

From the State Veterinary Serum Laboratory, Copenhagen, Denmark.

PARATUBERCULOSIS IN PIGS  
EXPERIMENTAL INFECTION BY ORAL ADMINISTRATION  
OF MYCOBACTERIUM PARATUBERCULOSIS

By

*J. Berg Jørgensen*

Infection with *Mycobacterium paratuberculosis* (*M. paratuberculosis*) in pigs has been described by *Ringdal* (1963), who isolated the organisms from slaughter pigs with lesions of the mesenteric lymph nodes resembling tuberculosis. The pigs were infected from cows with paratuberculosis, and the diagnosis was verified by culture and by inoculation experiments on two calves. *Runnels* (1955) reported a case of hyperplastic enteritis in a 10-month-old pig. Histological examination of the intestinal mucosa revealed heavy infiltration with histiocytic cells and giant cells with myriads of acid-fast rods. The macroscopical and histological changes resembled paratuberculosis of cattle, but since no cultures were made, it cannot be established with certainty that the changes were caused by infection with *M. paratuberculosis*. *Bergman* (1913) infected a 3-month-old pig by feeding it with intestinal mucosa from a cow with clinical paratuberculosis. The pig was slaughtered when about ten months old, and autopsy revealed thickening of the mucosa of the small intestine over a section of several metres. Histological examination showed thickening of the epithelium, cellular infiltration, and acid-fast rods almost everywhere. It was concluded that the pig had become affected with paratuberculosis as the result of having been fed with the infected material.

As it was thought to be of interest to see if lesions as those found by *Ringdal* in the mesenteric lymph nodes of pigs could be reproduced experimentally, pigs were inoculated orally with

culture of *M. paratuberculosis* and with intestinal mucosa and mesenteric lymph nodes from a cow with spontaneous paratuberculosis.

#### MATERIAL AND METHODS

A total of seven groups of pigs, comprising 8, 8, 9, 10, 11, 8 and 7 animals, respectively, were inoculated by the oral route. Six of the groups were given bacterial culture, while infected tissues were administered to one group.

Cultures for inoculation were suspended in physiological saline (100 ml to one tube of Löwenstein medium with dense growth of *M. paratuberculosis*). Tissues were ground in a blender until they had a consistency resembling gruel and thereafter stored in the frozen state.

Groups 1 and 3 were inoculated with a strain ("Nina") which was originally isolated by *Ringdal* (1963) from a pig with paratuberculous lesions of the mesenteric lymph nodes and later re-isolated from the faeces of a calf that had been infected with it experimentally.

Groups 2, 4, 6 and 7 were inoculated with four different strains isolated from cows with paratuberculosis. These strains are numbered 269/64, 610/64, 729/65 and 734/65.

The inoculum in the case of Group 5 was the intestinal mucosa and mesenteric lymph nodes of a cow with paratuberculosis.

Viable counts were made on the culture suspensions by inoculation of suitable dilutions on four tubes of Löwenstein medium with 5 % killed bovine tubercle bacteria added. Counting was performed after incubation for 6—8 weeks. An average was found of 2.55 million organisms per ml inoculum.

Pigs were slaughtered at various stages of the experiment and the gastro-intestinal canal, liver, and spleen removed for examination.

Group 1 consisted of eight pigs born at the experimental farm attached to the laboratory. These were 36 days old when the experiment started. Within 21 days, three inoculations of 1 ml suspension of the strain "Nina" were given to each pig by stomach tube. One pig was slaughtered on day 62 and another on day 95 after the start of the experiment. From day 108 to day 147 the remaining six animals were daily given 10 ml suspension of the same strain mixed with the fodder. The pigs were killed on day 155, i.e. at the age of 191 days, which corresponds ap-

Table 1. Survey of experiments.

