů chovů prasat na životní prostředí.

LITERATURA

Asman W. A. H., Sutton M. A., Schjorring J. K. (1998): Ammonia: emission, atmospheric transport and deposition. New Phytol., *1*, 27–48.

Canh T. T., Aarnik A. J. A., Verstegen M. W. A., Schrama J. W. (1998): Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. J. Anim. Sci., *7*, 1887–1895.

Bena E., Cihelka J., Müller J., Oppl L., Pulkrábek J., Roubal J., Souček B., Spurný K., Šula O., Teisinger J., Zelený F. (1955): Příručka vyšetřovacích metod v hygieně práce. Praha, SZN. 408 s.

Donham K. J. (1991): Association of enviromental air contaminants with disease and productivity in swine. Am. J. Vet. Res., *10*, 1723–1730.

Donham K. J., Reynolds S. J., Whitten P., Merchant J. A., Burmeister L., Popendorf W. J. (1995): Respiratory dysfunction in swine production facility workers: dose-response relationships of enviromental exposures and pulmonary function. Am. J. Ind. Med., *3*, 405–418.

Drummont J. G., Curtis S. E., Simon J., Norton H. W. (1980): Effects of aerial ammonia on growth and health of young pigs. J. Anim. Sci., *50*, 1085–1091.

Herzig I., Hampl J., Dočekalová H., Písaříková B., Vlček J. (1994): Vliv humátu sodného na ukládání kadmia v orgánech kuřat. Vet. Med. – Czech, *39*, 175–185.

Huang T. S., Lu F. J., Tsai C. W., Chopra I. J. (1994): Effect of humic acids on thyroidal function. J. Endocrinol. Invest., *17*, 787–791.

Klöcking R. (1994): Humic Substances as Potential Therapeutic. In: Senesi N., Miano T. M. (ed.): Humic Substances in the Global Enviroment and Implication on Human Health. Elsevier Science B. V., Amsterdam, 1245–1257.

Konopásek V. (1995): Vznik a snižování emisí amoniaku ze staveb pro živočišnou výrobu a skladování hnoje. Zeměd. Aktual., *8*, 15,18.

Kott J., Jelínek A., Plíva P. (1997): Jak omezit emise amoniaku a zápach. Náš chov, *1*, 32–33.

Lotosch T. D., Chaly A. S., Ternovskaza K. A. (1988): The bioregulating action of peat humic acid. In: Proc. 8th. Int. Peat Congress. Leningrad, 101–105.

Malayer J. R., Brandt K. E., Green M. L., Kelly D. T., Sutton A. L., Diekman M. A. (1988): Influence of manure gases on the onset of puberty of replacement gilts. Anim. Prod., *46*, 277–282.

Matoušková O., Chalupa J., Cígler M., Hruška K. (1992): Statický a grafický systém STAT Plus v. 1.01. Uživatelská příručka. Brno, Výzkumný ústav veterinárního lékařství. 168 s.

Noyes E. P., Pijoan C., Jacobson L. D. (1986): Air quality and swine management. Anim. Nutr. Hlth, *41*, 11.

Prokop V. (1994): Snížení exkrece dusíku u prasat ve výkrmu s využitím syntetických aminokyselin. Živoč. Výr., *39*, 421–428.

Robertson J. F., Wilson D., Smith W. J. (1990): Atrophic rhinitis: The influence of the aerial environment. Anim. Prod., *50*, 173–182.

Šimeček K., Zobač P., Kumprecht I. (1996): Aditiva ve výživě prasat a životní prostředí. In: Sbor. Ref. Výživa prasat '96. VÚVZ Pohořelice, 28. 11. 1996.

Valaja J., Alaviuhkola T., Suomi K. (1993): Reducing crude protein content with supplementation of synthetic lysine and threonine in barley-rapeseed meal-pea diets for growing pigs. Agr. Sci. Finland, 117–123.

Visek N. J. (1984): Ammonia: Its effects on biological systems, metabolic hormones and reproduction. J. Dairy Sci., *67*, 481–498.

Visser S. A. (1988): Some biological efects of humic substances on higher animals and man: the possible use of humic compounds in medical treatmens. In: Int. Humic Acid Soc. Meeting, Sevilla. 27 pp.

Zeman J. (1990): Studijní informace – zoohygiena. Pardubice, UVO. 180 s.

ČSN 46 7092 Metody zkoušení krmiv. 1986.

Vyhláška č. 222/1996 Sb. Ministerstva zemědělství, kterou se stanoví metody odběru vzorků, metody laboratorního zkoušení krmiv, doplňkových látek a premixů a způsob uchovávání vzorků podléhajících zkáze.

Received: 99–06–11

Accepted after corrections: 99–07–28

Kontaktní adresa:

MVDr. Pavel S u c h ý , Výzkumný ústav veterinárního lékařství, Hudcova 70, 621 32 Brno, Česká republika
Tel. +420 5 41 32 12 41, fax +420 5 41 21 12 29, e-mail: suchy@vri.cz, http: www.vri.cz

ADSORPTION PROPERTIES OF NATURAL ZEOLITE AND BENTONITE IN PIG SLURRY FROM THE MICROBIOLOGICAL POINT OF VIEW

ADSORPČNÉ VLASTNOSTI PRÍRODNÉHO ZEOLITU A BENTONITU V HNOJOVICI OŠÍPANÝCH Z MIKROBIOLOGICKÉHO HĽADISKA

J. Venglovský[1], Z. Pačajová[1], N. Sasáková[1], M. Vučemilo[2], A. Tofant[2]

[1]*Research Institute of Veterinary Medicine, Košice, Slovak Republic*
[2]*Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

ABSTRACT: Zeolites and bentonites are natural sorbents which, due to their adsorption properties and high affinity to ammonium ions, are capable of removing ammonium and undesirable odours from the environment and, because of that, can appear beneficial in the treatment of pig excrements. In our experiments we studied the influence of zeolite and bentonite on physical-chemical and microbiological parameters of pig slurry. After 28 days of contact of pig slurry either with zeolite or with bentonite, the plate counts of psychrophilic and mesophilic microorganisms decreased by 3 orders of magnitude in comparison with the control. The efficiency of removal of psychrophilic and mesophilic microorganisms was 98.9% and 100%, respectively. Coliform microorganisms were not detected after 14 days of the treatment of slurry either with zeolite or with bentonite. No fecal coliform microorganisms were detected in bentonite-treated slurry after 7 days of contact and in zeolite-treated slurry after 14 days of contact. During the experiment a decrease in the concentration of ammonium nitrogen ($N\text{–}NH_4^+$), total nitrogen (N_t), total phosphorus (P_t) and chemical oxygen demand (COD) was observed. The decrease in comparison with the control was significant for $N\text{–}NH_4^+$ ($P < 0.05$), N_t ($P < 0.05$), P_t ($P < 0.05$). The effectiveness of removal expressed in per cent was 59.2% for zeolite and 60.2% for bentonite. Both sorbents contributed to better sedimentation of suspended particles and decrease in odour. The results point to the possibility of utilization of zeolite and bentonite in the initial stages of pig excrement treatment.

pig slurry; zeolite; bentonite; microbial plate counts; physical-chemical parameters; ammonium removal

ABSTRAKT: Zeolity a bentonity sú prírodné sorbenty, ktoré sú schopné v dôsledku svojich adsorbčných vlastností a vysokej afinite k amónnym iónom odstraňovať amoniak a neželateľný zápach z prostredia, a vzhľadom na to sú perspektívne pri úprave exkrementov ošípaných. V našich pokusoch sme študovali vplyv zeolitu a bentonitu na fyzikálno-chemické a mikrobiologické parametre hnojovice ošípaných. Po 28 dňoch kontaktu hnojovice so zeolitom alebo bentonitom počty psychrofilných a mezofilných mikroorganizmov boli znížené o tri rády v porovnaní s kontrolou. Účinnosť odstraňovania dosahovala 98,9 % pre psychrofilné a 100 % pre mezofilné mikroorganizmy. Koliformné mikroorganizmy neboli zistené za 14 dní po ošetrení hnojovice, či už so zeolitom alebo bentonitom. Fekálne koliformné mikroorganizmy neboli detekované v hnojovici ošetrenej bentonitom po siedmich dňoch kontaktu. Počas pokusu bolo pozorované značné zníženie koncentrácie amoniakálneho dusíka ($N\text{–}NH_4^+$), celkového dusíka (N_t), celkového fosforu (P_t), ako aj chemickej spotreby kyslíka (CHSK). Zníženie v porovnaní s kontrolou bolo významné pre $N\text{–}NH_4^+$ ($P < 0,05$), N_t ($P < 0,05$), P_t ($P < 0,05$). Účinnosť odstraňovania $N\text{–}NH_4^+$ predstavovala 59,2 % pre zeolit a 60,2 % pre bentonit. Obidva sorbenty prispeli k lepšej sedimentácii suspendovaných častíc a zníženiu zápachu. Získané výsledky poukazujú na možnosť využitia zeolitu a bentonitu v počiatočných štádiách úpravy exkrementov ošípaných.

hnojovica ošípaných; zeolit; bentonit; počty mikroorganizmov; fyzikálno-chemické parametre; odstraňovanie amoniaku

INTRODUCTION

Agriculture is one of the important sources of environmental pollution. Considerable part of this pollution is released by large-capacity farms particularly those rearing pigs and poultry.

