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EXPERIMENTAL INFECTION WITH MYCOBACTERIUM AVIUM, SEROTYPE 2, IN PIGS

4. CONTACT INFECTION FROM ORALLY INOCULATED PIGS*

By

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JØRGENSEN, J. BERG: *Experimental infection with Mycobacterium avium, Serotype 2, in pigs. 4. Contact infection from orally inoculated pigs.* Acta vet. scand. 1978, 19, 58—72. — In 3 experiments, 13 pigs were inoculated orally with 0.5 mg Mycobacterium avium daily for 5 days (1 mg = $32\text{--}68 \times 10^6$ viable units). Five to 8 days after inoculation, 16 non-inoculated pigs were added to the inoculated pigs.

Cultures from faeces showed excretion of *M. avium* from all the inoculated pigs for some time within the period 16 to 65 days after the last inoculation; the greatest numbers of organisms (62000 per 100 g faeces) were found at about the middle of the excretion period (Table 2). Two of the contact pigs were found to excrete *M. avium* in small numbers, 1 at 15, the other at 37 and 44 days after contact.

All the inoculated pigs and 13 of the contact pigs showed positive intradermal tuberculin reactions. Post-mortem examination showed tuberculous lesions in all the inoculated pigs (Table 3). *M. avium* was transmitted to 15 of 16 pigs by contact. Five of these pigs showed gross lesions, 10 of them microscopic lesions only; in 9 of them the infection was proved by culture.

Mycobacterium avium, Serotype 2; oral inoculation; excretion; contact infection; pigs.

The significance of transmission of *Mycobacterium* (*M.*) *avium* infection among pigs is still doubtful. Reports have been published about contact infection (*Graham & Tunnicliff* 1926, *Gwatkin & Mitchell* 1952 and others) and descriptions been given

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of cases of tuberculous enteritis which may without doubt have involved an excretion of organisms with the faeces (*Griffith* 1911, *Følger* 1938, *Lautrup-Nielsen* 1975).

The present paper is a report of 3 experiments aiming at a quantitative study of the excretion of *M. avium* with the faeces of orally inoculated pigs, and at determining the duration of such excretion. Further, the significance of transmission of *M. avium* to non-infected pen mates was examined.

MATERIAL AND METHODS

Experimental animals. Table 1 gives a survey of the experimental pigs, which were either littermates or borne by different sows within a period of 5 days. The ages given in Table 1 are mean ages. If all pigs in a group have been necropsied within a period of 7 days, figures for age and weight and number of days in experiment are given as means for the whole group. In Experiment (Exp.) 13, 4 pigs were inoculated and 5 kept as non-infected controls; in Exp. 15: 5 inoculated and 6 controls; and in Exp. 16: 4 inoculated and 5 controls.

Inoculation material. Strain SSC 1336 (ATCC 25291) of chicken origin (*Engbæk et al.* 1971) was used in all 3 experiments. For culture preparation, viable unit counts and checking of colony morphology, see *Jørgensen* (1977 a). Viable unit counts per mg after 8 weeks were as follows: Exp. No. 13, 68×10^6 ; No. 15, 32×10^6 ; and No. 16, 51×10^6 . Colonies were mainly smooth transparent (SmT) with a few D and R variants (*Fregnan & Smith* 1962). The virulence of the strain was checked on rabbits by intravenous inoculation of 1 mg organisms, 1 rabbit per experiment. The animals died after 16, 19 and 15 days, respectively.

Inoculation. The culture, suspended in skimmilk, was administered individually by mouth, 0.5 mg organisms per pig daily for 5 days in all 3 experiments. The infected pigs were kept isolated for a further 5 to 8 days and then put together with the non-infected pigs in a clean room. During the period of contact it was endeavoured to keep the standard of hygiene, especially as regards removal of faeces, at the same level as in most Danish herds of swine.

Tuberculin tests with avian and human PPD tuberculins, 1000 t.u. per dose, were performed and evaluated as described previously (*Jørgensen* 1977 a). All pigs were tested before the experi-

ments. In Exps. 13 and 16 the pigs were tested 28 and 27 days after the first inoculation, respectively, and subsequently at 28-day intervals. In Exp. 15 tests were made 42 and 58 days after inoculation. The non-infected pen mates were tested on the same occasions as the infected pigs, beginning at 19, 31 and 14 days after contact had been established (Exps. 13, 15 and 16). In Exp. 16 an extra test was performed on the 3 contact pigs alive on Day 170.