Group	No. of pigs	No. of days in exp.	Macroscopical findings in mes.*)	Microscopical findings	Culture	Histological findings	Inoculum
1	1	62	—	—	+ mes.	—	culture "Nina"
	1	95	—	—	+ mes.	—	
	2	155	+	+ mes.	+ mes.	+ mes.	
	1	—	+	+ mes.	—	+ mes.	
	1	—	—	—	+ mes.	+ mes.	
	1	—	+	+ mes.	+ mes.	+ mes., + ileum	
	1	—	+	—	—	+ mes.	
2	1	68	—	—	—	+ ileum	culture Para 269
	1	110	—	—	—	—	
	3	166	—	—	+ mes.	—	
	2	—	—	—	+ mes., + ileum	—	
	1	—	—	—	+ ileum	—	
3	5	48	—	—	—	—	culture "Nina"
	4	62	—	—	—	—	
4	1	55	—	—	—	—	culture Para 610
	9	70	—	—	—	—	
5	1	55	—	—	+ mes.	—	mesente- ric lymph nodes intestinal mucosa.
	1	—	—	—	+ mes., + ileum	+ mes.	
	1	—	+	+ mes.	+ mes.	+ mes.	
	3	—	+	+ mes.	+ mes.	+ mes., + ileum	
	2	62	+	+ mes.	+ mes.	+ mes.	
	2	—	+	+ mes.	+ mes.	+ mes., + ileum	
	1	—	+	+ mes.	+ mes., + ileum	+ mes.	
6	1	96	—	—	+ mes., + jejunum, + ileum	+ ileum	culture Para 729
	1	158	—	—	+ mes.	+ mes.	
	1	—	—	—	+ mes., + jejunum, + ileum, + colon	—	
	1	—	—	—	+ mes., + liver, + ileum, + colon	—	
	1	165	—	+ mes.	+ mes., + jejunum	+ mes., + ileum	
	1	172	+	+ mes.	+ mes.	+ mes.	
	1	—	+	+ mes.	+ mes.	+ mes., + liver	
	1	—	+	+ mes.	—	+ mes.	
	1	—	—	—	—	—	
7	1	152	—	—	+ mes.	—	culture Para 734
	1	—	—	—	+ mes., + ileum	+ mes.	
	1	—	—	+ mes.	+ mes.	+ mes.	
	1	159	—	—	+ mes.	+ mes.	
	1	166	—	—	+ mes., + ileum	—	
	1	—	—	—	+ mes.	—	
	1	173	—	+ mes.	+ mes., + ileum	+ mes.	

\*) mes. = Mesenteric lymph nodes.

All pigs were subjected to macroscopical, bacterioscopical, cultural and histological examination.

— = no findings.

proximately to the age of a bacon pig of about 90 kg live-weight (Table 1).

Group 2 consisted of eight pigs born at the experimental farm. Within 14 days from the age of 41 days they were inoculated orally three times in the same way as Group 1 with culture of strain Para 269. One pig was slaughtered on day 68 and a second on day 110 after commencement of the experiment. The remaining six animals were given a daily dose of 10 ml suspension in their fodder from day 112 to day 158. They were killed eight days after the last inoculation, when they were 207 days old (Table 1).

Group 3 consisted of nine pigs, which were 146 days old at the start of the experiment. With the fodder they were given 10 ml daily of a suspension of the strain "Nina", five pigs for 40 days and four pigs for 54 days. The animals were 194 and 208 days old when killed 48 and 62 days after the start of the experiment (Table 1).

Group 4 consisted of ten pigs, which were 116 days old when the experiment started. Strain Para 610 was used as inoculum and the inoculation was made in the same way as in Group 3. One pig was inoculated daily for 47 days and killed on day 55, when it was 171 days old; nine pigs were inoculated for 62 days and killed on day 70, when they were 186 days old (Table 1).

Group 5 consisted of 11 pigs, six of which were 120 days and five 112 days old at the start of the experiment. All of them were inoculated by daily admixture to the fodder of 30 g of a mixture of infected tissues. Six pigs were fed for 47 days and slaughtered on day 55, when they were 175 days old; five pigs were fed for 54 days and slaughtered on day 62, when they were 174 days old (Table 1).

Group 6 consisted of eight pigs, which were 17 days old at the start of the experiment. Culture of strain Para 729 was inoculated by stomach tube in the same way as in Groups 1 and 2, seven times within 27 days. Thereafter they were given 10 ml culture daily in the fodder until they were slaughtered. One was slaughtered on day 96, when 113 days old; three on day 158, when 175 days old; one on day 165, when 182 days old; and three on day 172, when 189 days old (Table 1).

Group 7 consisted of seven pigs that were 20 days old when inoculated orally for the first time with culture of strain Para 734. Administration by stomach tube was performed six times

within 27 days, after which the pigs were given 10 ml culture suspension daily with the fodder. Three pigs were killed on day 152, when they were 172 days old; one on day 159, when it was 179 days old; two on day 166, when they were 186 days old; and one on day 173, when it was 193 days old (Table 1).

All pigs were killed at a slaughterhouse, and immediately after death the gastro-intestinal canal, spleen and liver were removed for macroscopical examination. The intestinal canal was opened and examined with special regard to hyperplasia of the mucous membrane. The mesenteric lymph nodes were sliced and carefully examined for lesions.