When properly treated and disposed of, the farm animal excrements are an important source of nutrients and should be returned to agricultural soil (Ondrašovič et al., 1996; Gajdoš, 1998). On the other hand, their improper handling can present risks to the environment resulting from their chemical and microbiological composition and the associated emissions.

Considerable portion of ammonia produced by bacterial and enzymatic activity during the decomposition of excrements is released into the air of animal housings and pollutes the surrounding area. It has an adverse effect on the housed animals, increases their susceptibility to aerogenic infections and when released to the atmosphere, it contributes to acidification of soil and pollution of water (Chiumenti et al., 1994). The adverse impact of large quantities of excrements on the environment can be decreased by their treatment in wastewater treatment plants. Wastewaters from large-capacity pig fattening are processed mostly by aerobic biological processes. In the first stage of this process the excrement solids are separated from the liquid fraction. The liquid fraction is treated chemically and biologically and the effluent is discharged into the recipient. The solid fraction is disposed of on field heaps or landfills and, after some storage, it is used for manuring purposes. However, the effective processing and subsequent utilization of animal excrements still remains an issue which attracts considerable attention of researchers in all countries with intensive animal production (Venglovský et al., 1994; Juriš et al., 1994; Králová et al., 1994; Kovanda and Růžek, 1996).

Some natural materials, such as zeolites and bentonites, have been used in the process of the treatment of animal wastes. Zeolites are crystalline hydrated aluminosilicates containing cations of alkaline metals and metals of alkaline earths. They exhibit considerable affinity to NH_4^+ ions and the combination of their adsorption and ion-exchange properties forms the basis for their use in the process of wastewater treatment. Bentonites are natural aluminosilicates. The essential component of bentonites is montmorillonite having crystals of the smallest size of all clay materials (Dvořák, 1989).

Our study focused on investigatio into the influence of natural zeolite (clinoptilolite) and bentonite, when added to the liquid fraction of pig slurry before its biological treatment, with regard to physical-chemical properties of the mixture and plate counts of selected groups of microorganisms after storage at ambient temperature.

MATERIAL AND METHODS

Our experiments were carried out using powder zeolite from Nižný Hrabovec, containing approximately 40–56% of clinoptilolite, mostly in the calcium-potassium form, and powder bentonite from Jelšový Potok, Slovak Republic.

Zeolite and bentonite were added to 5 l of slurry at the amount of 50 g per 1 l. The mixture obtained was mixed for 10 min and stored at laboratory temperature. Native slurry served as a control. During the storage the temperature of slurry ranged from 18 to 19 °C. Samples for microbiological and physical-chemical examination were taken after 30 min, 2, 5 and 24 h, and 7, 14 and 28 days of storage. They were examined for plate counts of psychrophilic, mesophilic, coliform and fecal coliform microorganisms, carried out according to STN 83 0531 and Štěpánek (1982). The physical-chemical examination included determination of pH, dry matter content (DM), ammonium nitrogen ($N\text{–}NH_4^+$), total nitrogen (N_t), total phosphorus (P_t), and chemical oxygen demand (COD). It was carried out according to STN 83 0540, Sedláček (1978), and the standard APHA methods (1985). The results were processed statistically using GraphPad PRISM *t*-tests.

RESULTS

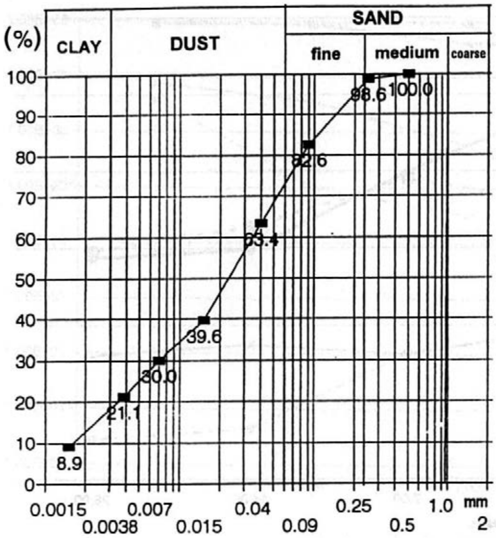
Tab. I shows chemical characteristics of zeolite and bentonite used in our experiments. Fig. 1 and 2 illustrate the distribution of grain size of the sorbents mentioned.

The effect of addition of zeolite and bentonite to pig slurry with regard to plate counts of selected microorganisms in comparison with the control is shown in Tab. II and Figs. 3, 4 and 5. Tab. III presents physical-chemical parameters of the slurries determined in the course of the experiment.

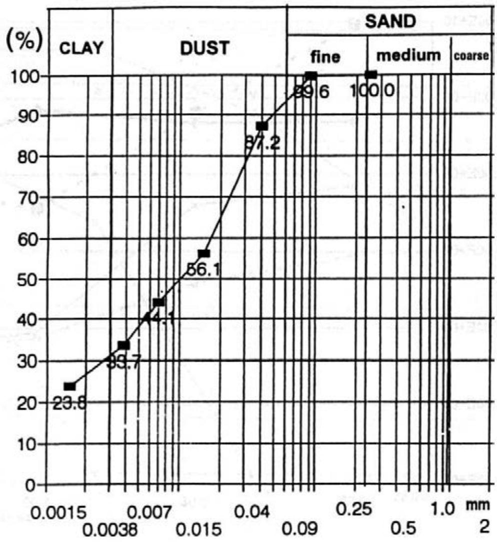
I. Chemical characteristics of sorbents

Parameter	Zeolite	Bentonite
	% dry matter	
SiO_2	71.090	64.970
Al_2O_3	12.110	17.780
Fe_2O_3	1.320	2.770
TiO_2	0.138	0.126
CaO	3.330	2.070
MgO	0.850	3.910
MnO	0.020	0.126
P_2O_5	0.050	0.040
Na_2O	0.430	0.770
K_2O	3.320	1.070
Loss on ignition (%)	7.440	7.050
H_2O (%)	4.620	9.550
	ppm dry matter	
As	0.600	109.000
Sb	0.200	14.800
Se	<0.1	<0.1
Cr	<5	20.000
V	5.000	<5
Be	0.800	2.500
Ni	<2	<2
Zn	35.000	45.000
Pb	8.000	18.000
Cu	5.000	10.000
Cd	<0.1	<0.1
Co	<1	<1
AEC* (0.15 M NH_4Cl)	0.774	0.579
	mol/kg	
Na^+ (%)	0.021	0.184
K^+ (%)	0.822	0.069
Ca^{2+} (%)	0.787	0.645
Mg^{2+} (%)	0.030	0.130

* anion exchange capacity



1. Grain-size distribution of zeolite

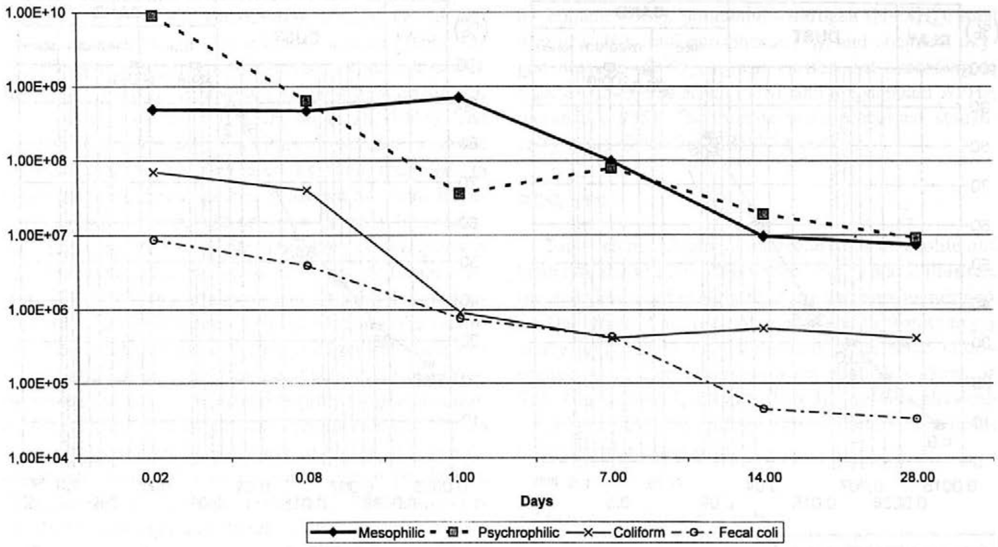


2. Grain-size distribution of bentonite

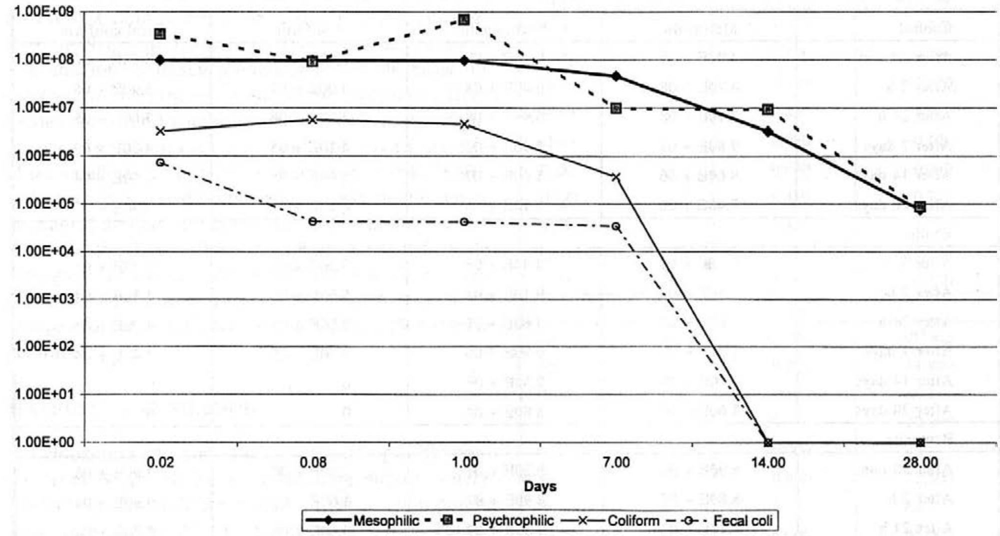
II. Microbiological parameters of slurry treated with zeolite and bentonite in comparison with the control

