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EXPERIMENTAL INFECTION WITH MYCOBACTERIUM AVIUM, SEROTYPE 2, IN PIGS

3. ORAL INFECTION WITH SMALL DOSES OF M. AVIUM*

By

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JØRGENSEN, J. BERG: *Experimental infection with Mycobacterium avium, Serotype 2, in pigs. 3. Oral infection with small doses of M. avium.* Acta vet. scand. 1978, *19*, 49—57. — Pigs were inoculated orally with Mycobacterium avium in doses ranging from 15.6×10^2 to 15.6×10^6 viable units daily for 15 days (Table 1). The animals were necropsied 31—32 days after the last inoculation.

Pigs given doses of 15.6×10^6 and 15.6×10^5 viable units showed delayed hypersensitivity to avian tuberculin 24 days after the last inoculation (Table 2). The pig inoculated with 15.6×10^6 viable units showed macro- and/or microscopic lesions of the intestines and the liver, and of the mandibular, mesenteric and hepatic lymph nodes. Cultures showed growth of M. avium from the same tissues and from the spleen and the left tracheobronchial lymph node. The pig inoculated with 15.6×10^5 viable units showed a pronounced granulomatous infiltration in the tonsils and the mesenteric lymph nodes. Growth of M. avium was obtained from the tonsils, the intestinal mucosa (Peyer patch) and the mandibular and mesenteric lymph nodes. Viable unit counts were high in the tonsils and in the mesenteric lymph nodes (Tables 3 and 4).

Lower doses gave rise to a minimal tissue reaction and/or very low viable unit counts, and are not considered to be capable of producing a progressive tuberculosis.

Mycobacterium avium, Serotype 2; pathogenicity; oral inoculation; large doses; pigs.

While literature about Mycobacterium (M.) avium infection in pigs includes a number of papers on oral infection experiments with large doses of cultured organisms (*Griffith* 1911, *Kauker &*

* This report is the result of a project planned and carried out in cooperation with dr. H. Chr. Engbæk and dr. A. Jespersen, both of the Tuberculosis Department, Statens Seruminstitut, Copenhagen, Denmark.

Zettl 1964, *Jørgensen* 1977 b), reports on experiments with small doses, i.e. doses of about 1 mg or less, are missing. The present work is a continuation of a series of experiments with doses ranging from 2.5 to 180 mg (*Jørgensen* 1977 b), in which it was found that even the smallest dose, i.e. 18.5×10^6 viable units daily for 5 days, caused microscopic tuberculous lesions and high viable unit counts in the tissues.

The chief purpose of the present work was to find the smallest oral dose of M. avium capable of causing tuberculous lesions and multiplication of the organisms.

MATERIAL AND METHODS

Experimental animals. Six SPF pigs, littermates, age, sex and weight as indicated in Table 1.

Inoculation material. M. avium, strain SCC 1336 = ATCC 25291 (*Engbæk et al.* 1971). The procedure for standard culture preparation, viable unit counts, and checking of colony morphology has been described earlier (*Jørgensen* 1977 a). Since previous experiments (*Jørgensen* 1977 b) had shown that divided doses were superior in effect to a single dose with the same total number of viable units, it was decided to give 15 inoculations over a period of 3 weeks. Dilutions were made from a standard culture with 66×10^6 viable units per ml, and the daily doses were: 15.6×10^6, 15.6×10^5, 15.6×10^4, 15.6×10^3 and 15.6×10^2 viable units. Colonies were almost exclusively smooth-transparent (SmT).

Inoculation. One pig was inoculated at each dosage level except 15.6×10^3, where 2 pigs were used. The culture, suspended in skimmilk, was administered individually by mouth. Table 1 gives the daily and total doses.

Tuberculin tests with avian and human PPD tuberculins, 1000 t.u. per 0.1 ml, were performed and evaluated as described previously (*Jørgensen* 1977 a). The animals were tested before the start of the experiment and 24 days after the last inoculation, i.e. 42 days after the first inoculation.

Clinical observations. The pigs were weighed once a week and their appetite and general condition noted daily.

Duration of the experiment. The pigs were killed and necropsied 31—32 days after the last inoculation, i.e. 49—50 days after the first inoculation.

Post-mortem examinations included macroscopic and histopathological examination (*Jørgensen* 1977 a) and culture (*Jørgensen* 1977 b). The materials examined are listed in Table 4.

RESULTS

Clinical observations. No clinical effect was observed. Weight gains are recorded in Table 1.

Table 1. Survey of experimental animals, dosage and weight gains.

