ACTA VET. BRNO, 54, 1985: 85–90

OCCURRENCE OF MYCOBACTERIA IN SAWDUST, STRAW, HAY AND THEIR EPIZOOTIOLOGICAL SIGNIFICANCE

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Received February 13, 1984

Abstract

Pavlas, M., Patloková, Vlasta: *Occurrence of Mycobacteria in Sawdust, Straw, Hay and Their Epizootiological Significance.* Acta vet. Brno, 54, 1985: 85—90.

The occurrence of mycobacteria in sawdust and straw used as bedding material in cattle and pig herds was investigated using 147 sawdust and straw samples from 47 farms.

The most important epizootiological finding was the demonstration of nine strains of *Mycobacterium intracellulare* (serotypes 4, 6, 8 and 9) and of one strain of *Mycobacterium avium* (serotype 1) in samples of sawdust and wood shavings, which accounted for a quarter of all these samples examined, no matter whether they were collected directly in wood-processing plants or from stores on the farms.

In view of the virulence of *M. intracellulare* for pigs, in which active excretion is very rare, the aforementioned bedding material should be regarded as one of the most important sources of mycobacteriosis in pigs.

A large number of atypical avirulent mycobacteria found in straw and hay suggests that this bedding material and roughage may be involved in the occurrence of non-specific reactions to avian or possibly mammalian tuberculin particularly in cattle.

Atypical mycobacteria, bedding material, swine mycobacteriosis.

The current favourable situation as to bovine tuberculosis in farm animals in our country suggests that a majority of sources of *Mycobacterium bovis* have been eliminated. A comprehensive investigation into the causes of reaction to mammalian and avian tuberculins in cattle, pigs and other farm animals showed that most importance among individual pathogenic mycobacterial species should be ascribed to *Mycobacterium avium* and atypical mycobacteria with biological and chemical properties similar to those of avian mycobacteria.

These atypical mycobacteria are, for the most part, of low virulence for a majority of farm animal species. Horizontal transmission of these mycobacteria is therefore very rare and is limited to a very short period of time after infection.

The importance of a typical mycobacteria emerges from the results of tuberculin testing in cattle in Czechoslovakia in 1979 where specific reaction was shown by 125 animals (17.8 per cent) out of 700 positive reactors to mammalian PPD tuberculin on 8 farms in 6 districts. On the other hand, the reactions to mammalian PPD tuberculin in 575 cattle in 34 districts were found to be caused not by *Mycobacterium bovis,* but by *Mycobacterium avium* or atypical mycobacteria present in the environment.

According to Kuker and Zettl (1964), Beerwerth and Popp (1971), Kauker and Rheinwald (1972, Kleeberg and Nel (1973) the major source of mycobacteria involved in the tuberculosis in pigs is sawdust. The presence of mycobacteria in bedding was also pointed out by Piening et al. (1972) who isolated 67 strains, three of them having the properties of *M. avium,* from sawdust used as bedding for pigs.

Beerwerth and Popp (1971), studying the mode of contamination of sawdust with mycobacteria, demonstrated a majority of group III and group IV atypical mycobacteria in samples originating from wood that had laid in wood stores for a considerable length of time before processing. On examination of 225 sawdust samples collected at local saw mills they isolated 3 strains of *Mycobacterium intracellulare* serotype 8 which is most important for pigs from the epizootiological point of view. Group III mycobacteria having the properties of *M. intracellulare* serotypes

4, 6, 8 and 9 were often demonstrated by the same investigators also in sawdust samples collected from stores on pig farms with evidence of tuberculosis.

A considerable degree of sawdust contamination with *M. avium* serotype 2 was demonstrated by Kauker and Rheinwald (1972). Having examined 52 sawdust samples, they isolated 7 strains of avian mycobacteria and suggested that the sawdust was contaminated by free-living birds particularly crows, starlings, sparrows, pigeons, ducks, herons, owls, hawks and eagles.

The possibility of *M. avium* and *M. intracellulare* replication and possibly survival in sawdust was investigated by Schliesser and Weber (1973). They found that *M. avium* retained its viability in sawdust at 37 °C for 42 days and *M. intracellulare* for 63 days. At the environmental temperature of 18—22 °C avian mycobacteria survived in sawdust for 160 days and *M. intracellulare* for 214 days. From these results it emerged that, in contrast to some opposite views, sawdust was not a suitable medium for the replication of *M. avium* and *M. intracellulare.*

From the pertinent literature it appears that sawdust may be a frequent source even of pathogenic mycobacteria for pigs, poultry and possibly other animal species. Information, however, is lacking on the incidence of mycobacteria in other kinds of bedding such as straw which is used as bedding material in some herds to improve the microclimatic conditions, particularly in conventional barns for breeding pigs. With this in view, we studied the incidence of mycobacteria not only in sawdust, but also in other bedding materials, namely straw and hay, to elucidate their involvement in the occurrence of tuberculosis among, pigs, cattle and other species of domestic animals.