Control	Mesophilic	Psychrophilic	Coliform	Fecal coliform
After 30 min	4.90E + 08	9.10E + 09	7.00E + 07	8.60E + 06
After 2 h	4.70E + 08	6.40E + 08	4.00E + 07	3.90E + 06
After 24 h	7.10E + 08	6.90E + 08	4.60E + 06	7.60E + 05
After 7 days	9.80E + 07	7.90E + 07	4.10E + 05	4.30E + 05
After 14 days	9.60E + 06	1.90E + 07	5.60E + 05	4.50E + 04
After 28 days	7.40E + 06	9.10E + 06	4.10E + 05	3.30E + 04
Zeolite				
After 30 min	9.80E + 07	3.40E + 08	3.30E + 06	7.30E + 05
After 2 h	9.60E + 07	9.10E + 07	5.60E + 06	4.30E + 04
After 24 h	9.60E + 07	3.60E + 07	9.20E + 05	4.20E + 04
After 7 days	4.60E + 07	9.90E + 06	3.70E + 05	3.40E + 04
After 14 days	3.30E + 06	9.30E + 06	0	0
After 28 days	7.60E + 04	8.80E + 04	0	0
Bentonite				
After 30 min	8.90E + 07	9.20E + 07	7.60E + 06	3.90E + 06
After 2 h	8.80E + 07	8.90E + 07	3.60E + 06	9.80E + 04
After 24 h	4.10E + 07	9.30E + 07	5.60E + 05	3.20E + 04
After 7 days	4.10E + 07	7.40E + 06	3.10E + 04	0
After 14 days	3.20E + 06	6.80E + 06	0	0
After 28 days	4.30E + 0	6.40E + 04	0	0

Fecal coliform counts decreased below the detectable level by day 7 of storage in the mixture with bentonite and by day 14 in the mixture with zeolite while in the control we detected fecal coliform microorganisms up to the end of the experiment. Coliform microorganisms were not detected in the slurries treated with bentonite or zeolite after 14 days of storage. The most marked differences in plate counts of psychrophilic and mesophilic microorganisms were observed after 28 days of storage when a decrease by 3 orders of magnitude was observed in the mixtures with zeolite and bentonite in comparison with the control. The effectiveness of decrease expressed in per cent ranged from 98.9 to 100 (Tab. II).



3. Plate counts of selected groups of microorganisms during the experiment in pig slurry – control

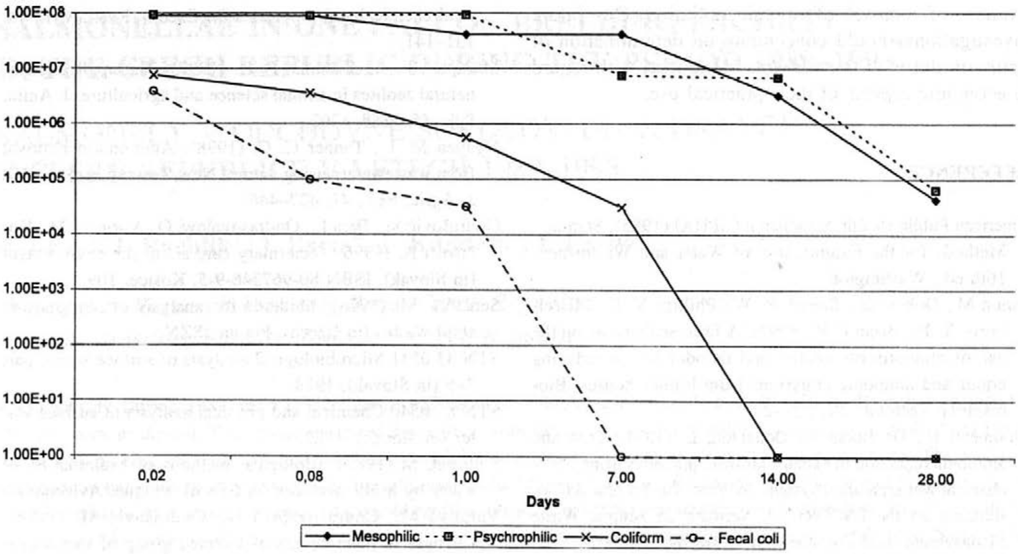


4. Plate counts of selected groups of microorganisms during the experiment in pig slurry amended with zeolite

The mixtures with zeolite and bentonite differed from the control in respect of sedimentation. Sedimentation of suspended solids in the zeolite mixture was rather rapid while the mixture with bentonite formed almost homogeneous gelatinous slurry without any supernatant. The latter separated to the solid and liquid fractions only after almost 24 h of standing. This was confirmed by the values of DM and other chemical parameters shown in Tab. III.

Tab. III indicates considerable differences in pH between mixtures with zeolite and bentonite as soon as after 24 hours of contact (7.06 versus 7.90). The difference increased always to the end of the experiment.

Significant differences were observed for the concentration of $N–NH_4^+$ ($P < 0.05$) in the supernatants. Its value in the mixture with zeolite decreased to 709.69 mg/l after 2 h of contact (control value = 1 123.6 mg/l) and to 453.83 mg/l after 14 days of contact (control



5. Plate counts of selected groups of microorganisms during the experiment in pig slurry amended with bentonite

value = 1 111.2 mg/l). The supernatant of the mixture with bentonite showed a decrease in this parameter after 5 h of contact. The decrease in the content of ammonia nitrogen for both mixtures in comparison with the control was significant on day 14 when the percentage of decrease reached 59.2% for zeolite and 60.2% for bentonite, which suggests high affinity of both sorbents for $N–NH_4^+$.

COD is a measure of chemically oxidizable organic portion of the waste. The values of COD showed an evident decrease for the mixture with zeolite as soon as after 5 h of contact while for bentonite 24 h elapsed before a decrease in this parameter was observed. Considerable decrease in COD for both sorbents was observed after 7 and 14 days.

The decrease in total nitrogen, observed between the supernatants of both mixtures and the control, significant after 7 and 14 days of contact, corresponded to the decrease in ammonia nitrogen ($P < 0.05$).

A marked influence of zeolite and bentonite was observed with regard to the values of total phosphorus in the supernatants. The greatest decrease was observed after 14 days of contact for both zeolite and bentonite (effectiveness 93.2% and 94.7%, resp.).

DISCUSSION AND CONCLUSION

Little attention has been paid to the evaluation of the influence of the sorbents mentioned on microbiological parameters and sedimentation/removal of suspended solids. According to Mumpton and Fishman (1977), the internal structure of zeolite, containing interconnected channels and voids of nanometer dimensions, allows entry of ammonium ions but not viruses or bacteria. The knowledge obtained in our experiments points to the influence of bentonite and zeolite on some selected groups of microorganisms important in hygienic evaluations, namely coliform and fecal coliform microorganisms, as the slurry treated with zeolite did not contain any fecal coliforms after 14 days of contact and that treated with bentonite after 7 days of contact.

The effectiveness of zeolite in the removal of ammonia and odours in relation to animal production was studied by many authors abroad (Amon et al., 1997, and others) and at home (Vargová et al., 1997). Nguyen and Tanner (1998) investigated the removal of ammonia from wastewaters using zeolite from New Zealand deposits and observed 87–98% effectiveness.

The possibilities of utilization of zeolite and bentonite in the treatment of farm animal excrements were described (Henriksen and Berthlsen, 1989; Vargová et al., 1996) already in the initial stages when they can contribute to better sedimentation of suspended solids, adsorption of nutrients and decrease in total organic contamination. The solid phase amended with zeolite can find better use in plant production as zeolites and bentonites favourably affect the structural properties of soil, retention of water and the release of nutrients.

The effect of zeolite on sedimentation properties of slurry was observed already after 30 min while the effect of bentonite was visible only after 24 h (separation of solid and liquid fractions). However, after the initial period, the contribution of bentonite to sedimentation of slurry was higher than that of zeolite, which is in an agreement with observations of Henriksen and Berthlsen (1989). We can expect that the simultaneous use of both sorbents could further enhance the effectiveness of removal of organic pollution. Our future investigations should concentrate on determination of optimum doses of the sorbents mentioned with regard to economic aspects of their practical use.

REFERENCE

American Public Health Association (APHA) (1985): Standard Methods for the Examination of Water and Wastewater. 16th ed., Washington.

Amon M., Dobeic M., Sneath R. W., Phillips V. R., Misselbrook T. H., Brian F. P. (1997): A farm-scale study on the use of clinoptilolite zeolite and de-odorase for reducing odour and ammonia emissions from broiler houses. Bioresource Technol., *61*, 229–237.