Cultures were made from faeces samples collected from the rectum. One sample from each pig was examined before the start of the experiments. In Exp. 13 samples were taken on Day 6 of the isolation period and 21 days after the last inoculation, and thereafter at 14-day intervals. In Exps. 15 and 16 samples were taken daily during the inoculation and isolation period, then on Day 9 after the last inoculation, and thereafter at 7-day intervals. The controls were tested on the same occasions as the infected pigs.

Technique. About 2 g faeces was decontaminated with 10 ml 4 % sodium hydroxide for 10 min. and with 10 ml 5 % oxalic acid with 0.1 % malachite green for 15 min. (Beerwerth 1967). The sediment was then suspended in 4 ml diluted Sauton medium and 0.1 ml of the suspension inoculated onto 4 slants of Löwenstein-Jensen (L-J) medium and 4 slants of L-J medium with 0.5 mg Actidione* per ml. Viable units were counted after incubation for 8 weeks at 37°C. To check the identity of the recovered organisms, subcultures were grown on L-J medium at 22°, 37° and 45°C. The criteria of *M. avium* were: nonchromogenic, smooth, dome-shaped colonies, growth at 22°C and 45°C in about 6 weeks and at 37°C in about 2 weeks. A few of the isolated strains were inoculated on rabbits, 1 mg i.v., and/or tested biochemically by amidase tests (Bönicke 1960) using amides Nos. 3 (urea), 5 (nicotinamide) and 6 (pyrazinamide), and by Tween 80 degradation test, catalase test and nitrate test (Wayne 1962 a, b).

Duration of the experiments is indicated in Table 1.

Post-mortem examinations included macroscopic and histopathological examination (Jørgensen 1977 a) and culture (Jør-

* Cycloheximide, Koch-Light Laboratories Ltd., Coinbrook, Bucks., England.

Table 1. Survey of experimental animals, duration of experiments, and weights at slaughter.

Experiment No.	Pig No. and sex	Mean age (days) at			Duration of experiment, mean number of days	Mean weight at slaughter, kg
		inoculation	contact	slaughter		
13 I	119, 121, 122, 123 ♂ ♂ ♀ ♀	69		218	149	98
13 C	124, 125, 126, 127, 128 ♀		79	232	153	109
15 I	134, 135, 136, 137, 140 ♂ ♂ ♀ ♀	54		119	65	33
15 C	138, 141, 142, 143, 144, 151 ♀ ♂ ♀ ♂ ♂ ♂		65	215	150	87
16 I	163, 164, 165 ♂ ♂ ♂			243	177	99
	166 ♀	66		220	154	90
16 C	155, 157, 159 ♂ ♂ ♀		79	256	177	119
	156, 158 ♂ ♀			217	138	91

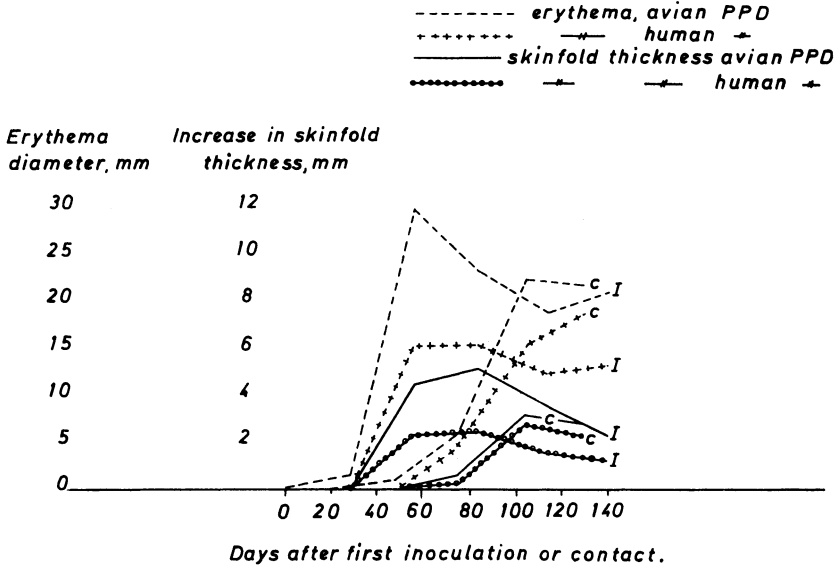
I = Inoculated by mouth.

C = Non-inoculated controls.

gensen 1977 b). The intestinal contents were decontaminated by the method of *Beerwerth*. The materials examined are listed in Tables 4 and 5.