Material for bacterioscopical, cultural and histological examination was taken from the mucous membranes of the jejunum, ileum and colon, and from the spleen, liver, and two mesenteric lymph nodes. If macroscopic changes were seen in the mesenteric lymph nodes, material was taken from the affected areas.

Bacterioscopy was carried out on smears stained by the Ziehl-Neelsen method. Cultures were made on Löwenstein medium to which 5 % killed bovine tubercle bacteria had been added. Four tubes of medium were used for each specimen, and the observation period was at least three months. Histological examination was performed after staining by the van Gieson and Ziehl-Neelsen methods.

## RESULTS

*Macroscopical examination.* Seventeen pigs (27.9 %) had changes in the mesenteric lymph nodes when slaughtered (Table 2). These pigs were distributed with five in Group 1 (62.5 %), nine in Group 5 (81.8 %) and three in Group 6 (37.5 %). The macroscopic changes were characterized by caseation, and resembled completely those found in mesenteric tuberculosis caused by avian tubercle bacteria. The affected lymph nodes were swollen and tuberculous to varying degrees, dependent on the size of the lesions, the largest of which were visible on the surface. On the cut surface well-defined caseous nodules were seen varying in size from millet seed to large peas. Some of the nodules were slightly calcified (Fig. 1). There was no perifocal haemorrhage or other signs of acute processes. Affected lymph nodes could be found in any part of the mesentery, but lesions seemed to be larger and more numerous in its posterior part.



Figure 1. Mesenteric lymph nodes with caseation.  
(E. Hermansen, fot.)

There were no definite changes in the gastro-intestinal canal resembling the hyperplastic enteritis seen in cattle with paratuberculosis.

The liver and spleen showed no macroscopical changes.

Table 2. Examination of mesenteric lymph nodes by different methods.

	Total of pigs	Macroscopical examination		Bacteri- scopy		Culture		Histological examination	
		+	-	+	-	+	-	+	-
No.	61	17	44	19	42	37	24	26	35
%	100	29.9	72.1	31.1	68.9	60.7	39.3	42.6	57.4

*Bacterioscopic examination.* Microscopy of Ziehl-Neelsen stained smears from the mesenteric lymph nodes revealed acid-fast rods in 19 pigs (31.1 %) (Table 2). In 16 of these pigs there were positive necropsy findings, while three showed no visible lesions (Tables 3 and 4).

Table 3. Numbers of positive bacterioscopic, cultural and histological findings in organs of 17 pigs with positive necropsy findings.

	Liver	Spleen	Mesenteric lymph nodes	Jejunum	Ileum	Colon
Bacterioscopy			16			
Culture			14		3	
Histology	1		17		5	

Table 4. Numbers of positive bacterioscopic, cultural and histological findings in organs of 44 pigs with negative necropsy findings.

	Liver	Spleen	Mesenteric lymph nodes	Jejunum	Ileum	Colon
Bacterioscopy			3			
Culture	1		22	3	10	2
Histology			8		3	

*Culture.* *M. paratuberculosis* was isolated from one or more organs of 14 pigs with positive necropsy findings and 23 pigs without visible lesions. In the group with positive findings, growth was obtained from the mesenteric lymph nodes in all of the 14 cases, and from the ileic mucous membrane in three cases (Table 3). In the group without visible lesions, growth was obtained in one case from the ileum alone, and in 22 cases either from the mesenteric lymph nodes alone or from these and other organs as well, viz. in one case from the liver, in three from the jejunum, in nine from the ileum, and in two from the colon (Table 4). In all, *M. paratuberculosis* was isolated from 37 (60.7 %) of the inoculated pigs (Table 2).

*Histological examination.* The histological findings in mesenteric lymph nodes with macroscopical changes consisted of larger or smaller areas of caseation with only slight tendency to calcification. The caseous tissue was surrounded by a layer of epithelioid and giant cells, which was separated from the normal lymphoid tissue by a more or less distinct capsule of connective tissue (Figs. 2 and 3). In some of the lymph nodes, large-celled hyperplasia with little tendency to caseation was the dominant feature. Acid-fasts were few.

In the ileum the changes were found in the Peyer patches, where larger or smaller granulomas with epithelioid cells could



Figure 2. Section of lymph node with caseation. Magnification: 12  $\times$ .  
Staining: van Gieson.  
(J. Müller, fot.)