Chiumenti R., Da Borso F., Donantoni L. (1994): Dust and ammonia reduction in exhaust air from piggeries using a mechanical wet separation system. In: Proc. 7th Technical Consultation on the ESCORENA Network on Animal Waste Management, Bad Zwischenahn, Germany. 301–307.

Dvořák M. (1989): The ability of bentonite and natural zeolite to adsorb aflatoxins from liquid media (in Czech). Vet. Med. (Praha), *34*, 307–316.

Gajdoš R. (1998): Bioconversion of organic waste by the year 2010: to recycle elements and save energy. Resources, Conserv. Recycl., *23*, 67–86.

Henriksen K., Berthlsen L. (1989): Slurry separation with a view to achieving a low nutrient content in the liquid portion. Meddelelse-Jordbrugsteknisk-Institut, *62*, 103.

Juriš P., Gulovič J., Lašanda V., Ružik V., Plachý P. (1994): Bacteriological, mycological and parasitological survey of slaughterhouse wastewaters contamination. Vet. Med. – Czech, *39*, 373–379.

Kovanda F., Ružek P. (1996): The release of ammonium-ions from the ammonium-enriched zeolites in the soil system (in Czech). Rostl. Výr., *42*, 149–154.

Králová M., Hrozinková A., Ružek P., Kovanda F., Koloušek D. (1994): Synthetic and natural zeolites affecting the physical-chemical soil properties (in Czech). Rostl. Výr., *40*, 131–141.

Mumpton F. A., Fishman P. H. (1997): The application of natural zeolites in animal science and agriculture. J. Anim. Sci., *45*, 1188–1203.

Nguyen M. L., Tanner C. C. (1998): Ammonium removal from wastewaters using natural New Zealand zeolites. NZ J. Agric. Res., *41*, 427–446.

Ondrašovič M., Para L., Ondrašovičová O., Vargová M., Kočišová A. (1996): Veterinary care about the environment (in Slovak). ISBN 80-967346-9-5, Košice, 109.

Sedláček M. (1978): Methods for analysis of sludges and solid wastes (in Czech). Prague, SZN.

STN 83 0531 Microbiological analysis of surface water, part 3–5 (in Slovak), 1978.

STN 83 0540 Chemical and physical analysis of surface water (in Slovak). 1982.

Štěpánek M (1982): Biological methods of examination of water by health services (in Czech). Prague, Avicenum.

Vargová M., Ondrašovičová O., Ondrašovič M. (1996): Changes in plate counts of selected group of microorganisms in the solid fraction of pig slurry stored with an addition of natural zeolite (in Slovak). In: Pro. Int. Conf. Ecology and Veterinary Medicine III., Košice. 193–198.

Vargová M., Ondrašovičová O., Ondrašovič M., Para L. (1997): Application of zeolite (clinoptilolite) in the process of stabilization and utilization of nutrients in farm animal wastes (in Slovak). In: Proc. Symp. Current and Prospective Possibilities of the use of Biotechnologies in Agriculture, Košice. 101–103.

Venglovský J., Juriš P., Sokol J. (1994): Survey of effectiveness of some water treatment plants operating on pig farms in Slovakia (in Slovak). Vet. Med. – Czech, *39*, 677–686.

Received: 98–09–03

Accepted after corrections: 99–07–23

Contact Address:

Ján Venglovský, DVM. PhD., Výskumný ústav veterinárnej medicíny, Hlinkova 1/A, 040 01 Košice, Slovenská republika
Tel. +421 95 632 18 72, fax +421 95 633 18 53, e-mail: venglov@tuke.sk

SALMONELLAE IN ONE FALCON BREEDING FACILITY IN THE CZECH REPUBLIC DURING THE PERIOD 1989–1993*

SALMONELY V ODCHOVNĚ SOKOLOVITÝCH DRAVCŮ V ČESKÉ REPUBLICE V LETECH 1989–1993

P. Lány[1], I. Rychlík[1], J. Bárta[2], J. Kundera[2], I. Pavlík[1]

[1]*Veterinary Research Institute, Brno, Czech Republic*
[2]*Falcon Breeding Facility, Milotice, Czech Republic*

ABSTRACT: During 1989 and 1993, a total of 837 samples of biological materials from the Falcon Breeding Facility in Milotice were examined. Twenty-six strains of Salmonella were isolated from 680 samples of eggs, young birds, faeces and the organs of kept animals, whereas 28 strains were isolated from 157 samples of animals used as food. The isolated Salmonellae belonged to the serotypes *Salmonella enteritidis* (25 strains), *S. typhimurium* (28 strains) and *S. typhimurium* var. *Copenhagen* (one strain). Analysis of plasmid profiles allowed the further classification of *S. enteritidis* strains into three subtypes and *S. typhimurium* into two subtypes. Chronologically it was possible to see the incidence of individual Salmonella subtypes. This result eliminated a long-term infection of falconid birds of prey with one Salmonella strain. In two cases the source of Salmonella for falcons originated from food animals (mice: source of *S. typhimurium*, chickens: source of *S. enteritidis*). In other cases the infection source was not found. Although Salmonellae were detected in the organs of one falconid bird and in 4 laid eggs of falconids, we failed to detect the circulating antibodies against *S. pullorum* antigen by using rapid slide agglutination test. Analysis of age resistance of the falconid birds revealed that Salmonellae were most frequently found in birds from the age of day 1 to 1 year or older than 3 years. Analysis of species sensitivity showed that Salmonellae were detected in 14 of 37 saker falcons (*Falco cherrug*), in 4 of 27 peregrine falcons (*F. peregrinus*), in both kestrels (*F. tinunculus*), in one of two hobby falcons (*F. subbuteo*), in 4 of 12 birds of a cross-breed (*F. cherrug* x *F. peregrinus*) and in one of four goshawks (*Accipiter gentilis*). Salmonella was isolated from two out of twenty birds which had diarrhoea. No Salmonella was isolated in faecal samples from 91 falcons (26 positive birds) and eggs from 30 females (4 females positive).

Falconidae; *Salmonella enteritidis*; *Salmonella typhimurium*; plasmid profile; rearing of falconid birds of prey

ABSTRAKT: V letech 1989 až 1993 bylo v Odchovně sokolovitých dravců v Miloticích vyšetřeno celkem 837 vzorků biologického materiálu. Z 680 vzorků vajec, mláďat, trusu i orgánů chovaných dravců bylo izolováno 26 kmenů a ze 157 vzorků krmných zvířat bylo izolováno 28 kmenů salmonel. Izolované salmonely patřily k sérotypům *Salmonella enteritidis* (25 kmenů), *S. typhimurium* (28 kmenů) a *S. typhimurium* var. *Copenhagen* (1 kmen). Analýzou plazmidových profilů bylo možné jednotlivé izolované kmeny *S. enteritidis* dále členit do tří a *S. typhimurium* do dvou subtypů. Časová posloupnost výskytu jednotlivých subtypů salmonel vyloučila dlouhodobou infekci dravců několika kmeny s následnou dlouhodobější cirkulací. Zdrojem salmonel dravců byly ve dvou případech krmná zvířata (myši: zdroj *S. typhimurium*, kuřata: zdroj *S. enteritidis*). V ostatních případech se zdroje infekce salmonel nepodařilo zjistit. Přestože salmonely byly prokázány v orgánech jednoho dravce a ve čtyřech snesených vejcích sokolovitých dravců, nepodařilo se nám prokázat při plošném vyšetření rychlou sklíčkovou aglutinací cirkulující protilátky v krvi proti antigenu *S. pullorum*. Při rozboru věkové rezistence dravců byly salmonely nejčastěji zjištěny u dravců do věku jednoho roku, nebo starších tří let. Při rozboru druhové vnímavosti byly salmonely prokázány u 14 ze 37 vyšetřovaných rarohů velkých (*Falco cherrug*), u čtyř ze 27 vyšetřovaných sokolů stěhovavých (*F. peregrinus*), u obou poštolek obecných (*F. tinunculus*), u jedoho ostříže lesního (*F. subbuteo*), čtyř ze 12 kříženců (*F. cherrug* x *F. peregrinus*) a u jednoho ze čtyř jestřábů lesních (*Accipiter gentilis*). Z celkového počtu dvaceti dravců s klinickými příznaky průjmu byly salmonely izolovány pouze u dvou z nich. Při opakovaném vyšetřování trusu od 91 dravců (26 pozitivních dravců) a od 30 samic (čtyři samice pozitivní) nebylo zjištěno opakované vylučování salmonel.

Falconidae; *Salmonella enteritidis*; *Salmonella typhimurium*; plazmidový profil; odchov sokolovitých dravců

* The submitted study was in 1991–1992 supported by the Ministry of Culture of the Czech Republic.

INTRODUCTION

The Falcon Breeding Facility has been established in Milotice, Czech Republic since 1979 to save falconid birds and reintroduce young birds to the wild, especially peregrine falcon (*Falco peregrinus*) and saker falcon (*F. cherrug*). The health status of the birds was systematically monitored through veterinary examinations during the period 1989–1993.