RESULTS

Tuberculin tests. None of the pigs showed reaction before inoculation. All the inoculated pigs showed delayed hypersensitivity, 1 pig 28 days after the first inoculation, the rest of the pigs after 55—58 days. The mean 24-hr. reactions of the 8 inoculated pigs in Exps. 13 and 16 are shown in Fig. 1. The strongest



I = pigs inoculated orally

c = pigs infected by contact

Figure 1. Mean reactions to PPD avian and human, 1000 t.u. per dose, in 8 pigs inoculated orally with 0.5 mg *M. avium* daily for 5 days, and in 5 pigs showing gross lesions after contact infection. Readings after 24 hrs.

reactions were found 55 (56) and 83 (84) days after inoculation. On Days 139 and 140 the reactivity was still present, but weaker. Among the contact pigs the most pronounced reactions were seen in the 5 pigs in Exp. 13 which showed gross lesions. The mean reactions of these pigs are shown in Fig. 1. On Days 103 and 131 their reactivity was at about the same level as that of the inoculated pigs. Eight of 11 contact pigs without gross lesions (Exps. 15 and 16) showed hypersensitivity. Table 2 shows the reactions of 7 of these pigs as read at 24 hrs. Pig 155, which reacted on Day 98 after contact, but at the 48-hr. reading only, is not included. The table shows that the onset of hypersensitivity (115—154 days) was slower in these pigs than in the contact pigs with gross lesions, and that the level of hypersensitivity was lower. Most reacting animals responded to both tuberculin, though as a whole with the strongest reaction to avian tuberculin. Further details about the tuberculin tests will be published in a subsequent paper (Jørgensen & Weis Bentzon, in preparation).

Table 2. Comparative tuberculin tests with PPD avian and human, 1000 t. u. per dose, on contact pigs with no gross lesions. Reactions as read at 24 hrs. Negative animals not listed.

Experi- ment No.	Pig No.	Days after first con- tact	Increase in skinfold thickness, mm		Erythema, diameter, mm	
			avian	human	avian	human
15	138	143	2.0	1.0	12.8	11.6
--	141	143	2.0	1.5	14.4	11.6
—	143	115	1.0	0	9.0	0
--	151	115	1.0	1.0	11.0	9.0
—	151	143	3.0	1.5	17.4	12.6
16	156	126	0	0	10.5	0
—	158	126	1.0	1.0	12.5	11.0
—	159	154	1.0	0	8.5	9.0
—	159	170	0	0	10.5	0

Explanations: In Exp. 15 tests were made on days 31, 59, 87, 115 and 143 after contact. Pigs 142 and 144 were negative at all tests. In Exp. 16 tests were made on days 14, 42, 70, 98 and 126 after contact (all pigs) and on days 154 and 170 (Pigs 155, 157 and 159). Pigs 155 and 157 were negative at all 24-hr. readings.

Cultures from faeces. All samples collected before the start of the experiments were negative. In Exp. 13 the samples collected on Day 6 after the last inoculation were negative. In Exp. 15 there was excretion from Day 2 of the inoculation period to Day 1 or 2 after the last inoculation, with viable unit counts from 500 to 62000 per 100 g faeces. In Exp. 16 irregular excretion was found during the infection period, but not during the rest of the isolation period. Table 3 shows the results of cultures from the inoculated pigs during their contact with non-inoculated pigs. In Exp. 13 excretion was found on Days 21 and 35, and in Exp. 15 on Day 16 to Day 58, with the heaviest excretion from Day 30 to Day 51. In Exp. 16, the first positive samples were found on Day 30 and the last on Day 65. The heaviest excretion was found on Days 37 and 44. Two of the contact pigs were positive. Thus, in Exp. 13, Pig 125 was positive on Day 15 after the first contact, with about 1000 viable units per 100 g faeces, and in Exp. 15 Pig 151 was positive on Days 36 and 43 with viable unit counts of about 500 on both days. In Exps. 15 and 16 growth appeared of slightly yellow, rough colonies, which differed from colonies of

Table 3. Pigs infected orally with *M. avium*, 0.5 mg daily for 5 days. Excretion of *M. avium* in faeces, viable units per 100 g faeces.

