Mycobacteria in peat used as a supplement for pigs: failure of different decontamination methods to eliminate the risk

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ABSTRACT: Peat used as a feed supplement for piglets has favourable dietetic qualities; however, its frequent contamination with potentially pathogenic mycobacteria (PPM) has been shown to pose a potential risk to piglet health. The purpose of the present study was to investigate possible ways of devitalising mycobacteria. Examination of 118 samples from various types of commercially available peat (natural peat, packed peat for horticulture and specially processed peat intended for piglet feeding) showed that PPM were present in 84 (71.1%) samples. *Mycobacterium avium* subsp. *hominissuis* (82.1%) was the most frequent mycobacterial isolate. In addition, from a natural locality where peat is mined and stored in large piles for up to four months, mycobacteria were detected in peat samples collected from the surface and from up to 25 cm in depth. We used different physical and chemical procedures for peat decontamination (peracetic acid, formaldehyde, steam, and microwave radiation) in attempting to devitalise the mycobacteria in peat. We found that PPM can be reliably devitalised with 1.0% peracetic acid, or 5.0% formaldehyde. However, under field conditions, when using bulk amounts of peat, none of the above procedures were shown to be suitable. Based on these results, the feeding of peat to piglets is confirmed as a high-risk practice.

Keywords: zoonosis; mycobacteriosis; feed safety; *Mycobacterium avium* complex

The use of peat holds many advantages for animals and is widely utilised in pig breeding. Besides its use as an absorbent bedding material, in the last couple of decades it has been supplemented into the diet of newborn piglets. Due to its richness in humic, fulvic and ulmic acids, peat favours the process of iron uptake by piglets. The high content of fibre, meanwhile, increases the overall uptake of food, thus improving the weight gaining process in piglets. Also, the low pH of peat has bactericidal effects on pathogenic bacteria in the intestinal tract of piglets helping to prevent or decrease the intensity of diarrhoea (Roost et al. 1990; Mesrogli et al. 1991; Fuchs et al. 1995; Trckova et al. 2005).

Although the positive effects of peat used for pig feeding are significant, after the intensification of its use on pig farms in the Czech Republic there was an increased incidence of the discovery of tuberculous lesions in slaughtered pigs. The losses per slaughtered pig with detected tuberculous lesions reached 22 to 24% of the original slaughter price in the Czech Republic (Pavlik et al. 2003). The aetiological agents causing these lesions were, in the majority of cases, members of the *Mycobacterium avium* complex (MAC), i.e., *M. a. hominissuis* and *M. a. avium* and some species of potentially pathogenic mycobacteria (PPM). Investigations into the external sources of infection causing the tuberculous lesions revealed that the primary source of MAC was peat fed as a supplement (Matlova et al. 2005; Trckova et al. 2006a,b). Various species of PPM have been detected in peat with *M. a. hominissuis* the most often isolated (Matlova et al. 2003, 2005; Krizova et al. 2010). In Germany, peat was also found to be a major source of mycobacteria in pigs as early as the mid-1970s (Engel et al. 1978). The current production of peat primarily involves mined peat, which is obtained during dry summer

Supported by the Ministry of Agriculture of the Czech Republic (Grants No. MZE 0002716202 and No. QH 91240) and the Ministry of Education, Youth and Sports of the Czech Republic (AdmireVet No. CZ.1.05/2.1.00/01.0006-ED 0006/01/01)

weather and stored over a period of 6 to 18 months in large stockpiles on a bog (Dykes et al. 1997). Storage in open spaces as well as transportation and storage of peat in perforated plastic bags could increase the risk of contamination (Pavlik 2009).

Mycobacteria, especially *M. avium*, have drawn increasing scientific and public attention in the past couple of decades, which is evident from the increasing number of papers published on them (Kaevska and Hruska 2010a,b). The *M. avium* subspecies causes avian tuberculosis, paratuberculosis and avian mycobacterioses in a wide variety of domestic and wild animals (Biet et al. 2005; Pavlik et al. 2005; Kaevska et al. 2010; Kriz et al. 2010a; Blahutkova et al. 2011).

Mycobacteria can thrive in the grey layer of sphagnum vegetation, due to the nutrient-rich and favourable temperature conditions found in this layer and can reach a concentration of up to 10^6/g of sphagnum. The numbers of detectable living mycobacteria are inversely proportional to the depth from which the sample is taken, and viable mycobacterial cells were not found in the profile of peat from 70 cm and deeper (Kazda 2000; Trckova et al. 2005, 2006a,b).