Materials and Methods

Samples of sawdust, straw and hay, weighing approximately 50 g, were thoroughly mixed with 200 ml of sterile saline and allowed to stand at room temperature overnight. The solution was then decanted into a centrifuge tube. After centrifugation 10 ml of a 4 per cent solution of sodium lye was added to the sediment and thoroughly mixed. The resultant suspension was contrifuged 15 minutes later. The supernatant was decanted and the sediment was mixed with 10 ml of 5 per cent oxalic acid. The suspension was centrifuged 15 minutes later and immediately afterwards the sediment was inoculated into Petragnani's and Stonebrink's solid egg media and Šula's liquid medium. The culture media were incubated at 25 and 37 °C for 8 weeks. The growth was read at 3 days and 2, 4 and 8 weeks.

The primary cultures of mycobacteria were transferred to Petragnani's, Šula's and Löwestein's media in which colonial morphology, pigmentations and growth rate were assessed. The mycobacterial cultures were examined by serotyping according to Schaefer (1965) and by bioassay on pullets, rabbits and white mice. Strains that could not be determined by serotyping were examined by biochemical tests (Kubica 1973). The nitrate reduction test was carried out according to Virtanen (1960) and Sytařová (1964). The picric acid reduction test was carried out according to Tsukamura (1961). The other biochemical tests employed were the quantitative catalase test (Pavlas 1975), the amidase activity test (Bönicke 1960) and the NaCl sensitivity test on solid egg media containing 5 per cent sodium chloride. The test for Tween 80 hydrolysis described by Wayne et al. (1964) and Wayne and Doubek (1965) was modified in that neutral red was added to the mycobacterial suspension immediately before the reaction was read, which facilitated the reading of the colour reaction due to neutral red.

The examination of the mycobacterial cultures by bioassay was carried out on pullets aged 4 to 6 weeks. They were inoculated intramuscularly with the mycobacterial suspension in 5 mg amounts of semiwet mass culture suspended in 0.5 ml saline. If the pullets did not die within 4 to 5 weeks, they were tuberculin-tested intradermally with avian PPD tuberculin and examined serologically with antigens prepared from *M. avium* and M. *intracellulare.* They were slaughtered 7 to 8 weeks after experimental infection and subjected to patho-anatomical and bacteriological examination.

Within the aforementioned study a total of 155 samples of sawdust, straw and hay were examined on 52 pig and cattle farms where positive reactions were found to avian or mammalian PPD tuberculins, while the comprehensive examination did not detect infection with mammalian pathogenic mycobacteria.

Results

Of 94 sawdust samples subjected to bacteriological examination for the presence of mycobacteria on 28 farms, 39 samples from 15 farms were positive. Straw samples

collected on 19 farms were positive for mycobacteria on 15 farms. Practically the same applied to hay samples where mycobacteria were demonstrated on 4 out of 5 farms examined (Table 1). The proportions of positive samples amounted to half of the straw and hay samples and to 41 per cent of the sawdust samples.

The most important finding from the epizootiological point of view was the demonstration of nine strains of *M. intracellulare* (serotypes 8, 4, 6 and 9) and of one strain of *M. avium* (serotype 1) in samples of sawdust and wood shavings, which accounted for a quarter of all these samples examined, no matter whether they were collected in wood-processing plants or from stores on the farms. The other mycobacterial species isolated from sawdust and wood shavings were 2 strains of *Mycobacterium scrofulaceum*, 5 strains of *Mycobacterium terrae* and 1 strain each of *Mycobacterium triviale*, *Mycobacterium chelonei* and *Mycobacterium gastri* (Table 2). Half of the mycobacterial strains could not be classified on the basis of serotyping and further examination. Most of these strains, however, sensitized laboratory animals

Table 1
Bacteriological examination of sawdust, straw and hay for mycobacteria

No.		Kind of sample	Results of culture for mycobacteria			
			No. positive		No. negative	
farms	samples		farms	samples	farms	samples
28	94	sawdust	15	39	13	19
19	53	straw	15	27	4	12
5	8	hay	4	4	1	2