Regarding the fact that a great proportion of the food of birds of prey in their natural habitat comes from sick animals, they acquire a high natural resistance against infectious diseases (Isenbügel and Rübel, 1987). Occurrence of Salmonellae and their impact on health has already been monitored in diurnal and nocturnal birds of prey. Various Salmonella serovars, e.g. *Salmonella typhimurium, S. typhimurium* var. *Copenhagen, S. enteritidis, S. newport, S. heidelberg, S. braenderup, S. havana, S. virchow* and *S. livingstone* were isolated. On the other hand, Needham et al. (1979) found no Salmonella in intestinal microflora of 40 birds of prey in nature. Therefore, the assumption of a very low probability of transmission of Salmonella infection from birds in nature to those living in captivity was made. However in captive bred falconid birds the living conditions differ markedly when compared to those of birds in their natural surroundings. A variety of stresses (way of rearing, composition and method of serving the food, concentration of birds in a small area, frequent disturbances, etc.) have a negative impact on their natural resistance to different infectious agents. In captive bred falconids, a viral infection causing Newcastle Disease and herpesvirus was detected (Gough et al., 1993; Samour and Cooper, 1993), mycoplasma and bacterial disease as avian tuberculosis, and staphylococcus infections caused by *Staphylococcus aureus* and *Staph. epidermidis* were also detected (Furr et al., 1977; Piechocki, 1981; Trommer, 1988; Bangert et al., 1988; Wernery et al., 1992; Štika et al., 1989). Secondary infections caused by *Escherichia coli* also often occur due to weak organism. Of fungal diseases aspergilosis and candidosis were described in falconid birds (Isenbügel and Rübel, 1987). Another bacterial and parasitic diseases might also be a severe complication in the rearing of captive falconids (Cooper, 1977; Lüthgen, 1978a, b; Sykes et al., 1981; Böer, 1982; Kirkpatrick and Trexler-Myren, 1986; Battisti et al., 1998).

The major portion of feed for captive falconid birds is composed from chickens and Salmonellosis is one of the most frequent bacterial infection in poultry (Badi et al., 1992). Hence, it is important to study the incidence and importance of Salmonellae in the falcon breeding facility. To investigate the epidemiology of Salmonella infections, molecular biology techniques beside serotyping are often used. Especially the analysis of plasmid profile is often used due to its rapidity and relative feasibility (Wachsmuth et al., 1991; Dorn et al., 1992; Rychlík et al., 1993). This is a study on Salmonella infections and source of Salmonella infection in falconid birds at the Falcon Breeding Facility at Milotice, Czech Republic during 1989 and 1993 by using the conventional bacteriological techniques as well as a molecular biology technique such as plasmid profile analysis.

MATERIALS AND METHODS

Bacteriological examinations of biological materials

Samples

The total of 837 biological materials (Tab. I) were examined for the presence of Salmonellae in the Falcon Breeding Facility in Milotice during the period 1989 to 1993. Four hundred and forty two faecal samples from 91 birds and 221 unfertilised eggs, or eggs with dead embryo, from 30 females falcons were examined (Tab. II).

Sampling of materials

Due to the accepted irregular excretion of Salmonellae by infected falconid birds, individual samples of faeces were collected three times in two-day intervals. Additional samples of faeces were collected based on the results of examination or under changed health status of the falconid birds. The eggs were aseptically opened prior to sampling, half of the yolk or embryos liver was sampled for the presence of xenobiotics and the other half was by culture examined for Salmonellae. Parenchymatous organs (liver, spleen kidney) from 15 dead falconid birds and 157 samples of animals used as food (chickens, mice, sewer-rats, quails) were collected at necropsy for culture examination. Two faecal samples were collected from the equipment used to introduce the birds back to nature.

Culture examinations for Salmonellae

Samples collected during necropsy were propagated in selective-propagating medium (selenite medium or Rappaport-Vassiliadis medium; Imuna, Šarišské Michalany, Slovakia) at 43 °C for 48 hrs. After 24 and 48 hrs of the growth they were plated onto selective-differential media (DC agar supplemented with 1% saccharose and XLD agar; Imuna, Šarišské Michalany, Slovakia). Solid media were assessed after 24 and 48 h of cultivation at 37 °C. Suspected colonies were further identified by biochemical reactions using Enterotest I (Lachema, Brno, Czech Republic). Serotyping of the isolated Salmonella was performed by agglutination tests using mixed serum against Salmonellae (Bioveta, Ivanovice na Hané, Czech Republic, monovalent sera, ÚSOL Prague, Czech Republic). Faecal samples were resuscitated overnight in a buffered peptone water at 37 °C prior to above procedure.

I. Biological material examined for the presence of Salmonellae in the period 1989–1993

Year	Materials examined	Number of samples	Number of strains	%	*S. enteritidis*	*S. typhimurium*
1989	eggs of raptors	9	0	0	0	0
1990	eggs of raptors	36	0	0	0	0
	organs of raptors (juv.)	4	0	0	0	0
	faeces of raptors	22	0	0	0	0
1991	eggs of raptors	61	0	0	0	0
	organs of raptors (juv.)	5	0	0	0	0
	organs of raptors (pul.)	2	0	0	0	0
	faeces of raptors	70	8	11.4	1	7
	mice organs	10	10	100	0	10
	chicks organs	40	10	25.0	10	0
	sewer-rat organs	3	0	0	0	0
1992	eggs of raptors	75	4	5.3	1	3
	organs of raptors (juv.)	3	0	0	0	0
	organs of raptors (pul.)	13	1	7.7	0	1*
	faeces of raptors	327	13	4.0	13	0
	chicks organs	50	8	16.0	0	8
	mice organs	26	0	0	0	0
	sewer-rat organs	2	0	0	0	0
	quail organs	5	0	0	0	0
	eggs of quails	17	0	0	0	0
	faeces from outlet equipment	2	0	0	0	0
1993	eggs of raptors	30	0	0	0	0
	faeces of raptors	21	0	0	0	0
	quail organs	4	0	0	0	0
Total (1989–1993)		837	54	6.5	25	29
Eggs of raptors		211	4	0.4	1	3
Faeces of raptors		442	21	4.8	14	7
Parenchymatous organs of raptors		27	1	3.7	0	1*
Other samples		157	28	17.6	10	18

Explanations:
* *S. typhimurium* var. *Copenhagen*
juv. = young bird up to 12 months
pul. = adult bird of prey

Analysis of plasmid profiles

The analysis of plasmid profiles of all the obtained Salmonella strains were used for epizootiological study (Rychlík et al., 1993, 1999). Shortly, the procedure consists of plasmid DNA purification, its digestion with restriction endonucleases *Taq*I and *Pst*I, electrophoresis, charge-coupled device camera scanning of the gels, and an analysis of the restriction patterns with the software Gel Manager.

Serological examination

Rapid slide agglutination of serum from 20 breeding falcons was done using *S. pullorum* antigen (Bioveta, Ivanovice na Hané, Czech Republic).

RESULTS

Serotyping and analysis of plasmid profiles of the isolated Salmonella strains

Isolated Salmonella strains and their serotypes (Tab. I)

Of 837 samples of biological materials, 54 Salmonella strains were isolated over the period 1989–1993: 25 *S. enteritidis* strains, 29 *S. typhimurium* strains. Twenty-one strains (14 *S. enteritidis* strains and 7 *S. typhimurium* strains) from the faecal samples of falcons and 4 strains (one *S. enteritidis* strain and 3 *S. typhimurium* strains) from 4 eggs with dead embryos were isolated. In addition, 10 *S. typhimurium* strains from the organs of mice and 18 strains (10 *S. enteritidis* strains and 8 *S. ty-*

II. Species proportion of the captive birds of prey of which in 1989–1993 biological material was examined for the presence of Salmonellae

Bird species	Number of faeces Number of raptors	Culture positive	%	Number of eggs Number of raptors	Culture positive	%
Peregrine falconm	124	5	4.0	75	1	1.3
Falco peregrinus	27	4	14.8	10	1	10.0
Saker falcon	172	14	8.1	129	3	2.3
Falco cherrug	37	14	36.8	17	3	17.7
Merlin	6	0	0	0	0	0
Falco columbarius	2	0	0	0	0	0
Kestrel	13	2	15.4	0	0	0
Falco tinunculus	2	2	100	0	0	0
Lanner falcon	5	0	0	3	0	0
Falco biarmicus	1	0	0	1	0	0
Hobby falcon	6	1	16.7	0	0	0
Falco subbuteo	2	1	50	0	0	0
Gyrfalcon	18	0	0	0	0	0
Falco rusticolus	2	0	0	0	0	0
Crosses gyrfalcon x	67	4	6.0	2	0	0
saker falcon	12	4	33.3	1	0	0
Goshawk	27	1	37.0	2	0	0
Accipiter gentilis	4	1	25.0	1	0	0
Sparrowhawk	1	0	0	0	0	0
Accipiter nisus	1	0	0	0	0	0
Eagle-owl	1	0	0	0	0	0
Bubo bubo	1	0	0	0	0	0
Total						
Number of samples	440	27	6.1	211	4	6.2
Number of birds	91	26	28.6	30	4	13.3

phimurium strains) from the organs of chickens were isolated.

Analysis of plasmid profiles (Tab. III)

Plasmid profiles of all isolated strains were examined. Among 24 *S. enteritidis* strains three different DNA subtypes SE-A ($n = 14$), SE-B ($n = 4$), SE-C ($n = 7$) and two different DNA subtypes STM-A ($n = 17$) and STM-B ($n = 11$) among 28 *S. typhimurium* strains were detected. One strain *S. typhimurium* var. *Copenhagen* had different DNA type STMvC-A.

