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Mycobacteria in the animal's environment in the Czech Republic

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ABSTRACT

The "atypical mycobacteria" are widely distributed in the natural environment and can produce infection in farm and wild animals bred in captivity. The objective of the study was to analyze the variety of mycobacterial species in the environment of breeding facilities, tanks and aquariums, as well as in samples of peat used as a feed supplement in the Czech Republic over the 2003-2004 period. A total of 1389 samples from environment were examined, collected from 29 sites throughout the Czech Republic. The samples were decontaminated and cultured at 25 °C and 37 °C in three culture media: Stonebrink's medium, Herrold's egg yolk medium and Sula's medium. Mycobacterial isolates grown were identified by the PCR method and growth and biochemical tests. Of the 1389 environmental samples, mycobacteria were demonstrated by culture in 400 (28.8%) of them originating from different substances. In the samples of farm environment and in peat, mycobacteria were isolated in 185 of 1064 (17.4%) samples, and in 201 of 325 (61.8%) samples from aquarium and breeding tank environment. The results show significant difference between the findings from the stable environment (17.4%) and those from the aquarium environment (61.8%). A wide range of species was found in all constituents of the environment. The following species were most frequently isolated from the stable environment: *M. avium* (6.5%), *M. fortuitum* (3.2%), *M. flavescens* (3.2%), and the aquarium environment harboured most frequently *M. fortuitum* (13.9%), *M. marinum* (8.0%) and *M. gordonae* (4.5%). The results confirm the frequent occurrence of various mycobacterial species in the environment, predominantly in aquariums, some of which can produce infection in animals and in man following contact with the infected environment.

Key words: zoonosis, epidemiology, *IS901*, *IS1245*, mycobacteriosis, tuberculosis

Introduction

Research in human and veterinary medicine has been focused on mycobacteria,

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the causative agents of tuberculosis, predominantly *Mycobacterium tuberculosis* and *M. bovis*. Lately, research workers have addressed themselves to the issue of what are known as "atypical mycobacteria" which are widely distributed in nature, pose health risk to humans and animals and can also cause huge economic losses in farm animals. Atypical or what are sometimes called "potentially pathogenic mycobacteria" are able to utilize nutrients present in natural substances such as the soil, water, straw, leaves etc. (HORVATHOVA et al., 1997; KAZDA, 2000). Frequent findings of various mycobacterial species in the environment suggest their importance in the final stage of mineralization of organic substances in nature (BEERWETH and KESSEL, 1976; KAZDA, 2000).

Feed, drinking water and the environment of farm facilities for domestic and wild animals, they can all be the source of mycobacterial infections (MATLOVA et al., 2003). These infections can cause great economic losses (HORVATHOVA et al., 1997; PAVLIK et al., 2002, 2003). The sources of atypical mycobacteria in pigs were found to be among peat supplemented to feed (MATLOVA et al., 2005), china clay (MATLOVA et al., 2004a) and bran (FISCHER et al., 2003) and sawdust used as litter (MATLOVA et al., 2004b). Various mycobacterial species have also been found in breeding tanks and fish aquariums (PATTYN et al., 1971; BERAN et al., 2006). The high incidence of mycobacteria in aquariums can affect the health status of fish and pose the risk of skin infections in humans handling contaminated aquariums (JERNIGAN and FARR, 2000).

This paper analyses the spectrum of mycobacterial species in various constituents of the environment in farm facilities, fish tanks and aquariums, and in peat specimens used as a feed supplement in the Czech Republic over the 2003-2004 period.

Materials and methods

A total of 1389 samples of environment were collected from 29 sites of the Czech Republic. The samples were classified into two groups: a) samples from the farm facilities environment and peat, b) samples from the aquarium and fish tank environment. Peat, which is used as a feed additive, originated from different producers and distributors in the Czech Republic. The aquariums and tanks were used for both breeding and experimental purposes.

