Acta vet. scand. 1974, *15*, 507—518.

From The State Veterinary Serum Laboratory, Copenhagen, Denmark.

MYCOPLASMA SUIPNEUMONIAE AND MYCOPLASMA FLOCCULARE IN COMPARATIVE PATHOGENICITY STUDIES

By

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FRIIS, N. F.: *Mycoplasma suipneumoniae and Mycoplasma flocculare in comparative pathogenicity studies.* Acta vet. scand. 1974, *15*, 507—518. — Three field strains of Mycoplasma suipneumoniae each inoculated into 3 gnotobiotic piglets produced macro- and microscopic lung lesions typical of enzootic pneumonia in 8 of the animals. Under similar conditions 3 strains of M. flocculare produced typical macroscopic lung lesions in just 1 out of 9 animals. It is therefore concluded that M. flocculare is not of primary etiologic importance in the porcine enzootic pneumonia complex.

The frequency of successful reisolation from nasal cavities, lungs, and other tissues indicated that the lungs are the sole natural habitat for M. suipneumoniae, while for M. flocculare lungs as well as nasal cavities should be regarded as the natural habitat.

None of the organisms apparently spread via the blood stream. M. flocculare, but not M. suipneumoniae, induced histologic alterations of the nasal mucosa.

Mycoplasma suipneumoniae; Mycoplasma flocculare; gnotobiotic piglets; porcine enzootic pneumonia; porcine rhinitis.

Mycoplasma suipneumoniae (M. suip.) was first demonstrated in 1965 by *Goodwin et al.* and *Maré & Switzer.* Its ability to induce macroscopic pneumonia in pigs was established by the same authors and has later on been confirmed (*Goodwin et al.* 1967, *Hodges et al.* 1969, *Huhn* 1971, *L'Ecuyer* 1969). The histologic lesions (*Whittlestone* 1972) may be characterised as a catarrhal-interstitial bronchopneumonia. By a number of investigators M. suip. has been recovered from pulmonary tissue of both naturally diseased and experimentally infected pigs. Seemingly, only *Goodwin* (1972) has recovered it from nasal cavities.

Mycoplasma flocculare (M. floc.), culturally and morphologically hardly distinguishable from M. suip., was first demonstrated in 1972 (*Friis, Meyling & Friis*). In transmission experiments with a single strain (*Friis* 1973) it produced no true macroscopic signs of pneumonia in pigs. On the other hand, slight interstitial changes in the lungs were noticed histologically. Minor histologic alterations were noted also in the nasal mucosa. M. floc. was recovered from lung and nasal mucosa.

The cited information may indicate that a difference exists between M. suip. and M. floc. in their primary pneumonogenic capability for swine and in their readiness to colonise the nasal mucosa. The present study was undertaken in order to bring some light on this problem.

MATERIAL AND METHODS

Experimental animals

Hysterotomy-produced, colostrum-deprived gnotobiotic piglets* housed 3 together in isolators (*Friis* 1971b) were used. Each animal was given 110 mg Fe^{+++} (Imferon) i.m. on the day of hysterotomy, and a few days later a culture of Lactobacillus was given in the trough. The animals were fed sterilised canned milk and infected with mycoplasmas by aerosol for 7 min. once at about 10 days old. Each strain was tested on 3 animals housed together. Rectal temperatures were recorded every second day. The animals were sacrificed in the 3rd to 5th weeks p.i. by bleeding from a brachial artery in Mebumal anesthesia.

Strains of mycoplasma

Three strains of M. suip. and 3 of M. floc. were used. They were all newly isolated from field material. All the strains of M. suip. and 1 of M. floc. (My811) originated from pneumonic tissue. The remaining strains of M. floc. were from nasal mucosa, 1 of them, Mp326, from a distinct case of atrophic rhinitis in a 3-month-old pig. The number of passages in broth varied from 5 to 9. The dilution of original tissue was at least 10^{12}. All the strains were cloned once on solid medium.

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* Kindly supplied by Dr. M. Mandrup, The Danish Meat Research Institute, Roskilde, Denmark.