Chronological examination of biological materials and spreading of Salmonella infections

Examinations during 1989 and 1990 (Tab. I)

No Salmonellae were detected in the bacteriological examinations of 71 samples of biological materials (45 eggs, 26 samples of faeces).

Examinations in 1991 (Tab. I)

Salmonellae were isolated from faeces of 8 birds only at examination in November (one *S. enteritidis* strain and 7 *S. typhimurium* strains). From the organs of all 10 examined mice (5 mice showing loss of appetite and somnolence, 5 mice without clinical signs) 10 *S. typhimurium* strains were isolated. Analysis of plasmid profile was performed on all *S. typhimurium* strains and it was designated as STM-A.

Nine strains of *S. enteritidis* were isolated from the organs of 20 chickens (>10 days old) which had a high mortality rate. Additional examination of 10 chickens (killed at 2 days of age and then kept frozen) from the same supply were negative. Plasmid profiles of *S. enteritidis* strains from chickens and an infected falcon were the same and these were designated as subtype SE-A (Tab. III). In December 1991, ten chickens from another hatchery were examined, and one strain of *S. enteritidis* with the same subtype SE-A was identified.

No Salmonella was isolated from the parenchymatous organs (liver, spleen kidney) and intestinal contents (small intestine, caecum) of 7 dead falconids and their progenies. In December, serological examinations of 20 adult falconid birds with antigen *S. pullorum* were negative.

Examinations in 1992 (Tab. III)

In January pre-nesting seasons, *S. enteritidis* was isolated from the faecal samples of four breeding birds. Plasmid profiles of three isolates were classified as subtype SE-C, and one strain as subtype SE-B.

III. Occurrence of individual plasmid subtypes *S. enteritidis* a *S. typhimurium* in Falcon Breeding Facility in Milotice (1991–1992)

Year	1991		1992					
Plasmid type	November	December	March	April	May	June	July	September
SE-A	faeces of raptor (1) organs of chicks I.(9)	organs of chicks II.(1)						faeces of raptors (7)
SE-B			faeces of raptor (1)					faeces of raptors (3)
SE-C			faeces of raptors (3)	eggs of raptors (1)		faeces of young bird I	faeces of young bird II	
STM-A	faeces of raptors (7) organs of mice (10)							
STM-B				eggs of raptors (3)	organs of chicks (8)			
STMvC-A						liver of raptor (1)		

Explanations:
SE-A to C = *S. enteritidis* plasmid subtype A to C
STM-A to B = *S. typhimurium* plasmid subtype A to B
STMvC-A = *S. typhimurium* var. *Copenhagen* plasmid subtype A
(3) number of isolated and examined strains

Culture of four falcon eggs laid in March with dead embryos resulted in isolation of three strains of *S. typhimurium* belonging to plasmid profile subtype STM-B and one strain *S. enteritidis* belonging to subtype SE-C (Tabs. I and III). No Salmonella was isolated from the faeces or another 12 unfertilised eggs or eggs with dead embryos from the same females (which laid infected eggs).

During examination of parenchymatous organs and intestinal contents from 16 dead birds and their progenies, Salmonellae were isolated only from the liver of a young peregrine falcon (*F. peregrinus*) in June. This peregrine falcon was killed by another falcon. The isolated strain was determined as *S. typhimurium* var. *Copenhagen* belonging to subtype STMvC-A (Tabs. I and III).

S. enteritidis strain subtype SE-C was isolated at the end of July from the faeces of one of two young peregrine falcons which had diarrhoea. Two weeks later one *S. enteritidis* strain of the same plasmid subtype SE-C (Tab. III) was isolated from the other bird which had a more severe clinical symptoms. Both affected birds were successfully treated with chloramphenicol (50 mg/kg wt) in the same dosage as is used in poultry). However, two months later *S. enteritidis* subtype SE-A (Tab. III) was isolated from the dropping of the second bird.

In September, another 10 strains of *S. enteritidis* were isolated from the droppings of 10 falconid birds. Seven of the 10 isolated strains were of plasmid subtype SE-A and three of subtype SE-B (Tab. III).

During 1992, a total of 40 chickens from 5 different supplies (8/supply) were examined for Salmonellae at the age of 7–10 days. *S. typhimurium* subtype STM-B (Tab. III) was isolated from the second supply of 8 new chicks in May. Additional examination of 10 frozen one day-old chicks from the same supply proved negative. Examination of 26 mice from two new supplies bought in March and November was also Salmonella negative.

Examination in 1993 (Tab. I)

In January and February 21 faecal samples were cultured and in March 4 quails used for feeding were cultured, no Salmonella was isolated from these samples. The examination of 30 eggs during the nesting season revealed no Salmonella.

Retrospective study of infected falconid birds sent for the examination for the presence of Salmonellae

Species proportion of the infected falconid birds of prey (Tab. II)

Analysis of species representation of the infected falconid birds showed that 4 out of 27 (14.8%) peregrine falcons (*Falco peregrinus*), 14 out of 37 (37%) saker falcons (*F. cherrug*) and 4 out of 12 (33.3%) crosses of saker falcon and peregrine falcon were infected.

Age composition of the infected falconid birds (Tab. IV)

The age of the infected falconid birds was analysed based on breeding data. It was found that most infected falconid birds were one day to one year old or more than four years old.

IV. Age composition of birds infected by Salmonellae

Year	Month	≥ 1 year	≥ 2 years	≥ 3 years	< 3 years
1991	November	5	1	1	1
1992	March	1	0	0	7*
1992	September	7**	0	1	2
Total		13	1	2	10

Explanations:
* included also 4 females with Salmonella isolation from eggs
** included Salmonella isolation from liver of dead raptor

Repeated culture examination for Salmonellae of materials coming from individual falconid birds

Repeated examination of faeces from individual falconid birds and eggs laid from one female found no repeated Salmonella isolation in any of the birds.

Clinical assessment of falconid birds and examination for Salmonellae

Assessment of clinical state of every falconid bird during sampling of faeces over the period 1990–1993 showed that 19 birds suffered from of diarrhoea and impaired nutrition. However, severe infection of *S. enteritidis* was only detected in the faeces of two young peregrine falcons.

Treatment of clinically ill falconid birds of prey infected with Salmonellae

Salmonellae isolated from clinically ill peregrine falcons were sensitive to chloramphenicol and streptomycin. Chronicin (chloramphenicol) was chosen for the treatment (50 mg/1 kg wt). Following a 5-day peroral antibiotic course, probiotic (Microbion tablets, Bioveta, Ivanovice na Hané, Czech Republic) was administered for a more rapid restoration of physiological microflora as it contains cultural bacteria (*Bacillus subtilis, Streptoccocus faecium* var. *durans* and *Streptococcus faecalis* var. *zymogenes*).

DISCUSSION

Salmonella sources in the Falcon Breeding Facility

The assumption that falconid birds at the Falcon Breeding Facility were infected with Salmonellae from animals used as a food source was confirmed. The same conclusions were also reached by Battisti et al. (1998) who isolated *S. havana* and *S. livingstone* with the same biochemical characteristics and sensitivity to antibiotics from diurnal and nocturnal falconid birds and also from day-old chicks used for feeding.

Salmonella sources in 1991

In 1991 Salmonellae of the same serotype and plasmid subtype were isolated simultaneously from the organs of animals for feeding (chickens and mice) and from the faeces of infected falconid birds. Therefore it is evident that the source of Salmonella infection at the Falcon Breeding Facility in 1991 was from mice and chickens used for feeding.

Salmonella sources in 1992

No Salmonella sources were found at the Falcon Breeding facility in 1992. For financial reasons not all the supplies of chicken used for feeding of the falconid birds were examined. Due to the unfavourable epizootiological situation that exists in poultry flocks of the Czech Republic and other European states, it is possible that the falconid birds acquired the Salmonella infection from poultry source fed to the falconids.

Clinical course of Salmonellosis in falconid birds of prey

Clinical Salmonellosis was detected only in two young peregrine falcons (*F. peregrinus*). Clinical signs appeared in these birds after their feeding by improperly stored killed chickens during the summer months. The falcons were successfully treated by using oral antibiotics chosen based on antibiogram. Only one of the treated falcons was reinfected with Salmonella without clinical signs. In the other infected falconid no clinical signs were observed. From the view point of view of differential diagnostics it is necessary to emphasize that there are other causes leading to affection of the intestinal tract with diarrhoeal signs. Coccidiosis caused by the species *Caryospora neofalconis* and *Caryospora kutzeri* was also recorded in the same Falcon Breeding Facility (Pavlík et al., 1998). It can be a dietary cause especially after ingestion of frozen food. Diarrhoea lasting several days occurred in some of these birds (Pavlík et al., 1997).

Colonisation of the intestinal and reproductive tracts

Colonisation of the intestinal tract

Regarding the colonisation of the intestinal tract with Salmonellae we found that in three subsequent samples of faeces (sampling interval 3 says) Salmonella was isolated in one sample taken from each of the birds. This can be explained by irregular shedding of Salmonellae in faeces. In falconid birds without clinical signs who had already excreted Salmonellae, no reinfection with the same or other serovar was detected. Based on these findings we suggest that after the colonisation of falconid birds with Salmonellae, immunity against Salmonellae is developed and can persist even for several years. However, antibiotic treatment does not allow the bird to develop a complete resistance to reinfection and therefore reinfection may occur as was found in the case of a young falcon mentioned above.