Pig No.	Sex	Age, days	Weight, kg	Weight gain, kg	Dosage, in viable units total 234 ×	Dosage, in viable units daily for 15 days 15.6 ×
113	♂	91	32.5	32.5	10^2	10^2
114	♂	91	33.5	28.5	10^3	10^3
115	♀	91	31.0	24.0	10^3	10^3
116	♀	91	25.0	29.5	10^4	10^4
117	♀	91	31.0	32.0	10^5	10^5
118	♀	91	29.5	29.0	10^6	10^6

Tuberculin tests. The pigs showed no reaction before inoculation. At 24 days after the last inoculation the 2 pigs on the largest doses were sensitive. Pig 117, dose 15.6×10^5, reacted to avian tuberculin only, while Pig 118, dose 15.6×10^6, reacted to human tuberculin as well, though with the strongest reaction to avian tuberculin. The reactions, especially the erythema, were strongest after 24 hrs. (Table 2).

Table 2. Comparative tuberculin tests with PPD avian and human, 1000 t.u. per dose, 24 days after the last inoculation.

Pig No.	Increase in skin-fold thickness, mm 24 hrs.* avian	24 hrs.* human	Erythema, diameter in mm 24 hrs. avian	24 hrs. human	48 hrs. avian	48 hrs. human	72 hrs. avian	72 hrs. human
117	1.5	0	13.1	0	13.0	0	0	0
118	1.0	1.0	15.2	10.6	14.2	9.2	12.0	8.5

* No reactions were observed after 48 and 72 hrs.
Pigs Nos. 113, 114, 115 and 116 showed no reaction.

Pathology. Gross lesions were seen only in Pig 118, dose 15.6×10^6. A few pin-point to miliary whitish nodules were found in the mandibular and mesenteric lymph nodes and in the liver.

The Peyer patches of the ileum were hyperplastic, with scattered miliary, caseated nodules.

Histopathology (Table 3). Pig 113 (dose 15.6×10^2) had a few typical epithelioid and giant cell granulomas in the mandibular lymph nodes (Fig. 1). Pigs 114 and 115 (dose 15.6×10^3) were negative. In a Peyer patch of Pig 116 (dose 15.6×10^4) a single giant cell was found. Pig 117 (dose 15.6×10^5) had tuberculous granulomas in the tonsils and in the mesenteric lymph nodes,

Table 3. Pathology and histopathology.

	Pig No. and daily dosage					
Tissue	113 15.6×10^2	114 15.6×10^3	115 15.6×10^3	116 15.6×10^4	117 15.6×10^5	118 15.6×10^6
Lymph node No.*	(1)	0	0	0	(11)	1, 11 (1, 11, 13)
Liver	0	0	0	0	0	+ (+)
Tonsil	0	0	0	0	(+)	0
Intestinal mucosa (Peyer patch)	0	0	0	(+)	0	+ (+)
Spleen, lung and kidney	0	0	0	0	0	0

* For lymph node No., cf. Table 4.
Figures indicate lymph nodes with lesions.
Figures in brackets indicate microscopic lesions.
\+ = Macroscopic lesions.
(+) = Microscopic lesions.

most pronounced in the latter. Pig 118 (dose 15.6×10^6) had tuberculous granulomas in the mandibular, mesenteric and hepatic lymph nodes and in the liver. Areas of caseation with connective tissue demarcation were found in the mesenteric and mandibular lymph nodes. The lymphoid tissue of the Peyer patches showed granulomatous infiltration, with scattered areas of caseation, and even ulcers, in which numerous acid-fast organisms were found (Fig. 2).

Culture. The results are shown in Table 4. Pig 113 (dose 15.6×10^2) and Pig 114 (dose 15.6×10^3) were negative. In Pig 115 (dose 15.6×10^3) the result was positive for the posterior mesenteric lymph nodes, in Pig 116 (dose 15.6×10^4) for the hepatic