Biological material was examined according to methods described previously (MATLOVA et al., 2003). About 1 g of sample was homogenized in water and decontaminated using HCl/NaOH (FISCHER et al., 2001). 40 µl of inoculum and subjected to culture at temperatures 25 °C and 37 °C on three different media: solid Stonebrink's medium, Herrold's egg yolk medium without Mycobactin J, and one liquid medium according to Sula. The growth of mycobacteria was monitored during the first week (detection of

rapidly growing species) and then every two weeks for a two-month period (detection of slowly growing species).

Mycobacterial isolates were examined by the PCR method for the detection of the dnaJ gene specific for the *Mycobacterium* genus (NAGAI et al., 1990). Specific insertion sequences for *M. avium* were identified by the previously described PCR technique for the detection of IS*901* and IS*1245* (BARTOS et al., 2006). Mycobacterial isolates lacking IS*901* and/or IS*1245* were assessed by growth and biochemical tests (WAYNE and KUBICA, 1986).

Results

Of 1389 environmental samples, mycobacteria were isolated by culture in 400 (28.8%) samples. In farm facilities and peat, mycobacteria were isolated in 185 of 1064 (17.4%) samples. Mycobacteria were evenly distributed in all the studied constituents of the environment. In this group of samples, mycobacteria were most frequently isolated from biofilm (43.3%), peat (39.6%), drinking water (32.4%), and litter (31.1%). The most frequently isolated species were *M. avium* (6.5%), *M. fortuitum* (3.2%) and *M. flavescens* (3.2%). A high percentage of the isolates were not determined or determination was not successful (76.2%) (Tab. 1).

Table 1. Identification of mycobacterial isolates originating from farm facilities and peat producers in the Czech Republic (2003-2004)

		Mycobacterium									
	No. of isolates	*avium*	*fortuitum*	*flavescens*	*chelonae*	*gordonae*	*scrofulaceum*	*terrae*	*triviale*	other species	spp.
Swabs	34	0	0	3	0	1	0	0	2	0	28
Litter	20	0	0	0	0	0	1	0	0	2	17
Plants	24	9	1	1	0	1	1	0	0	0	11
Compost	3	0	0	0	0	0	0	1	0	0	2
Soil	41	2	3	0	1	0	3	1	1	0	30
Water	25	0	1	0	1	0	0	0	0	0	23
Dust, spider webs	2	0	0	0	0	0	0	0	0	1	1
Food and feed	19	0	1	2	0	0	0	0	1	1	14
Earthworms	4	0	0	0	0	0	0	0	0	0	4
Peat	13	1	0	0	0	0	0	0	1	0	11
Total	185	12	6	6	2	2	5	2	5	4	141

In the aquarium environment and fish tank environment, mycobacteria were demonstrated in 201 of 325 (61.8%) samples. In this group of samples, mycobacteria were most frequently isolated from biofilm (77.1%), plants (68.0%) and sediment (65.9%). In contrast, mycobacteria were only isolated from 12.5% of feed specimens. The prevailing species were *M. fortuitum* (13.9%), *M. marinum* (8.0%) and *M. gordonae* (4.5%). We did not determine or failed to determine 56.7% of the isolates (Tab.2).

Table 2. Identification of mycobacterial isolates originating from aquariums and breed tanks in the Czech Republic (2003-2004)

	Mycobacterium										
	No. of isolates	*fortuitum*	*marinum*	*kansasii*	*gordonae*	*szulgai*	*terrae*	*chelonae*	*abscessus*	other species	spp.
Water	53	14	2	0	3	0	1	2	2	6	23
Biofilm, algae	35	5	6	1	4	0	1	1	0	2	15
Sediment	45	2	2	1	2	0	0	3	0	2	33
Plants	35	5	3	1	0	2	1	1	1	3	18
Snails	13	2	2	0	0	0	0	0	0	1	8
Absorption pellets	11	0	0	0	0	0	0	1	0	0	10
Feed	7	0	0	0	0	0	0	0	0	0	7
Invertebrates	2	0	1	0	0	0	0	0	0	1	0
Total	201	28	16	3	9	2	3	8	3	15	114