Table 2
Typing of mycobacteria isolated from sawdust, straw and hay

No. isolated from	mycobacterial strains Species as determined by serotyping								Biochemical examination of strains not classified by serotyping	
		M. avium	M. intracellulare	M. sorofulaceum	M. fortuitum	M. terrae	not classified	Auto-agglutination	total	classified as
sawdust and wood shavings	39	1	9	2	1	4	16	6	4	1 M. triviale 1 M. terrae 1 M. chelonei 1 M. gastri
straw	27		1			13	6	7	11	1 M. triviale 6 M. terrae 3 M. gordonae 1 M. flavescens
hay	4					3	1		1	1 M. gordonae
	70 100 %	1 1.4 %	10 14.3 %	2 2.8 %	1 1.4 %	20 28.6 %	23 32.9 %	13 18.6 %		

to avian and mammalian PPD tuberculins without producing lesions in the organs.

Straw samples yielded 27 mycobacterial strains, half of which showed the properties of *M. terrae* and one was classified on serotyping as *M. intracellulare* serotype 8. Of the remaining 11 mycobacterial strains that could not be determined by serotyping 6 strains showed the properties of *M. terrae*, 3 those of *Mycobacterium gordonae*, 1 those of *M. triviale* an 1 exhibited the properties of *Mycobacterium flavescens*.

In elucidating positive reactions to avian PPD tuberculin in young cattle in a large-capacity rearing enterprise, we examined 4 samples of hay from this farm. Bacteriological examination of these samples for the presence of mycobacteria yielded 4 strains, three of which showed the properties of *M. terrae* and 1 strain those of *M. gordonae*. When tested for allergenic properties, the isolated strains were found to sensitize experimental animals to avian PPD tuberculin.

On the other hand, isolation attempts from 17 samples of water collected directly from the water pipeline on the farms yielded only one strain of atypical mycobacteria which could not be classified.

Discussion

From the present study of the involvement of the external sources of tuberculosis and of atypical mycobacteria affecting the results of tuberculin testing in cattle it appears that sawdust is of the greatest epizootiological significance among the bedding materials examined. The fact that one quarter of the samples of sawdust and wood shavings yielded *M. intracellulare* serotypes 4, 6, 8 and 9 and one strain of *M. avium* serotype 1 suggests that sawdust poses a hazard particularly on pig farms where it is used for improvement of animal hygiene conditions. The results are in keeping with the observations reported by Kauker and Zettl (1964), Berwerth and Popp (1971), Kauker and Rheinwald (1972) a.o.

In view of the virulence of *M. intracellulare* for pigs, in which active excretion in the faeces is very rare, external source of these mycobacteria should be regarded as one of the most important causes of the incidence of caseous findings in the submandibular, postpharyngeal or intestinal lymph nodes. On the other hand, cattle and poultry infected naturally with *M. intracellulare* or *M. terrae* and other atypical mycobacteria generally show reactions to avian or mammalian tuberculin.

As to the epizootiological role of straw as a possible external source of tuberculosis, it can be concluded that straw is very rarely involved in the incidence of mycobacteriosis in pigs. Nevertheless, the large number of atypical avirulent mycobacteria in straw as well as in hay suggests that both of them may be involved in the occurrence of non-specific reactions to avian tuberculin particularly in cattle.

Výskyt mykobaktérií v pilinách, slámě, seně a jejich epizootologický význam

Při studiu výskytu mykobaktérií v pilinách a slámě používaných ke stlaní v chovech prasat a skotu bylo vyšetřeno 147 vzorků shora uvedeného steliva ve 47 lokalitách.

Z epizootologického hlediska byl nejzávažnější nález devíti kmenů *Mycobacterium intracellulare* (serotyp 4, 6, 8 a 9) a jednoho kmene *Mycobacterium avium* (serotyp 1) ve vzorcích pilin a hoblin, což představovalo 1/4 všech uvedených vyšetřovaných

vzorků a to bez ohledu, zda byly odebrány přímo ve dřevozpracujících závodech nebo ze skladů na farmách.

Vzhledem k virulenci *Mycobacterium intracellulare* pro prasata, u nichž aktivní vylučování je zcela ojedinělé, je třeba uvedené stelivo pokládat za jeden nejzávažnějších zdrojů mykobakteriózy prasat.