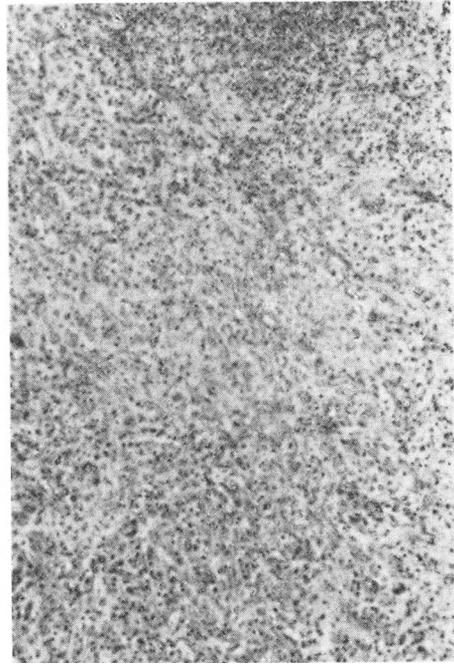


Figure 3. Same as Fig. 2. Infiltration with epithelioid and giant cells can be seen at the edge of the caseous tissue. Magnification: 30  $\times$ . Staining: van Gieson.  
(J. Müller, fot.)

be seen in the lymphoid tissue (Fig. 4). In one case caseation could be seen centrally in such a granuloma.

Pig no. 5 from Group 6 had infiltrations with epithelioid and giant cells in the ileic mucosa, with many intracellular acid-fast rods (Fig. 5). There was large-celled hyperplasia in the Peyer patches, and it appeared as if the cellular infiltrations had spread from the lymphoid tissue of these into the propria mucosae. The resulting histological picture was hardly distinguishable from that seen in cattle with paratuberculosis (Fig. 6). In one pig a small accumulation of epithelioid cells was found in the liver.

Histological changes were demonstrated in altogether 26 pigs, or 42.6 % (Table 2). In all of the 17 pigs with positive necropsy findings histological lesions were found in the mesenteric lymph nodes, and, in addition, five had changes in the ileum and one in the liver (Table 3). Of the 44 pigs without gross lesions, seven

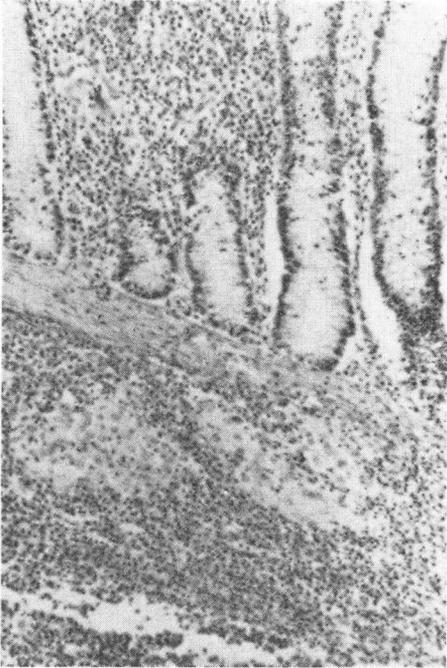


Figure 4. Section of ileum, Peyer patch with epithelioid-cell infiltration. Magnification: 30  $\times$ . Staining: van Gieson.  
(J. Müller, fot.)

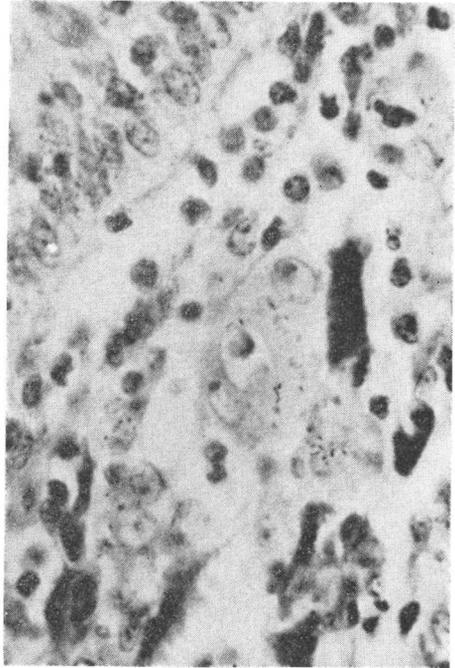


Figure 5. Section of ileum, epithelioid-cell infiltration in propria mucosae and many acid-fast rods in epithelioid cells. Magnification: 187.5  $\times$ . Staining: Ziehl-Neelsen.  
(J. Müller, fot.)

had histological changes in the mesenteric lymph nodes, one in the mesenteric lymph nodes and ileum, and two in the ileum (Table 4).