Colonisation of the reproductive tract

The cause of embryo death in four eggs of falconid birds was most probably due to their infection with Salmonellae. The same finding was also observed by Battisti et al. (1998). They recorded embryonal death in the eggs of an eagle-owl (*Bubo bubo*), peregrine falcon, buzzard (*Buteo buteo*) and saker falcon due to the infection with *S. havana* and *S. virchow*.

The finding of one salmonella positive egg out of 15 eggs laid by one female might indicate the possible vertical transmission of Salmonella from infected female to its progeny. No infection was found either in previously or in consequently laid egg of any of the females. These results indicate that repeated culture examination ought to be made in order to eliminate the infected birds or eggs laid by a single birds.

Influence of the age on the susceptibility of falconid birds to the infection

Analysis of age proportion of the infected falconid birds revealed that the most frequent Salmonella shedders are young birds up the age of one year and then birds older than 4 years (Tab. IV). It should be mentioned that all the falconid birds were kept under the same conditions. Great positivity in birds younger than one year can be explained by insufficiently formed mucosal immunity protecting their intestinal tract against Salmonella colonisation from food. However, in older falconid birds those facts were not elucidated.

Detection of serum antibodies after infection of falconid birds

The question whether Salmonellae penetrate through the intestinal tract and into organs or whether they only colonise the intestine can be answered basis of the presence of serum antibodies. In poultry *S. pullorum* antigen reacts not only with homologous antibodies but also with serum antibodies in a crossed reaction after infection by *S. enteritidis* and *S. typhimurium* (Nicholas, 1992; Furrer et al., 1993) in the rapid slide agglutination test. Negative serological examinations of 20 breeding falconid birds using rapid slide agglutination with *S. pullorum* antigen indicate that there is a lack of serum antibodies in these birds. This may be due to insufficient concentrations of antiserum against Salmonella. However, the rapid slide agglutination test does not detect serum antibodies against certain Salmonella species which are detectable only by using a more sensitive ELISA method (Nicholas, 1992).

Infection sources for falconid birds and possibilities to reduce risk of introduction of the infection into the breeding facility

Study of infection spreading in the breeding facility

The epizootiology of Salmonellae was studied using the methods of serotyping and analysis of plasmid profile. Three Salmonella serotypes with six plasmid subtypes were detected by typing all isolated Salmonella strains. This strain heterogeneity consequently enabled us in some cases to identify the sources of infection (Tab. III). A specific variety of individual Salmonella serotypes and their subtypes was determined in the Falcon Breeding Facility. Therefore no long-term prevalence of Salmonella infection with one or two Salmonella strains was detected in the monitored breeding facility but instead of it, single, time-limited Salmonella infections coming from different sources.

Sources of Salmonella infections from chicks determined for feeding

A negative Salmonella finding in frozen one-day old chicks for feeding compared with positive findings in the same group aged 7–10 days is interesting from the epizootiological view. Since Battisti et al. (1998) found frozen one-day old chicks as Salmonella sources in captive diurnal and nocturnal birds of prey, our negative culture examinations can be explained in two ways. One cause could be a small percentage of infected one-day old chicks at the time of collecting in the hatcheries, another cause could be the reduction of the Salmonella count to an undetectable number necessary for bacteriological exàmination due to the freezing-thawing of chicks.

CONCLUSIONS

Based on presented results it is possible to recommend the following preventive measures:

1. To use one-day old chickens for feeding from farms with a good quality of sanitation, which had received the laid eggs from the examined flocks for Salmonella infection.
2. To examine samples of bird feed (chickens, mice etc.) for the presence of Salmonella before feeding, especially during the breeding season.
3. To prepare and store the feed in such a manner that will minimize the propagation of Salmonella.
4. All new incoming birds should be tested for the presence of Salmonella in three subsequent samples of

faeces (sampling interval 3 days). Shedding birds should be isolated until Salmonella excretion disappears and should be treated only of clinical signs are present.

Acknowledgement

We wish to thank to Pyone Pyone Aye from Department of Veterinary Preventive Medicine (The Ohio State University, Columbus, USA) and Rebecca Norton (University of Wales, Aberystwyth, UK) for the critical reading of the manuscript. Our thanks belong to Iva Ryšavá for technical assistance at bacteriological examinations.

REFERENCES

Badi M. A., Iliadis N., Sarris K., Artopios E. (1992): Salmonella – Infektionsquellen in Geflügelbeständen Nordgriechenlands. Berl. Münch. Tierärztl. Wschr., *105*, 236–239.

Bangert R. L., Ward A. C., Stauber E. H., Cho B. R., Widders P. R. (1988): A survey of the aerobic bacteria in the feces of captive raptors. Avian. Dis., *32*, 53–62.

Battisti A., Guardo G. D., Agrimi U., Bozzano A. (1998): Embryonic and neonatal mortality from salmonellosis in captive bred raptors. J. Wildlife. Dis., *34* (1), 64–72.

Böer B. (1982): Untersuchungen über das Vorkommen von Kokzidien bei Greifvögeln und über die Entwicklungen von zwei Caryospora Arten der Falken (*Caryospora neofalconis* n. sp. und *Caryospora kutzeri* n. sp.). [Inaugural--Dissertation.] Hannover, Institut für Parasitologie der Tierärztlichen Hochschule.

Cooper J. E. (1977): Veterinary problems of captive breeding and possible reintroduction of birds of prey. International Zoo Year Book, *17*, 32–38.

Dorn R., Silapanuntakul R., Angrick E. J., Shipman L. D. (1992): Plasmid analysis and epidemiology of *Salmonella enteritidis* infection in three commercial layer flocks. Avian Dis., *36*, 844–851.

Furr P. M., Cooper J. E., Taylor-Robinson D. (1977): Isolation of mycoplasmas from three falcons (*Falco* spp.). Vet. Rec., *100*, 72–73.

Furrer B., Baumgartner A., Bommeli W. (1993): Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Salmonella enteritidis* in chicken blood or egg yolk. Zbl. Bakt., *279*, 191–200.

Gough R. E., Drury S. E. N., George A. D., Randall C. J. (1993): Isolation and identification of a falcon herpesvirus. Vet. Rec., *132*, 220–221.

Isenbügel E., Rübel A. (1987): Greifvögel. In: Gabrish K., Zwart P. (eds.): Krankheiten der Wildtiere. Hannover, Schlütersche Verlagsanstalt. 191–252.

Kirkpatrick C. E., Trexler-Myren V. P. (1986): A survey of free-living falconiform birds for Salmonella. J. Am. Vet. Med. Assoc., *189*, 997–998.

Lüthgen von W. (1978a): Salmonelleninfektion oder Salmonellose bei Greifvögeln. Prakt. Tierarzt., *9*, 663–666.

Lüthgen von W. (1978b): Ergebnisse fünfjähriger Untersuchungen von Greifvögeln und Eulen. Prakt. Tierartz., *9*, 666–670.

Needham J. R., Kirkwwod J. K., Cooper J. E. (1979): A survey of the aerobic bacteria in the droppings of captive birds of prey. Res. Vet. Sci., *27*, 125–126.

Nicholas R. A. J. (1992): Serological response of chickens naturally infected with *Salmonella typhimurium* detected by ELISA. Brit. Vet. J., *148*, 241–248.

Pavlík I., Bárta J., Lány P., Fischer O., Černík J., Zendulková D., Kundera J. (1997): Health problems in captive birds of prey. I. Parasitic, bacterial, viral and noninfectious diseases of adult birds (in Czech). Veterinářství, *47*, 385–388.

Pavlík I., Černík J., Bárta J., Kundera J., Pecka Z. (1998): Occurrence of coccidia (*Caryospora neofalconis* and *Caryospora kutzeri*) in birds of prey in Falcon Breeding Facility in Milotice in the Czech Republic. Vet. Med. – Czech, *43*, 301–306.

Piechocki R. (1981): Tuberkulose bei wildlebenden Greifvögeln (*Falconiformes*) und Eulen (*Strigiformes*). In: Erkrankungen der Zootiere. Verhandlungsbericht des XXIII. Internationalen Symposiums über die Erkrankungen der Zootiere. Halle/Saale. Academie-Verlag Berlin. 55–66.

Rychlík I., Šišák F., Lány P. (1993): Differentiation of *Salmonella enteritidis* and *S. typhimurium* by plasmid profile analysis and restriction endonuclease analysis of chromosomal DNA. Vet. Med. – Czech, *38*, 433–439.

Rychlík I., Karpíšková R., Faldynová M., Šišák F. (1999): Computer-assisted restriction endonuclease analysis of plasmid DNA in field strains of *Salmonella enteritidis*. Can. J. Microbiol., *44*, 1183–1185.

Samour J. H., Cooper J. E. (1993): Avian pox in birds of prey (order *Falconiformes*) in Bahrain. Vet. Rec., *132*, 343–345.

Sykes G. P., Murphy C., Hardaswick V. P. (1981): Salmonella infection in a captive peregrine falcon. J. Am. Vet. Med. Assoc., *179*, 1269–1271.

Štika V., Čermák S., Rachač V. (1989): Tuberculosis in diurnal birds of prey (in Czech). Veterinářství, *39*, 86–88.

Trommer G. (1988): Lokalisierte Tuberkulose bei Greifvögeln. Tierärztl. Prax., *16*, 55–56.