Experi- ment No.	Pig No.	Days after last inoculation								
		21	35	49	63	77	91	105	119	133
13	119	1000	3500	0	0	0	0	0	0	0
	121	4500	10000	0	0	0	0	0	0	0
	122	500	10667	0	0	0	0	0	0	0
	123	0	2000	0	0	0	0	0	0	0
		Days after last inoculation								
		16	23	30	37	44	51	58	65	72-135
15	134	500	500	4500	4000	9000	2000	500	0*	
	135	0	0	41000	11500	10000	18000	7000	8000*	
	136	667	0	26500	19000	62000	6500	2000	0*	
	137	1500	500	6670	57000	19000	20000	1500	0*	
	140	500	500	9500	6500	1500	22000	2667	1000*	
16	163	0	0	0	4000	0	3000	500	0	0
	164	0	0	3500	40000	54667	0	1000	0	0
	165	0	0	7000	4000	29000	500	500	500	0
	166	0	0	2000	3500	31000	18000	500	1000	0

* Culture from rectal contents at necropsy 61 days after the last inoculation.

M. avium by faster growth at 22°C (7—14 days) and 37°C (7 days) and by not growing at all at 45°C. Being non-virulent for rabbits, positive in the nitrate, catalase and Tween-80 degradation tests, and negative in the amidase tests (amides 3, 5 and 6) they were identified as *M. triviale*.

Necropsy. Table 4 shows the distribution of macro- and microscopic lesions. All the inoculated pigs showed gross lesions. Pigs necropsied 65 days after the first inoculation (Exp. 15) showed caseated lesions, 0.5—3 mm in diameter, with signs of acute inflammation. Two of these pigs (137, 140) showed tuberculous ulcers in the Peyer patches. In pigs necropsied 149—177 days after inoculation the lesions were well-defined, caseo-calcareous. Lesions of the same type were found in the contact pigs in Exp. 13. In Exps. 15 and 16 the contact pigs showed no gross lesions.

Histopathology. Animals examined 65 days after the first inoculation showed epithelioid tubercles with or without casea-

tion and slight calcification, and with a distinct tendency to demarcation and organization by fibrous tissue.

In the Peyer patches microgranulomas were seen in all 5 pigs and ulcers in 2. At 149—177 days after inoculation the lesions were caseo-calcareous and lined with epithelioid cells, lymphocytes and eosinophiles, and surrounded by a capsule of fibrous tissue. Besides such chronic lesions microgranulomas were seen with or without fibrous tissue demarcation. Similar lesions were seen in the contact pigs in Exp. 13 (with gross lesions). Of the contact pigs in Exps. 15 and 16 (with no gross lesions) all except Pig 144 showed microgranulomas in the tissues listed in Table 4. These granulomas consisted almost entirely of giant cells.

Culture. The results are given in Table 5.

Inoculated pigs. Five pigs (Exp. 15) examined 65 days after the first inoculation were positive, mostly with high viable unit counts. Four pigs (Exp. 13) examined 149 days after inoculation, and Pig 166 (Exp. 16) examined after 154 days, were positive with low viable unit counts, while 3 pigs (Nos. 163, 164 and 165, Exp. 16) examined after 177 days were negative.

Contact pigs. Nine of 16 pigs were positive, viz. the 5 pigs in Exp. 13, 3 of 6 pigs in Exp. 15, and 1 of 5 pigs in Exp. 16 (Table 5).

DISCUSSION

Previous experiments have proved that on oral inoculation in doses ranging from 100 to 200×10^6 organisms divided over 5 to 15 days, *M. avium* is capable of producing manifest tuberculous infection (Jørgensen 1977 b, 1978). The results of the oral inoculations in the present experiments have confirmed this finding and offered an opportunity to study the pathogenesis of the infection in its later stages.

In the tissues of pigs killed 65 days after the first inoculation, histological and bacteriological examinations showed about the same distribution of lesions and the same number and distribution of acid-fast organisms as found previously in 2 pigs inoculated with, respectively, 92.5×10^6 and 234×10^6 organisms, and killed 35 and 50 days after the first inoculation, respectively. In pigs killed on Day 35 the lesions were of granulomatous nature; caseation was found on Day 50, and on Day 65 calcification was apparent. Connective-tissue demarcation was found in pigs killed on Days 50 and 65.

Table 4. Results of pathological examination.

