

DYNAMICS OF EXCRETION OF *M. AVIUM* AND *M. INTRACELLULARE* IN  
FAECES OF EXPERIMENTALLY INFECTED PIGS

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Received June 23, 1987

Abstract

Pavlas M.: Dynamics of Excretion of *M. avium* and *M. intracellulare* in Faeces of Experimentally Infected Pigs. Acta vet. Brno, 56, 1987: 337-342.

The dynamics of the excretion of mycobacteria in faeces was investigated in 44 pigs of the breed Large White x Landrace, infected orally with a single dosis of the mycobacterial cultures of *M. avium* and *M. intracellulare*. Out of 17 animals infected with *M. avium* serotype 2, 3, pathogenic mycobacteria were found in the faeces of 14 animals as early as 24 hrs p. i. Passive excretion lasted two days. After this period, bacteriological examination for the presence of mycobacteria showed negative results for the next three weeks. The highest rate of active excretion was recorded 6 weeks p. i. when the mycobacteria were excreted by 10 animals /59 %/. Three months after the infection of animals with *M. avium*, the pathogens were excreted by 17.6 % of animals.

In the infection of pigs with *M. intracellulare*, the active excretion in the faeces lasted for 3 - 8 weeks. Having investigated the excretion of mycobacteria in the faeces we found that both the amount and the period of active excretion depended on the virulence and infective dosis of mycobacteria.

*Mycobacterium avium*, *Mycobacterium intracellulare*, experimental infection, pigs, mycobacteria in faeces.

Apart from external sources of tuberculosis, horizontal transmission can be involved in pig infections, too. Active excretion of pathogenic mycobacteria in pig faeces can occur especially in specific inflammations of the intestinal mucosa, in sporadic cases in animals with an organ form of tuberculosis, e. g. in diseases of the liver and kidney.

The possibilities of active excretion of mycobacteria in the pig faeces were pointed out by Zunker (1962), Brehmer

(1964) and Beerwerth (1967). Pathogenic mycobacteria were in the pig faeces mostly represented by *Mycobacterium avium* and *Mycobacterium intracellulare* (Piening et al. 1982). The excretion of mycobacteria after oral infection of avian tuberculosis in pigs with a suspension of poultry organs and the possibility of horizontal transmission of the infection in pigs were demonstrated by Hejliček and Tremel (1975). Sixteen normal control pigs were in contact with experimentally infected ones and positive allergic reaction to tuberculin was recorded in the majority of them three months after the exposure (Tremel 1975). Out of 14 mycobacterial strains isolated from the pig faeces (Engel et al. 1978) demonstrated the properties of *M. avium* serotype 3,9 in 2 strains, those of *M. intracellulare* serotype 8 in 11 strains and those of *M. intracellulare* serotype 4 in 1 strain.

Active excretion of *M. avium* was also demonstrated by Jørgensen (1978) in 13 pigs infected experimentally with a dose of 0.5 mg of mycobacterial culture administered orally in the succession of 5 days. Having examined the faeces by cultivation, the author demonstrated the excretion of *M. avium* in all experimental animals during days 16-65 after the last infection. The highest occurrence of mycobacteria in the faeces was on day 40 after the beginning of excretion.

On examination of the faeces during 17-135 days after the last infection no mycobacteria were found in 15 out of 16 experimental pigs. Morphological changes were in orally infected animals situated mainly on the intestinal mucosa in the site of Peyer's patches (in the form of ulceration) and in the mesenteric lymph nodes (in the form of caseification).

## Materials and Methods

The investigation of the dynamics of excretion of the strains of *M. avium* serotype 2, 3 and *M. intracellulare* serotype 6, 8 was conducted using 44 pigs of the BU x L breed. The animals were infected by a single oral dose of suspension of the above-mentioned mycobacteria in saline (0.01 - 0.1 and 1 mg of semiwet weight of mycobacterial culture per 1 kg live weight). The doses were administered in the volume of 2-3 ml on the root of the tongue using a syringe in which the needle had been replaced by a thick rubber tube 15 cm long and 10 mm in diameter.