#### DISCUSSION

Oral infection experiments with *M. paratuberculosis* were carried out on 61 pigs, divided into seven groups, six of which were inoculated with culture material and one with infected bovine intestinal mucosa and mesenteric lymph nodes. Changes in the mesenteric lymph nodes of the type described by Ringdal (1963) were produced in 17 pigs in three groups. One of these groups had been inoculated with organ material, the other two with culture. The percentage of takes was 27.9 in relation to the total number of pigs in the experiment, and 42.9 in relation to the number of groups. In Group 1, five out of eight pigs were

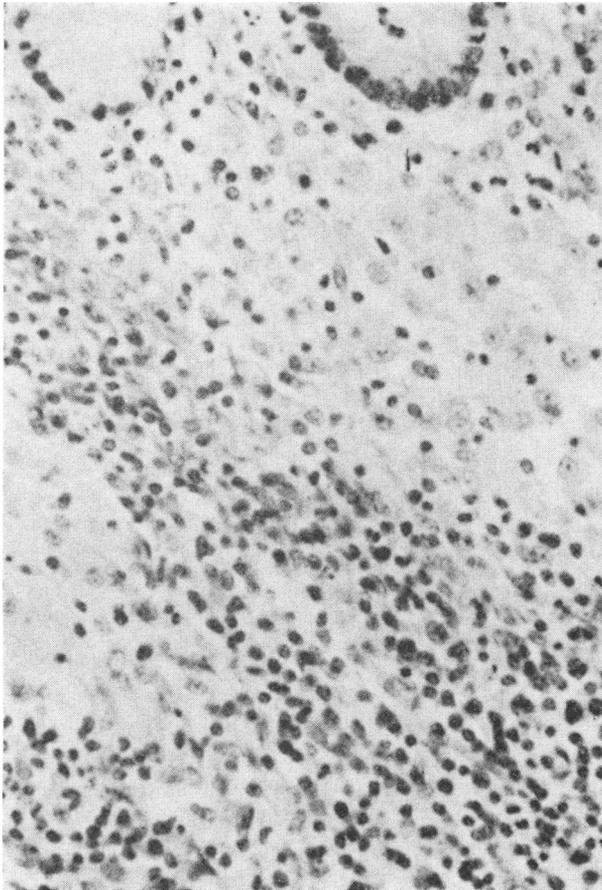


Figure 6. Section of ileum, epithelioid-cell infiltration in Peyer patch and propria mucosae. Magnification: 100  $\times$ . Staining: van Gieson.

(J. Müller, fot.)

positive (62.5 %), in Group 5 nine out of 11 (81.8 %), and in Group 6 three out of eight (37.5 %). Groups 1 and 3 were inoculated with the same strain ("Nina"), but while there were 62.5 % positive pigs in Group 1, there were none in Group 3. Both groups were given culture in the fodder from the time the pigs were 144 and 146 days old, and for a total of 39 and 40—54 days, respectively. The only difference between the two groups was that, between 36 and 57 days of age, the Group 1 pigs had been given three initial inoculations by stomach tube. The com-

pletely negative result in the one group is not readily explainable, though presumably the age resistance of the pigs is of great significance.

Groups 1 and 2 were inoculated by stomach tube at a time when the pigs were too small for trough-feeding. Two animals were selected from each group and slaughtered before the daily inoculations commenced. In none of these pigs were macroscopical changes demonstrated. For establishing infection it would appear to be necessary to administer daily doses of organisms during a protracted period, but the material is too small to permit a definite conclusion. Group 5 is an example of pigs inoculated at an age of 112 and 120 days with highly virulent material, viz. intestinal mucosa and mesenteric lymph nodes from a cow with paratuberculosis. The pigs were inoculated daily for 47 or 54 days, and the percentage of takes was 81.8. Groups 3 and 4 were the only two groups that were negative by all the methods of examination used, viz. necropsy, bacterioscopy, culture and histological examination. Since these two groups are rather like Group 5 as regards inoculation technique, age of pigs at the start of the experiment, and period of observation, the completely negative outcome is probably due to lack of pathogenicity of the strains used for inoculation.