No.	Tissue	Experiment 13												
		Pig No.	119	121	122	123	124	125	126	127	128	134	135	136
		Mode of infection	I	I	I	I	C	C	C	C	C	I	I	I
	Days in experiment	149	149	149	149	153	153	153	153	153	65	65	65	
1	Ln. mandibularis						+		+	+				
				(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	
2	Ln. parotideus													
4	Ln. retrophar. med.				(+)							(+)		
11	Ln. mesentericus I		+	+	+			+			+		+	
			(+)	(+)	(+)			(+)			(+)	(+)	(+)	
11	Ln. mesentericus II	+	+	+	+	+	+		+	+	+	+	+	
		(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)		(+)	(+)	(+)	
11	Ln. mesentericus III	+	+	+	+		+				+		+	
		(+)	(+)	(+)	(+)		(+)			(+)	(+)	(+)	(+)	
12	Ln. tracheobronchal.											(+)		
13	Ln. hepaticus	(+)			(+)		(+)				(+)	(+)	(+)	
14	Spleen													
15	Liver	+									+		+	
		(+)		(?)							(+)		(+)	
16	Lung													
17	Kidney											+		
19	Musc. long. dorsi													
25	Tonsil			(+)	(+)					(+)	(+)		(+)	
27	Intestinal mucosa (Peyer patch)			(+)						(+)	(+)	(+)	(+)	

Explanations:

I = Inoculated by mouth. C = Non-inoculated, in-contact.

Figures without brackets indicate gross lesions.

Figures in brackets indicate microscopic lesions.

In the present experiments a reduction in the number of tissues with macro- or microscopic lesions occurred between Day 65 and Days 149—177. Cultures showed a drastic reduction of viable acid-fast organisms from Day 65 to Day 149 (154) and at 177 days after inoculation no viable acid-fasts were found, i.e. the pigs had apparently overcome the infection.

Griffith (1911) and *Graham & Tunnicliff* (1926) reported on excretion of *M. avium* in the faeces of infected pigs, as proved by, respectively, microscopy and inoculation on chickens. Trans-

Experiment 15								Experiment 16								
137	140	138	141	142	143	144	151	163	164	165	166	155	156	157	158	159
I	I	C	C	C	C	C	C	I	I	I	I	C	C	C	C	C
65	65	150	150	150	150	150	150	177	177	177	154	177	138	177	138	177
(+)	(+)		(+)	(+)				(+)		+	+					
										(+)	(+)	(+)				
													(+)	(+)		
+																(+)
(+)	(+)							(+)		+	+	+				
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(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
(+)	(+)							(+)								(+)
(+)	(+)	(?)						(?)		+					(?)	(?)
			(+)												(?)	(?)
	(+)															(+)
(+)	(+)															(+)
(+)	(+)									(?)						(+)

Blank space = No abnormalities.
 (?) = Lymphocyte accumulations.

mission of *M. avium* or *M. intracellulare* among pigs after experimental infection has been described by *Graham & Tunnicliff*, *Gwatkin & Mitchell* (1952), *Kauker & Zettl* (1964), *Ray* (1966), *Tuffley et al.* (1973) and *Thoen et al.* (1976).

So far, no data are available on the duration of the excretion or the quantity of organisms excreted. In the present work all the artificially infected pigs excreted *M. avium* for periods of varying length between Day 16 and Day 65 after the last of 5 inoculations. The heaviest excretion was found at about the middle of the excretion period with maximum 62000 organisms per 100 g faeces. In Exp. 13, the excretion would seem to have been heavier than

Table 5. Results of culturing.

		Experiment 13									
		119	121	122	123	124	125	126	127	128	
Pig No.		I	I	I	I	C	C	C	C	C	
Mode of infection											
Days in experiment		149	149	149	149	153	153	153	153	153	
No.	Tissue										
1	Ln. mandibularis	40		160		∞	3560	610	10	400	
2	Ln. parotideus										
4	Ln. retrophar. med.	10	490		130						
11	Ln. mesentericus I	40	200		20			19700			
11	Ln. mesentericus II		10	300	20		1000				
11	Ln. mesentericus III	30	470	480	260						
12	Ln. tracheobronchal. sin.								10		
13	Ln. hepaticus	10	20	140	90	170	10	10	20	30	
14	Spleen										
15	Liver										
16	Lung										
17	Kidney										
19	Musc. long. dorsi										
25	Tonsil	60	1100	110	1450			1000			
27	Intestinal mucosa (Peyer patch)										
28 ^I	Intestinal contents										
28 ^{II}											

Explanations:

I = Inoculated by mouth. C = Non-inoculated, in-contact.

Blank space = No growth.

indicated by the results of cultures from faeces (Table 3) in that all of the 5 contact pigs in this experiment showed gross lesions (Table 4). The short excretion period observed in this experiment is difficult to explain, but may be due to shortcomings of the cultural procedure.