The strain of *M. avium* serotype 2, 3 was administered to 17 pigs as a single dose of 0.1 mg of mycobacterial culture per kg live weight. *M. intracellulare* serotype 4, 8 was administered to 27 pigs divided into 3 groups of nine. The individual groups were infected by semiwet weight of mycobacterial culture administered in a single dose of 0.01 - 0.1 and 1 mg of bacterial mass in the saline suspension per kg live weight. The faeces of experimentally infected pigs were collected from the rectum using cotton swabs which were following the collections macerated in sterile saline. The supernatant of the suspension was decanted into a centrifugation tube. After centrifugation 7 ml of a 4 per cent solution of sodium lye were added and thoroughly mixed through shaking. The resultant suspension was centrifuged 15 minutes later. The supernatant was decanted and the sediment

was mixed with 5 per cent oxalic acid (Beerwerth 1967). After a 15 minute exposure the suspension was centrifuged and the upper layer of the sediment was inoculated into culture media, namely into Petraghani's and Stonebrink's solid egg media. The culture media were incubated in a thermostat at 37 °C for two months. The isolated cultures were examined by serotyping (Schaefer 1965). The biochemical tests employed were the quantitative catalase test (Pavlas 1975), the nitrate reduction test (Virtanen 1960), the Tween 80 hydrolysis test (Wayne and Doubek 1965), and the NaCl sensitivity test on solid egg media containing 5 per cent sodium chloride.

When assessing the patho-morphological changes in the experimentally infected animals, our attention focused on those in the lymph nodes, parenchymatous organs, intestinal mucosa and wall. Samples for cultivation were collected from both macroscopically changed and unchanged lymph nodes including parenchyma of the organs. The tissue was homogenized in a mortar and then decontaminated using 1 n HCl and neutralized using NaOH.

## Results

On investigation of the excretion dynamics of *M. avium* serotype 2, 3 in the faeces of experimentally infected pigs we found that passive excretion of mycobacteria started as early as 24 hrs after infection. The pathogens were demonstrated in the faeces of 14 (82.3 %) out of 17 animals infected with avian mycobacteria, namely as early as 24 hrs after infection. The amount of passively excreted mycobacteria decreased markedly during two days. In this period, *M. avium* was demonstrated by cultivation in examined samples of faeces originating from one pig only. From the third day p.i., the cultivation finding on examination of faeces samples was negative for mycobacteria for three weeks. Active excretion of *M. avium* in the faeces of experimentally infected animals was recorded in 11.6 % of animals after 4 weeks, with a peak at 6 weeks p.i., when the mycobacteria were excreted by 10 pigs (59 %) (Fig. 1). Three months p.i. the pathogens were excreted by 3 pigs (17.6 %) in their faeces. Tuberculous changes in the mesenteric submandibular and lymph nodes were recorded in the pigs infected with *M. avium*, in one animal also in the liver parenchyma. Besides these, specific changes of the intestinal mucosa in the site of Peyer's patches were found in 4 pigs excreting *M. avium* in their faeces.

Further stage of the experiments was designed to reveal to which extent the pigs infected with less virulent mycobacteria (*M. intracellulare* being their major representative) contribute to horizontal transmission. In order to judge the active excretion of *M. intracellulare* serotype 4, 8, three groups of pigs were orally infected. The first group received a dosis of 0.01 mg of mycobacterial culture per kg body mass. Passive excretion of *M. intracellulare* in the faeces was demonstrated within 3 days p.i. with a peak on the first day p.i. Then a resting stage followed which lasted 3 weeks similarly to that after infection with *M. avium*. Two out of 27 infected animals excreted mycobacteria in

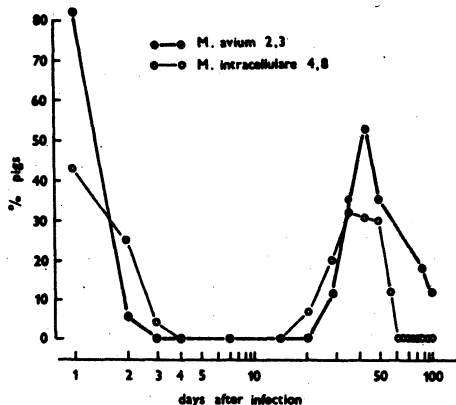


Fig. 1. Excretion of mycobacteria in swine faeces

their faeces after 21 days. The highest percentage of positive results were recorded 5 weeks p.i., when the mycobacteria were demonstrated in one third of animals infected with *M. intracellulare*. Opposite to the group infected by *M. avium*, the excretion of *M. intracellulare* was completed as early as 8 weeks p.i., when three animals were found to be positive for *M. intracellulare*.