Serological identification of mycoplasmas

For identification purposes, the growth inhibition (g.i.) and the metabolic inhibition (m.i.) tests (*Friis* 1971a) were used with rabbit hyperimmune antisera for the type strain (J*) of M. suip. and the type strain (Ms42**) of M. floc. In both tests, the strains of mycoplasma used for infection were indistinguishable from their respective type strain and different from the other type strain. The mycoplasmas reisolated from the experimental pigs were identified by g.i. only.

Necropsy

After inspection, material for cultivation was taken from 3 lung lobes, from the nasal cavity (ethmoturbinates), cerebrum, spleen, liver, right stifle joint, right hock joint, and from the peritoneal, pleural, and pericardial cavities. For histologic examination material was taken from lungs and nasal mucosa (naso- and maxilloturbinates) and stained with haemalum-eosin and after van Gieson.

Media and cultivation

Material was ground in a mortar and cultivated for mycoplasmas in 10-fold dilutions of broth to 10^{-8}. The medium, here referred to as FF II, has been described (*Friis* 1971c). Solid medium was prepared with Ionagar No. 2 (Oxoid) 0.8 % and supplemented with DEAE-Dextran (*Tauraso* 1967) for improvement of growth. The material was further examined for bacteria on ordinary blood-agar plates incubated aerobically. Material from localisations with macro- or microscopic evidence of disease was examined for virus in pig-kidney monolayers, 3 passages each of 1 week's duration being performed.

RESULTS

Clinical

Whether infected with M. suip. or M. floc., none of the animals displayed any overt signs of disease, i.e., neither coughing nor sneezing was observed. The rectal temperatures of the animals were found within normal limits for piglets. The highest value

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* and ** Type NCTC 10110 and NCTC 10143, respectively, Mycoplasma Reference Laboratory, Colindale, England.

noted was 40.1 C, which was recorded just once (Pig 191, 13 days p.i.).

Necropsy

Gross lesions (Diagram 1) were found in lungs, but nowhere else. They appeared as soft, reddish, greyish-reddish, or dark-reddish areas of consolidation. On incision no excessive amount of fluid came forth.

All 3 strains of M. suip. were found to induce gross lesions (Table 1). Of the 9 piglets inoculated only 1 (144) was without lesions, and this one had been killed already 15 days p.i. In 6 of the animals widespread lesions were noted.

In the experiments with M. floc. 2 of the 3 strains produced gross changes, each in 1 of 3 animals. In 1 of these (Pig 190 of Exp. V) the areas of consolidation were distinct though small, while in the other one (Pig 195 of Exp. VI) 2 hardly discernible areas of consolidation were found, located 1 on each tip of the cardiac lobes.

Histologic examination

All 3 strains of M. suip. produced similar microscopic alterations in the lungs (Fig. 2). Thus, there was emigration of histiocytic cells, large macrophages, and a few neutrophils to the bronchial and alveolar lumina. Scattered areas with an increased number of neutrophils were noted in a few of the animals. Macrophages were protruding into the alveolar lumina from the interalveolar septa. Distinct accumulations of lymphocytic and histiocytic cells were present as circular cuffings of the bronchi and vessels. Similar cells were often found in the interalveolar septa, which were then thickened, and sometimes also in the propria mucosae of the bronchi. Atelectasis and emphysema were also seen.

No histologic alterations of the nasal mucosa were found in any of the animals infected with M. suip., regardless of the presence of the organism in the nasal cavity.

The histologic lung lesions produced by 2 strains of M. floc. (My811 and Mp334, Exps. IV and VI) appeared. similar. They were mainly localised to the bronchi, especially the larger ones, and consisted of scattered proliferations of lymphocytic and histiocytic cells in the propria mucosae (Figs. 3 and 4). These