The low pH and high concentration of organic nutrients in peat are optimal conditions for the growth and multiplication of mycobacteria (Kazda et al. 1989). It is of great importance that, prior to use as a feed supplement, peat should be disinfected or decontaminated from mycobacteria. The devitalisation of mycobacteria using chemical and physical processes has been studied extensively; however, decontamination processes have most often been applied to bacterial cultures or food (Taylor et al. 2000; Altic et al. 2007; Rademaker et al. 2007). Chlorine-containing agents are commonly used to disinfect materials from pathogenic mycobacteria (Pelletier et al. 1988). Stabilised buffered peracetic acid solution (0.35%, 'Nu-Cidex') was successfully used to disinfect bronchoscopes experimentally infected with *M. tuberculosis*, *M. avium-intracellulare* and *M. chelonae* (Middleton et al. 1997). In an experimental study, Collins (1986) found that 2% alkaline glutaraldehyde solution displayed bactericidal activity against a number of PPM species. *M. marinum*, *M. smegmatis* and *M. fortuitum* were the most susceptible. The bactericidal effects of chemical or physical decontamination methods on mycobacteria in peat have not yet been studied.

The objective of the present study was to identify and confirm the source of mycobacterial contamination of peat, which causes increased rates of tuberculous lesions in pig lymph nodes. To this end, various peat types were analysed for the presence of mycobacteria along with the potential contamination of natural peat, stored in unfenced stockpiles on a bog in the open. The second aim was to investigate different methods of peat decontamination, under both laboratory and field conditions on a pig farm.

MATERIAL AND METHODS

Peat origin

A total of 118 samples of commercial, garden and natural peat were examined. The commercial peat was imported from France and designated for use as a feeding supplement for piglets. Also, commercially available peat, treated against mycobacteria from the producer was tested. Garden peat was obtained from a supermarket and was intended for use in households or in the garden (sometimes it is used

Table 1. Contamination of different types of peat with mycobacteria

Sources	Examined samples			Mycobacterial isolates (n = 84)	
	No.	positive	%	*M. a. hominissuis.*	other sp.*
Natural peat	22	14	63.6	9	5
Garden peat	16	16	100	14	2
Commercial peat	71	54	76.1	46	8
Commercially sterilised peat	9	0	0		
Total	118	84	77.1	69	15
%		100		82.1	17.9

*other species were identified as *M. fortuitum* (n = 3), *M. gordonae* (n = 3), *M. chelonae* (n = 2), *M. terrae* (n = 1), *M. xenopi* (n = 1), *M. flavescens* (n = 1), *M. phlei* (n = 1), *M. scrofulaceum* (n = 1) and *Mycobacterium* sp. (n = 2)

Table 2. Contamination of peat collected at different depths from a mine in the Czech Republic

Depth under the surface (cm)	Examined samples			Mycobacterial isolates	
	No.	positive	%	*M. a. hominissuis*	*M. fortuitum*
0–5	20	15	75.0	15	0
10–15	3	2	66.0	2	0
20–25	3	2	66.0	1	1
30–35	3	0	0	0	0
40–45	3	0	0	0	0
Total	32	19	59.4	18	1
%		100		94.7	5.3

by farmers as feeding supplement). Natural peat was obtained from a surface peat mine in the South Bohemian region of the Czech Republic (Table 1).

From the stockpiles of natural peat, samples were taken from different sections of the pile (bottom, middle and top). Then, from the middle and upper parts of the stockpile, samples were collected from up to 45cm of depth (Table 2).

Mycobacterial culture and identification of isolates

Peat samples were collected with a sterile wooden stick into a sterile plastic bag and stored at 6 °C. Peat samples (approx. 5 g) were processed in accordance with a method described previously (Fischer et al. 2000). All positive isolates were examined by PCR which targets specific fragments of the genus *Mycobacterium* and *M. avium* subspecies (Moravkova et al. 2008).

Decontamination methods under laboratory and field conditions

Different decontamination methods were tested on four samples of contaminated peat under laboratory conditions and routine field conditions. In the laboratory, peracetic acid (0.1 to 1.0%) for 60 min, formaldehyde (2 and 5%) for 60 min and Citrex (citric acid with glycerine, commercially available product, Citrex Inc., USA), were tested for their ability to chemically decontaminate peat. Steam (100 °C/10min) and microwave radiation (approx. 700 W, 5 min) were selected as physical treatments. The tests were done with approximately 5 g of material. Subsequently, treatment with 1% peracetic acid (Persteril, Overlack Plzen, Czech Republic) for 60 min was applied to larger amount of material (up to 10 kg) to test its applicability in field conditions.