During the entire period that followed, i.e. up to 99-116 days after experimental infection, no excretion of mycobacteria was recorded on examination of the faeces.

Our investigation of the *M. intracellulare* excretion in the individual groups of pigs revealed that the excretion of pathogens decreased markedly together with a decreased infective dosis. While maximal excretion of *M. intracellulare* was at the dosis of 0.1 mg of culture per kg body mass demonstrated in 11.1 % of experimentally infected pigs, at the dosis of 0.01 mg of bacterial mass per kg body mass the examination of faeces did not reveal any active excretion throughout the entire experiment.

### Discussion

In agreement with the results by Jorgensen, the maximal active excretion in the faeces was demonstrated after oral infection of pigs with the mycobacteria of the group *M. avium* - *M. intracellulare*, administered in a single dosis, after 35 - 42 days p.i. The achieved results contributed to our hitherto knowledge in that the intensity of active excretion depends on the infective dosis and the virulence of mycobacteria for pigs. The virulence varies markedly especially in the individual strains of *M. avium*. In the practice, when usually smaller infective doses operate (compared with the amount of mycobacteria administered in experimental infections), the opportunity of excretion in spontaneously infected pigs will also vary to a considerable extent.

Our results suggest that the success of health-control schemes introduced in pig herds (using the method of elimination) will depend on whether the possibility of massive infection has been excluded and, at the same time, on the zoohygienic standard. In infections of pigs caused by pathogenic mycobacteria in the amount on the border of threshold infective dosis, the active excretion of mycobacteria in the faeces does not - as a rule - occur, even if the results of allergenic reactions to tuberculin are positive. On the other hand, the excretion of mycobacteria in pig herds with an occurrence of changes in the parenchymatous organs is usually not limited by time. Thus, the possibility to free the herd from tuberculosis using the elimination method remains without radical depopulation and thorough sanitation unsuccessful.

#### Dynamika vylučování *Mycobacterium avium* a *M. intracellulare* trusem experimentálně infikovaných prasat

Dynamika vylučování mykobaktérií trusem byla sledována po jednorázové perorální infekci 44 prasat plemene BU x L kmeny mykobaktérií komplexu *M. avium*-*intracellulare*. Ze 17 zvířat infikovaných *M. avium* sérotyp 2, 3 byly prokázány patogenní mykobaktérie v trusu 14 prasat již za 24 hodin po infekci. Pasivní vylučování trvalo 2 dny. Po této době byl výsledek bakteriologického vyšetření na mykobaktérie negativní po dobu 3 týdnů. Maximum celkového vylučování bylo zaznamenáno za 6 týdnů po infekci, kdy mykobaktérie vylučovalo 10 prasat, tj. 59 % zvířat. Za 3 měsíce po infekci prasat *M. avium* bylo aktivní vylučování trusem zjištěno u 17,6 % zvířat.

Při infekci prasat *M. intracellulare* bylo zaznamenáno aktivní vylučování trusem v období 3 - 8 týdnů. Při sledování vylučování mykobaktérií trusem bylo zjištěno, že množství i doba aktivního vylučování u prasat závisela na virulenci a infekční dávce mykobaktérií.

#### Динамика выделения *Mycobacterium avium* и *Mycobacterium intracellulare* испражнениями экспериментально инфицированных свиней

Проводились исследования динамики выделения микобактерий после однократной пероральной инфекции 44 свиней породы BU x L 17 животных, инфицированных *M. avium* серотип 2, 3, патогенные микобактерии были установлены в испражнениях 14 свиней уже через 1 сутки после инфекции. Пассивное выделение длилось 2 суток. По истечении указанного периода результаты бактериологических исследований на микобактерии были негативным в течение 3 недель. Максимум общего выделения было установлено через 6 недель после инфекции, когда микобактерии выделяли 10 свиней, т.е. 59% животных. Через 3 месяца после инфекции свиней *M. avium* было установлено активное выделение испражнениями у 17,6% животных.

При инфекции свиней *M. intracellulare* активное выделение испражнениями отмечалось в период 3-8 недель. В ходе исследований микобактерий испражнениями было установлено, что количество и время активного выделения у свиней зависели от вирулентности и инфекционной дозы микобактерий.

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