A comparison of the suitability of the various methods for revealing the presence of *M. paratuberculosis* or changes caused by this organism shows (see Table 2) that macroscopical examination gave 27.9 %, bacterioscopy 31.1 %, culture 60.7 %, and histological examination 42.6 % positive results. It is not surprising that culture revealed about twice as many infected animals as macroscopical and bacterioscopical examination, since many animals may harbour organisms without showing pathological changes. This phenomenon is well-known also in paratuberculosis in cattle. It is more remarkable that 42.6 % of the pigs showed histological changes indicating infection with *M. paratuberculosis*. Had the period of observation been prolonged, it is possible that more pigs would have shown macroscopical changes.

In one case, histological examination revealed changes in the intestinal mucosa analogous to the cellular infiltrations found in cattle with paratuberculosis. The cellular infiltrations were found in a Peyer patch and had apparently from there invaded the propria mucosae (Fig. 6). Had this pig lived long enough, it is

not improbable that changes as those described by *Runnels* (1955) and *Bergman* (1913) might have developed.

*M. paratuberculosis* was isolated from the liver of one pig, and in the liver of another pig there was an accumulation of epithelioid cells which indicated infection with this organism. Organisms must have been present at some time or other in the blood stream, probably in the portal system.

The experiments have shown that pigs can be infected relatively easily with *M. paratuberculosis*. Since the macroscopical changes in the mesenteric lymph nodes cannot be distinguished from tuberculosis without cultural examination, it is strongly recommended that pigs should not be housed together with paratuberculous cows. Furthermore, the fact that a high percentage of takes (in these experiments 81.8 % in Group 5) can be obtained by inoculating pigs with highly virulent strains of *M. paratuberculosis* would seem to open a possibility of using pigs as test animals, for instance for evaluation of anti-paratuberculous drugs. The short observation period, in these experiments 55—62 days, and the very marked pathological changes in the mesenteric lymph nodes will offer great advantages.

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#### SUMMARY

Oral administration of *Mycobacterium paratuberculosis* to 61 pigs resulted in the development of caseous lesions of the mesenteric lymph nodes in 27.9 % of the animals. Positive results were found by bacterioscopy in 31.1 %, by culture in 60.7 % and by histological examination in 42.6 % of the animals.

Histological changes typical of infection with *M. paratuberculosis* were found in the ileum in eight pigs. These changes, which consisted of infiltrations with epithelioid cells, were usually limited to the Peyer patches, but in one case such infiltrations were also present in the propria mucosae, similarly as in paratuberculosis in cattle.

## ZUSAMMENFASSUNG

*Paratuberkulose beim Schwein.**Infektionsversuche mit peroraler Zufuhr von Paratuberkulosebakterien.*

Bei peroraler Zufuhr von Paratuberkulosebakterien an 61 Ferkel wurden bei 27,9 % der Ferkel paratuberkulöse Veränderungen als kaseöse Prozesse in den Mesenteriallymphknoten hervorgerufen. Bei einer mikroskopischen Untersuchung wurden 31,1 % positive, bei einer Züchtungsuntersuchung 60,7 % und bei einer histologischen Untersuchung 42,6 % positive gefunden.

Bei 8 Ferkeln wurden im Ileum histologische Veränderungen — hervorgerufen durch Paratuberkulosebakterien — festgestellt. Die Veränderungen zeigten sich als Epitheloidzelleninfiltrationen in den пейerschen Platten. Bei einem der Ferkel wurden Zelleninfiltrationen gefunden, die sich von den пейerschen Platten in Propria mucosa hinaus verbreitet hatten und hier Veränderungen hervorgerufen hatten, die der Paratuberkulose beim Rind ähnlich waren.

## SAMMENDRAG

*Paratuberkulose hos svin.**Infektionsforsøg ved peroral tilførsel af paratuberkelbakterier.*

Ved peroral tilførsel af paratuberkelbakterier til 61 grise er fremkaldt paratuberkuløse forandringer i form af forostninger i mesenteriallymfekirtlerne hos 27,9 % af grisene. Ved mikroskopisk undersøgelse fandtes 31,1 % positive, ved dyrkningsundersøgelse 60,7 % og ved histologisk undersøgelse 42,6 %.

Hos 8 grise er i ileum påvist histologiske forandringer fremkaldt af paratuberkelbakterier. Forandringerne viste sig som epitheloidcelleinfiltationer i Peyer-pletterne, dog fandtes hos een af disse grise celleinfiltationer, der fra Peyer-pletten bredte sig ud i propria mucosae og fremkaldte forandringer, der lignede paratuberkulose hos kvæg.

*(Received February 6, 1969).*