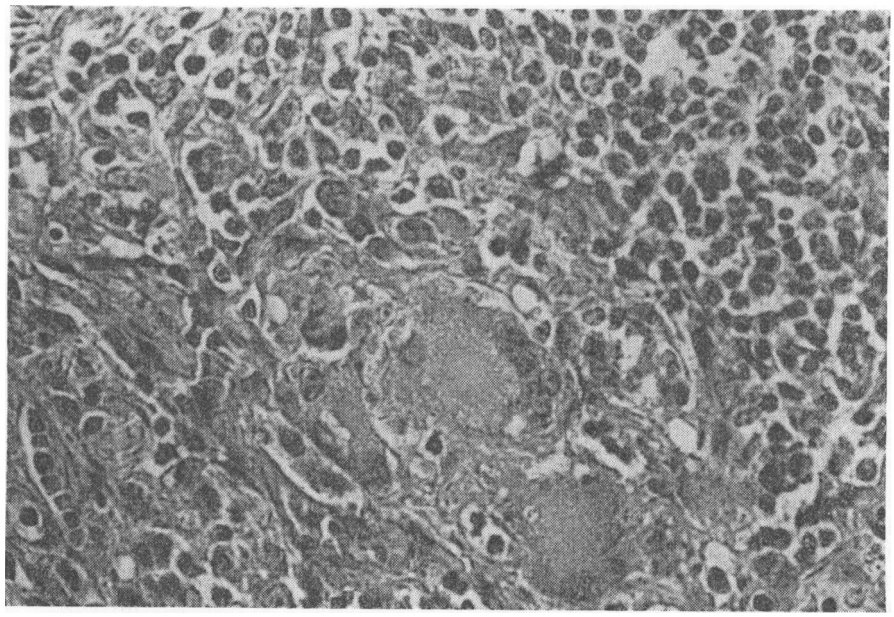

Figure 1. Pig 113. Section af mandibular lymph node. Micrograuloma consisting of typical epithelioid and giant cells. Magnification: approx. 480 ×. Staining: Haemalum-eosin.

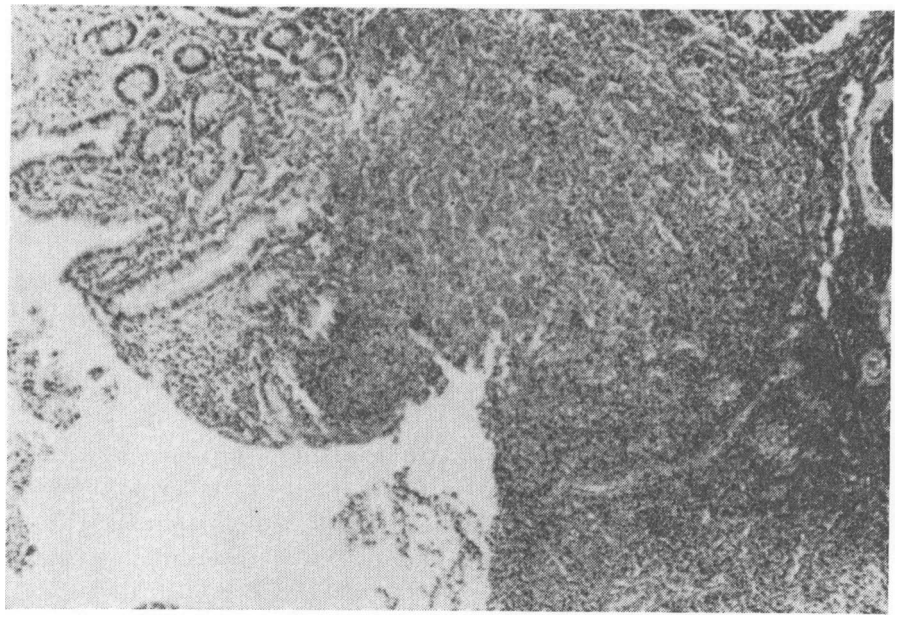

Figure 2. Pig 118. Section of Peyer patch with caseation and ulceration. Magnification: approx. 60 ×. Staining: van Gieson.

Table 4. Results of culture 31—32 days after the last inoculation.

No.	Tissue	Pig No. and daily dosage					
		113 15.6×10^2	114 15.6×10^3	115 15.6×10^3	116 15.6×10^4	117 15.6×10^5	118 15.6×10^6
1	Ln. mandibularis					20	80
2	„ parotideus						
4	„ retropharyngeus medialis						
11	„ mesentericus I					300	20000
11	„ „ II						143000
11	„ „ III			100		∞	19000
12	„ tracheobronchal. sin.						20
13	„ hepaticus				720		2670
14	Spleen						10
15	Liver						30
16	Lung						
17	Kidney						
19	Musculus longissimus dorsi						
25	Tonsil					367000	
27	Intestinal mucosa (Peyer patch)					40	20
28^{I}	Intestinal content						
28^{II}	Intestinal content						

Blank space = No growth.
Figures indicate viable units per 0.5 g tissue.
∞ = > 400000 units.

lymph nodes. In Pig 117 (dose 15.6×10^5) cultures were positive from tonsils and Peyer patches, and from mandibular and mesenteric lymph nodes, corresponding to a complete primary complex. Pig 118 (dose 15.6×10^6) showed infection of the Peyer patches, spleen, liver, and the mandibular, mesenteric, tracheobronchial and hepatic lymph nodes. The intestinal contents were negative in culture.