M. avium was transmitted by contact to 15 of 16 pigs, as indicated by the results of pathological and cultural examination and tuberculin tests. Most convincing, of course, was the isolation of *M. avium* from 9 pigs. In 5 of these, gross lesions were found. A cellular reaction (epithelioid cells and giant cells) indicative of tuberculous infection was found in 10 pigs without gross lesions (Table 5). Thirteen of the contact pigs showed delayed hypersensitivity to tuberculin, most pronounced in the pigs with gross

Experiment 15										Experiment 16				
								143		163		155		
								144		164		157		
134	135	136	137	140	138	141	142	151		165	166	159	156	158
I	I	I	I	I	C	C	C	C		I	I	C	C	C
65	65	65	65	65	150	150	150	150		177	154	177	138	138
2320										70				
	∞	2360	1720	∞		∞	∞							
	480		3160											
2240	22600	21080	200									230		
34400	416000	444000	33000	494000								270		
108600	340000	560000	283000	231000										
40	120		40	360	10									
1240	520	2160	2080	600										
	10													
	20	20												
1320		133000	80	∞		10	10					10		∞
20	2960	10	10	40										
	10													
	160			20										

Figures indicate viable units per 0.5 g tissue or 2 g intestinal contents.
 ∞ = > 400000 units.

lesions, the reactions of which were comparable to those of the inoculated pigs, though with a delay of 1 to 2 months in the onset of hypersensitivity, as reckoned from the first day of exposure to infection. Of the 11 contact pigs without gross lesions 8 showed hypersensitivity, but with weaker reactions and an even greater delay in the onset of hypersensitivity than in the contact pigs with gross lesions.

The results indicate that among pigs exposed to contact infection sensitivity to tuberculin may be expected from about 75 to about 150 days after exposure, presumably depending on the dose of infection.

Only 1 pig was negative at all examinations.

Two of the contact pigs were found to excrete *M. avium* in the faeces, 1 at 15 days, the other at 37 and 44 days after the first

contact. Since it is hardly credible that a tuberculous enteritis should have developed within 15 days, the finding in the first-mentioned pig was probably due to a simple passage of ingested organisms. In the last-mentioned pig a slight and transient tuberculous enteritis may have developed.

In a previous experiment *Jørgensen* (1978) found that the minimum oral dose of *M. avium* capable of producing manifest tuberculous infection in pigs was between 23.4×10^5 and 23.4×10^6 viable units. In the present experiments 5 of 16 contact pigs ingested enough organisms for gross lesions to develop, while 10 pigs developed microscopic lesions only. No doubt the environmental burden of infective organisms was so low that minor fluctuations in that burden might determine whether manifest infection would be induced in the exposed pigs or not. *Graham & Tunnicliff* concluded that "the transmission from infected to healthy pigs through association appears to be slower than from infected chickens to pig following comparable contact".

Cultures from faeces showed that there was little or no excretion of *M. avium* from the contact pigs. Maintenance of the infection in a herd of swine would therefore seem to be impossible, unless infection is continuously introduced from the outside.

According to experiences from natural cases, *M. avium* infection among swine has a definite tendency to disappear within 4—12 months (*Biering-Sørensen* 1959, *Jørgensen et al.* 1972) and the present results confirm these findings.

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SAMMENDRAG

*Infektionsforsøg på svin med Mycobacterium avium, Serotype 2.
4. Kontaktsmitte fra svin podet per os.*

I 3 eksperimenter blev ialt 13 grise podet peroralt med 0,5 mg *M. avium* daglig i 5 dage (1 mg = $32-68 \times 10^6$ bakterier).

Fem til 8 dage efter podningen blev 16 ikke-podede grise sat sammen med de podede.

Ved dyrkning fra gødningsprøver kom vækst af *M. avium* fra alle de podede grise. Udskillelsesperioden lå mellem 16. og 65. dag efter podningen, og den maksimale udskillelse (62000 bakterier pr. 100 g gødning) fandtes ca. midt i perioden (Tabel 2). To af kontaktgrisene viste udskillelse af *M. avium* i ringe antal, den ene gris 15. dag og den anden 37. og 44. dag efter kontaktsmittens begyndelse.

Post mortem undersøgelser viste, at alle de inficerede grise havde tuberkuløse forandringer.

Femten af 16 kontaktgrise viste tegn på smitteoverførsel enten ved tilstedeværelse af makroskopiske processer (5 grise) eller mikroskopiske processer (10 grise). Ni kontaktgrise var positive ved dyrkning.

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