RESULTS AND DISCUSSION

Mycobacteria were isolated from all the different types of peat (Table 1). From the samples of commercially available peat sterilised by the producer (ionisation; information on intensity and duration was not available), there were no mycobacteria recovered.

The risk that peat poses with regard to mycobacterial infections has been confirmed in this study. The rate of mycobacterial contamination ranged from 63 to 100% with the predominant isolate being *M. a. hominissuis* (82.1%), followed by *M. fortuitum* and *M. gordonae* (3.5%). This finding concurs with previous studies in which *M. a. hominissuis* was detected most frequently (Matlova et al. 2003, 2005; Krizova et al. 2010). *M. a. hominissuis* as well as other PPM and environmentally saprophytic mycobacteria (which were also found in peat), can induce the development of tuberculous lesions in mesenteric lymph nodes in pigs (Pavlik et al. 2003; Matlova et al. 2005; Shitaye et al. 2006). It is important to bear in mind that peat represents a risk also when used as a bedding material and not only as a feeding supplement (Pavlik 2009).

Peat samples taken from the surface of different sections of the stockpile and from a depth of up to 25 cm were heavily contaminated with mycobacteria (66 to 75%). In contrast, samples from deeper parts of the stockpile (30 to 35 cm and 40 to 45 cm) were found to contain no detectable mycobacteria by culture examination (Table 2). Sterile, underground peat for commercial use is mined from a depth of more than 2 m and stored on stockpiles

in open spaces for a few months; therefore, it may become contaminated from a variety of sources during subsequent storage. These include: rainwater, dust, soil and the faeces of infected domestic and free living birds or mammals (Kazda et al. 2009; Pate et al. 2009; Shitaye et al. 2009; Kriz et al. 2010a,b; Skoric et al. 2010; Kaevska et al. 2011). However, we have not determined the exact sources of contamination in these cases.

The chemical decontaminates tested in the laboratory were effective at higher concentrations (1.0% peracetic acid and 5.0% formaldehyde). None of the four tested samples were positive after these treatments. The treatment with Citrex was ineffective, as we obtained isolates from all of the samples tested. Microwave radiation and exposure to steam caused a reduction in the level of mycobacterial contamination when applied for at least 5 and 10 min, respectively (Table 3). Similar results were also reported by Rosaspina et al. (1994), who studied the bactericidal effect of microwaves on *M. bovis*. After 4 min of microwave exposure, complete destruction of the mycobacteria was achieved.

Under field conditions, the tested method of decontamination (1.0% Persteril for 60 min) applied to a large volume of peat (approx. 10 kg) was shown to be inadequate (15 positive from 20 treated samples). Although ionisation effectively devitalised mycobacteria (performed by the producer), this type of radiation is not currently allowed under EU legislation. Thus, it will be necessary to investigate other procedures for the safe devitalisation of mycobacteria in peat.

Table 3. Efficacy of different decontamination procedures of peat under laboratory conditions

Treatment	Exposition conditions	Samples No./positive
Laboratory conditions	5 g of sample	
Peracetic acid*	0.1%, 60 min	4/3
	0.3%, 60 min	4/2
	0.6%, 60 min	4/2
	0.9%, 60 min	4/1
	1.0%, 60 min	4/0
Formaldehyde	2.0%, 60 min	4/4
	5.0%, 60 min	4/0
Citrex (glycerin)	1000 ppm, 60 min	4/4
	100 ppm, 60 min	4/4
Steam	100 °C, 10 min	10/1
Microwave	700 W, 2.5 min	4/4
	700 W, 5.0 min	4/0

*commercially available (Persteril, Overlack s.r.o, Plzen, Czech Republic)

In conclusion, we found peat to be extremely susceptible to contamination with PPM, and the currently used methods for its decontamination were ineffective under field/farm conditions. Therefore, feeding peat as a supplement to piglets remains a highly risky practice.

Acknowledgement

The authors wish to thank Mgr. Lenka Bartosova, PhD and P. Bejcek for the excellent technical assistance.

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Received: 2011–06–09
Accepted after corrections: 2012–04–16

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