DISCUSSION

In many cases of natural M. avium infection in pigs, the animals have presumably been exposed to small or moderate doses of infection over a shorter or longer period through contact with tuberculous poultry, wild birds (*Plum* 1942), tuberculous pigs, or infected feed and litter. *Jørgensen* (1977 b) demonstrated the enhancing effect of extending the inoculations over 5 days. In the present work 5 inoculations were given weekly for 3 weeks at 5

different dosage levels, corresponding to a total dose of, respectively, 234×10^6, 234×10^5, 234×10^4, 234×10^3 and 234×10^2 viable units.

Post mortem, the pig infected with the largest dose showed gross lesions of the Peyer patches, the mandibular and mesenteric lymph nodes, and the liver. Histological examination verified these findings and showed tuberculous granulomas in the hepatic lymph nodes too. The lesions of the Peyer patches were of granulomatous-caseating nature with ulcer formation in the propria mucosae. The finding of numerous acid-fast rods in these lesions by histological examination is suggestive of an excretion of M. avium with the faeces. Viable unit counts were high for the mesenteric lymph nodes, but very low for the intestinal mucosa, possibly because of the decontaminating treatment, which is apt to reduce the number of viable organisms by 50—90 %.

The second largest dose caused a granulomatous inflammation of the tonsils and the mesenteric lymph nodes, with high viable unit counts, and culture showed infection of the intestinal mucosa and the mandibular lymph nodes as well.

The 3 smallest doses, having caused a minimal tissue reaction (Table 3) and a very low-grade infection of the organs (Table 4), would hardly have been capable of producing a tuberculous infection with gross lesions even after a prolonged period of observation. It should be noted that even the smallest dose (15.6×10^2 units) gave rise to microscopic epithelioid tubercles in the mandibular lymph node. However, the failure to recover the organisms in culture would seem to indicate that this dose was too small for a persistent infection to be established.

That only the 2 pigs with high viable unit counts showed reaction to tuberculin (Table 2) is consistent with the view (*Rich* 1951) that one of the conditions for the development of delayed hypersensitivity is the presence of a certain, relatively large, amount of antigen in the organism concerned.

The present experiment showed that although doses ranging from about 1500 to about 150000 viable units, given daily for 15 days, may give rise to either slight tuberculous inflammation or slight infection of the organs, 15 daily doses of about 1.5 million viable units (total dose about 23 million) represent the lowest dosage capable of producing manifest infection with tuberculous inflammation, high tissue counts of organisms and hypersensitivity. With a longer period of observation, gross lesions might

perhaps have developed in the pig given that dose, especially in the posterior mesenteric lymph nodes, in which the number of organisms was very high.

Both this and the previous oral infection experiment (*Jørgensen* 1977 b) would seem to indicate that for M. avium to produce gross lesions a total dose of 100—200 million viable units, divided over 5—15 days, is appropriate.

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SAMMENDRAG

Infektionsforsøg på svin med Mycobacterium avium, Serotype 2. 3. Peroral infektion med små doser af M. avium.

Med det formål at bestemme den mindste dosis af M. avium, der ved peroral podning kunne fremkalde tuberkulose hos svin, blev der podet med doser fra $15{,}6 \times 10^2$ til $15{,}6 \times 10^6$ viable units 5 gange om ugen i 3 uger, ialt 15 podninger (Tabel 1). Grisene blev slagtet 31—32 dage efter sidste podning.

Dyrene podet med doserne $15{,}6 \times 10^5$ og $15{,}6 \times 10^6$ viste følsomhed for aviært PPD tuberkulin (1000 enh. pr. dosis) 24 dage efter sidste podning (Tabel 2). Grisen podet med den højeste dosis ($15{,}6 \times 10^6$) havde makro- eller mikroskopiske forandringer i tarmslimhinden (Peyerplet) og lever samt i lnn. mandibulares, mesenterici og hepa-

tici, og ved dyrkning kom vækst af M. avium fra de samme væv samt fra milt og ln. tracheobronchalis sin. Grisen podet med $15{,}6 \times 10^5$ viable units havde udtalt granulomatøs, tuberkuløs infiltration i tonsil og lnn. mesenterici, og der kom vækst af M. avium fra tonsil, tarmslimhinde og lnn. mandibulares og mesenterici. Kimtallene var høje for tonsil og lnn. mesenterici (Tabel 3 og 4).

De mindre doser forårsagede minimal vævsreaktion og/eller meget lav infektion i vævene og menes ikke at være i stand til at fremkalde en progressiv tuberkulose.

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