Wernery U., Remple J. D., Neumann U., Alexander D. J. - Manvell R. J., Kaaden O. R. (1992): Avian paramyxovirus serotype 1 (Newcastle disease virus) – infections in falcons. J. Vet. Med. B, *39*, 153–158.

Wachsmuth I. K., Kiehlbauch J. A., Bopp C. A., Cameron D. N., Strockbine N. A., Wells J. G., Blake P. A. (1991): The use of plasmid profiles and nucleic acid probes in epidemiologic investigations of foodborne, diarrheal diseases. Int. J. Food Microbiol., *12*, 77–90.

Received: 98-07-11
Accepted after corrections: 99-08-31

Contact Address:

MVDr. Ivo Pavlík, CSc., Výzkumný ústav veterinárního lékařství, Hudcova 70, 621 32 Brno, Česká republika
Tel. +420 5 41 32 12 41, fax +420 5 41 21 12 29, e-mail: pavlik@vri.cz, http://www.vri.cz/wwwrflptext.htm

POKYNY PRO AUTORY

Časopis uveřejňuje původní vědecké práce, krátká sdělení a výběrově i přehledné referáty, tzn. práce, jejichž podkladem je studium literatury a které shrnují nejnovější poznatky v dané oblasti. Práce jsou uveřejňovány v češtině, slovenštině nebo angličtině. Rukopisy musí být doplněny krátkým a rozšířeným souhrnem. Časopis zveřejňuje i názory, postřehy a připomínky čtenářů ve formě kurzívy, glosy, dopisu redakci, diskusního příspěvku, kritiky zásadního článku apod., ale i zkušenosti z cest do zahraničí, z porad a konferencí.

Autoři jsou plně odpovědni za původnost práce a za její věcnou i formální správnost. K práci musí být přiloženo prohlášení o tom, že práce nebyla publikována jinde.

O uveřejnění práce rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a přínosu a kvalitě práce. Redakce přijímá práce imprimované vedoucím pracoviště nebo práce s prohlášením všech autorů, že se zveřejněním souhlasí.

Rozsah původních prací nemá přesáhnout 10 stran psaných na stroji včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI.

Rukopis má být napsán na papíře formátu A4 (30 řádek na stránku, 60 úhozů na řádku, mezi řádky dvojité mezery). K rukopisu je vhodné přiložit disketu s textem práce, popř. s grafickou dokumentací pořízenou na PC s uvedením použitého programu. Tabulky, grafy a fotografie se dodávají zvlášť, nepodlepují se. Na všechny přílohy musí být odkazy v textu.

Pokud autor používá v práci zkratek jakéhokoliv druhu, je nutné, aby byly alespoň jednou vysvětleny (vypsány), aby se předešlo omylům. V názvu práce a v souhrnu je vhodné zkratek nepoužívat.

Název práce (titul) nemá přesáhnout 85 úhozů a musí dát přesnou představu o obsahu práce. Jsou vyloučeny podtitulky článků.

Krátký souhrn (Abstrakt) musí vyjádřit všechno podstatné, co je obsaženo v práci, a má obsahovat základní číselné údaje včetně statistických hodnot. Nemá překročit rozsah 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě.

Rozšířený souhrn prací v češtině nebo slovenštině je uveřejňován v angličtině, měly by v něm být v rozsahu cca 1–2 strojopisných stran komentovány výsledky práce a uvedeny odkazy na tabulky a obrázky, popř. na nejdůležitější literární citace. Je vhodné jej (včetně názvu práce a klíčových slov) dodat v angličtině, popř. v češtině či slovenštině jako podklad pro překlad do angličtiny.

Literární přehled má být krátký, je třeba uvádět pouze citace mající úzký vztah k problému. Tato úvodní část přináší také informaci, proč byla práce provedena.

Metoda se popisuje pouze tehdy, je-li původní, jinak postačuje citovat autora metody a uvádět jen případné odchylky. Ve stejné kapitole se popisuje také pokusný materiál a způsob hodnocení výsledků.

Výsledky tvoří hlavní část práce a při jejich popisu se k vyjádření kvantitativních hodnot dává přednost grafům před tabulkami. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce, diskutuje se o možných nedostatcích a výsledky se konfrontují s údaji publikovanými (požaduje se citovat jen ty autory, jejichž práce mají k publikované práci bližší vztah). Je přípustné spojení v jednu kapitolu spolu s výsledky.

Literatura citovaná v textu práce se uvádí jménem autora a rokem vydání. Do seznamu se zařadí jen publikace citované v textu. Citace se řadí abecedně podle jména prvních autorů.

Klíčová slova mají umožnit vyhledání práce podle sledovaných druhů zvířat, charakteristik jejich zdravotního stavu, podmínek jejich chovu, látek použitých k jejich ovlivnění apod. Jako klíčová slova není vhodné používat termíny uvedené v nadpisu práce.

Na zvláštním listě uvádí autor plné jméno (i spoluautorů), akademické, vědecké a pedagogické tituly a podrobnou adresu pracoviště s PSČ, číslo telefonu a faxu, popř. e-mail.

Úplné znění pokynů pro autory s dodatky najdete na URL adrese http://www.clark.cz/vri/Pokyny.htm

For full text of instruction for authors see http://www.clark.cz/vri/Pokynya.htm

INSTRUCTIONS FOR AUTHORS

Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short or a longer summary. The journal also publishes readers' views, remarks and comments in form of a text in italics, gloss, letter to the editor, short contribution, review of a major article, etc., and also experience of stays in foreign countries, meetings and conferences.

The authors are fully responsible for the originality of their papers, for its subject and formal correctness. The authors shall make a written declaration that their papers have not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper. The editors accept papers approved to print by the head of the workplace or papers with all the authors' statement they approve it to print.

The extent of original papers shall not exceed ten typescript pages, including tables, figures and graphs.

Manuscript should be typed on standard paper (quarto, 30 lines per page, 60 strokes per line, double-spaced typescript). A PC diskette with the paper text or graphical documentation should be provided with the paper manuscript, indicating the used editor program. Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes.

The **title** of the paper shall not exceed 85 strokes and it should provide a clear-cut idea of the paper subject. Subtitles of the papers are not allowed either.

Abstract. It must present information selection of the contents and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes and comprise base numerical data including statistical data.

Introduction has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form. This introductory section also provides information why the study has been undertaken.

Review of literature should be a short section, containing only literary citations with close relation to the treated problem.

Only original method shall be described, in other cases it is sufficient enough to cite the author of the used method and to mention modifications of this method. This section shall also contain a description of experimental material and the method of result evaluation.

In the section **Results**, which is the core of the paper, figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

Discussion contains an evaluation of the study, potential shortcomings are discussed, and the results of the study are confronted with previously published results (only those authors whose studies are in closer relation with the published paper should be cited). The sections Results and Discussion may be presented as one section only.

References in the manuscript are given in form of citations of the author's name and year of publication. A list of references should contain publications cited in the manuscript only. References are listed alphabetically by the first author's name.

Key words should make it possible to retrieve the paper on the basis of the animal species investigated, characteristics of their health, husbandry conditions, applied substances, etc. The terms used in the paper title should not be used as keywords.

If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telephone and fax number, or e-mail.

VETERINARY MEDICINE – CZECH

Volume 44, No. 11, November 1999

CONTENTS

Dvorská L., Parmová I., Lávičková M., Bartl J., Vrbas V., Pavlík I.: 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 of 1996–1998 (in English) 321

Suchý P., Herzig I., Písaříková B.: The use of sorbents on the basis of humic acids to reduce ammonia levels in stable environment 331

Venglovský J., Pačajová Z., Sasáková N., Vučemilo M., Tofant A.: Adsorption properties of natural zeolite and bentonite in pig slurry from the microbiological point of view (in English) 339

Lány P., Rychlík I., Bárta J., Kundera J., Pavlík I.: Salmonellae in one falcon breeding facility in the Czech Republic during the period 1989–1993 (in English) 345

VETERINÁRNÍ MEDICÍNA

Ročník 44, č. 11, Listopad 1999

OBSAH

Dvorská L., Parmová I., Lávičková M., Bartl J., Vrbas V., Pavlík I.: Izolace *Rhodococcus equi* a atypických mykobakterií z mízních uzlin prasat a skotu z chovů s výskytem tuberkuloidních nálezů v České republice v letech 1996 až 1998 321

Suchý P., Herzig I., Písaříková B.: Využití sorbentů na bázi huminových kyselin ke snížení hladiny amoniaku ve stájovém prostředí 331

Venglovský J., Pačajová Z., Sasáková N., Vučemilo M., Tofant A.: Adsorpčné vlastnosti prírodného zeolitu a bentonitu v hnojovici ošípaných z mikrobiologického hľadiska 339

Lány P., Rychlík I., Bárta J., Kundera J., Pavlík I.: Salmonely v odchovně sokolovitých dravců v České republice v letech 1989 až 1993 345

Vědecký časopis VETERINÁRNÍ MEDICÍNA ● Vydává Ústav zemědělských a potravinářských informací ● Redakce: Slezská 7, 120 56 Praha 2, tel.: 02/24 25 79 39, fax: 02/24 25 39 38, e-mail: edit@uzpi.cz ● Sazba: Studio DOMINO – ing. Jakub Černý, Plzeňská 145, 266 01 Beroun, tel.: 0311/62 29 59 ● Tisk: ÚZPI Praha ●

Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2