Discussion

Mycobacteria were more or less evenly distributed in all the environmental components under study that originated from different sites. However, a significant difference was observed in detection rates by culture in stable environment samples (17.4%) and those originating from aquarium environment (61.8%). Most likely, the aquarium environment offers more favorable conditions for propagation of mycobacteria (temperature, water containing enough oxygen etc.). In contrast, dry pelleted feed for fish is not suitable for mycobacteria and hence the percentage of mycobacteria is lower (12.5%). Analysis of peat specimens revealed difference in samples originating from different producers and distributors and showed the effect of peat origin and method of treatment. Major differences were observed between treated and untreated peat (unpublished data).

Distribution of mycobacterial species in stable environment was similar to that found in previous studies by the authors as well as by other authors (PAVLAS and PATLOKOVA, 1985; PAVLAS et al., 1991; PAVLIK, 1992; MATLOVA et al., 2003). The identified mycobacterial species were not of clinical importance, except for *M. avium*. The incidence of *M. marinum* and *M. kansasii* in the aquarium environment is of importance. *M. marinum* can be found in skin lesions in humans causing what are known as "swimming pool granuloma" or "fish tank granuloma", according to the source of infection (KULLAVANIJAYA et al., 1993). The finding of clinically important *M. kansasii* is interesting for the reason that its occurrence is endemic in the north Moravian region (KAUSTOVA et al., 1995).

Both animals in the wild and those reared in captivity are exposed to mycobacterial infections. However, it has to be considered that the risk of infection in animals reared in captivity is significantly higher. This is due to transmission of mycobacterial infection from peat, sawdust and wood shawings that are used as litter. Another cause of infection is feeding with meat and laboratory animals that have not passed through veterinary inspection and the use of drinking water contaminated with potentially pathogenic mycobacteria.

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SAŽETAK

Takozvane "atipične mikobakterije" naveliko su prisutne u prirodnom okolišu te mogu uzrokovati zaraze farmskih životinja i divljih životinja držanih u zatočeništvu. Svrha istraživanja bila je odrediti prisutnost pojedinih vrsta mikobakterija u području uzgojnih objekata, rezervoara vode i akvarija, kao i u uzorcima korištenima kao dodatak krmivima na području Češke u razdoblju od 2003. do 2004. godine. Ukupno je prikupljeno i pregledano 1389 uzoraka s 29 lokaliteta na području Češke. Uzorci su dekontaminirani i kultivirani u tri različita medija (Stonebrinkov, Herroldov i Sula-medij) na 25 °C i 37 °C. Izrasle kolonije identificirane su na temelju karakteristika rasta, biokemijskih osobitosti te lančanom reakcijom polimeraze (PCR). Od ukupno 1389 uzoraka, mikobakterije su dokazane u njih 400 (28,8%), i to u 185 od 1064 uzorka treseta (17,4%) i u 201 od 325 uzoraka prikupljenih u uzgojnom području i akvarijima (61,8%). Rezultati pokazuju značajnu razliku između stajskih uzoraka (17,4% pozitivnih) i onih prikupljenih u akvarijima (61,8%). Najčešće izdvajane vrste u uzorcima prikupljenima u staji su *M. avium* (6,5%), *M. fortuitum* (3,2%), *M. flavescens* (3,2%), a iz akvarijskih uzoraka *M. fortuitum* (13,9%), *M. marinum* (8,0%) i *M. gordonae* (4,5%). Rezultati istraživanja potvrđuju prisutnost različitih vrsta mikobakterija u okolišu, a posebice u akvarijima. Neke od navedenih bakterija mogu uzrokovati infekcije ljudi i životinja.

Ključne riječi: zoonoza, epidemiologija, *IS901*, *IS1245*, mikobakterioze, tuberkuloza