Vysoký počet atypických avirulentních mykobaktérií ve slámě a seně svědčí pro možnost podílu tohoto druhu steliva a objemné píce na výskytu nespecifických reakcí na ptačí, případně savčí tuberkulin a to zejména u skotu.

Наличие микобактерий в опилках, соломе и сене и их эпизоотологическое значение

В ходе исследований наличия микобактерий в опилках и соломе, используемых в свиноводстве и скотоводстве для подстилки было обследовано в 47 местах 147 образцов вышеприведенной подстилки.

С эпизоотологической точки зрения важнейшим было выявление девяти штаммов *Mycobacterium intracellulare* (серотип 4, 6, 8 и 9) и одного штамма *Mycobacterium avium* (серотип 1) в образцах опилок и стружки, что представляло собою 1/4 всех приведенных исследуемых образцов несмотря на то, проходил ли их отбор непосредственно на деревообрабатывающих заводах или на складах ферм.

Учитывая вирулентность *Mycobacterium intracellulare* для поросят, у которых активное выделение весьма редко, необходимо приведенную подстилку считать одним из важнейших источников заболевания микобактериозом у поросят.

Большое число нетипичных, авирулентных микобактерий в соломе и сене свидетельствует о возможности определенной роли данного вида подстилки и грубого корма в наличии неспецифических реакций на туберкулин птиц или млекопитающих, в особенности у крупного рогатого скота.

References

BEERWERTH, W. — POPP, K.: Zur epizootologischen Bedeutung der Sägemehleinstreu für das Auftreten der Schweinetuberkulose. Zbl. Vet. Med., B, **18,** 1971: 634—645.

BÖNICKE, R.: Classification of Mycobacteria by means of chemical tests. Bull. Un. Intern. Tuberc., **28,** 1960: 153—156.

KAUKER, E. — RHEINWALD, W.: Untersuchungen über das Vorkommen atypischer Mykobakterien der Gruppe III nach Runyon in Einstreu (Sägemehl) und Futter von Schweinen in Nordhessen. Berl. Münch. tierärztl. Wschr., **85,** 1972: 384—387.

KAUKER, E. — ZETTL, K.: Beitrag zur käsigen Lymphknotentzündung der Schweine. Berl. Münch. Tierärztl. Wschr., **77,** 1964: 173—176.

KLEEBERG, H. — NEL, E.: Occurrence of environmental atypical Mycobacteria in South Africa. Ann. Joc. belge Med. trop., **53,** 1973: 405—418.

KUBICA, G.: Differential identification of mycobacteria VII. Key features for identification of clinically significant mycobacteria. Am. Rev. resp. Dis., **107,** 1, 1973: 9—21.

PAVLAS, M.: Aktywnosc katalazy Mycobacterium avium niefotochromogennych mykobakterii izolowanych od swin. Zeszyty problem. postep. nauk rolnicz., **164,** 1975: 151—158.

PIENNING, G. — ANZ, W. — MEISSNER, G.: Serotyp Bestimmung und ihre Bedeutung für epidemiologische Untersuchungen der Schweinetuberkulose in Schleswig-Holstein. Dte tierärztl. Wschr., **13,** 79, 1972: 316—321.

SCHAEFER, W. B.: Serologic identification and classification of the atypical mycobacteria by their agglutination. Am. Rev. resp. Dis., **92,** 1965: 85—93.

SCHLIESSER, TH. — WEBER, A.: Untersuchungen über die Tenazität von Mykobakterien

der Gruppe III nach Runyon in Sägemehleinstreu. Zbl. VetMed., B, **20,** 1973: 710–714.
SYTAŘOVÁ, J.: Použití nitrátového testu k identifikaci Mycobacterium bovis. Rozhl. Tuberk., **24,** 1964: 582–592.
TSUKAMURA, M.: Enzymatic reduction of picric acid by mycobacteria. Am. Rev. resp. Dis., **84,** 1961: 87–89.
VIRTANEN, J.: A study of nitrate reduction by mycobacteria. Acta tuberc. scand., **48,** 1960: 1–5.
WAYNE, L. G. – DOUBEK, R. J. – RUSSEL, R. L.: Classification and identification of mycobacteria: I. Tests employng Tween 80 as substrate. Am. Rev. resp. Dis., **90,** 1964: 588–597.
WAYNE, L. G. – DOUBEK, R. J.: Classification and identification of mycobacteria. Am. Rev. resp. Dis., **91,** 1965: 738–745.