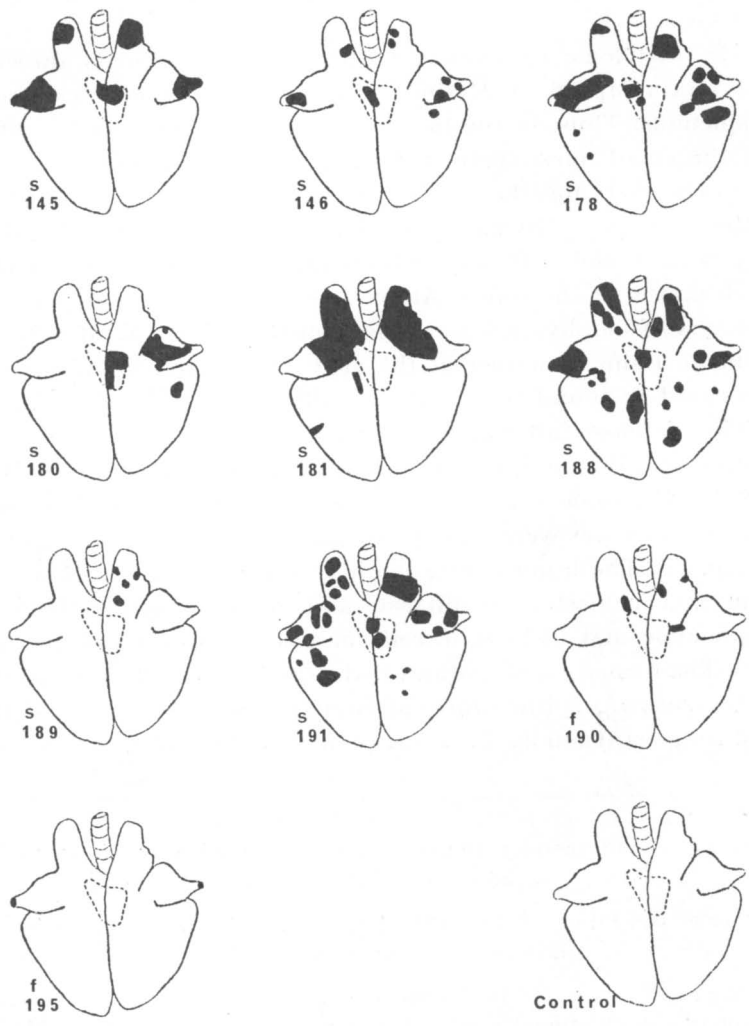

Diagram 1. Distribution of gross lesions in experimental piglets infected with M. suipneumoniae (s) and M. flocculare (f). For further details, see text.

proliferations might be diffuse or nodular. Sometimes the epithelium over the nodules was flattened, with some tendency to disruption. The macroscopic lesions noted in Pig 195 of Exp. VI were purely atelectatic, probably representing persisting fetal atelectasis.

The histologic alterations found in the 3 animals infected with strain Mp326 of M. floc. (Exp. V) were somewhat more conspicuous. Thus, in the lumina of many of the bronchi, often also the small ones, there were aggregates of lymphocytic and histiocytic cells together with some neutrophils. In some areas of the lung parenchyma (Figs. 5 and 6) a number of similar cells were found diffusely infiltrating the interalveolar septa, which appeared thickened. Atelectasis was a prominent feature, but signs of emphysema were also noted. In Pig 193 of this experiment a small number of the above-mentioned inflammatory cells could be found free in the alveoli.

The histologic alterations (Figs. 7 and 8) noted in the nasal cavities of all 9 animals infected with M. floc. were similar, though with some variation in intensity between the 3 strains. Thus, the changes were most pronounced in Exp. VI and slightest in Exp. IV. The lesions were mainly located in the propria mucosae and consisted of diffuse and nodular infiltrations of lymphocytic and histiocytic cells and sometimes a few neutrophils. The formation of nodules had usually resulted in flattening of the covering epithelium and complete or partial loss of the cilia, and occasionally in a complete disintegration of the epi-

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Figure 1. Pulmonary tissue of a non-infected gnotobiotic piglet, 28 days of age. × 265.

Figure 2. Lung of Pig 191, Exp. III, with pneumonia. Strain Mp270 of M. suipneumoniae. × 265.

Figures 3 and 4. Bronchus of Pig 196, Exp. VI, with proliferation of lympho-histiocytic cells in the propria mucosae. Strain Mp334 of M. flocculare. × 65 and 265, resp.

Figures 5 and 6. Lung of Pig 190, Exp. V, with bronchitis and interstitial proliferations, atelectasis and emphysema. Strain Mp326 of M. flocculare. × 65 and 265, resp.

Figures 7 and 8. Nasal mucosa of Pig 195, Exp. VI, with proliferation of lympho-histiocytic cells in the lamina propria. Strain Mp334 of M. flocculare. Note epithelial damage. × 65 and 265, resp.

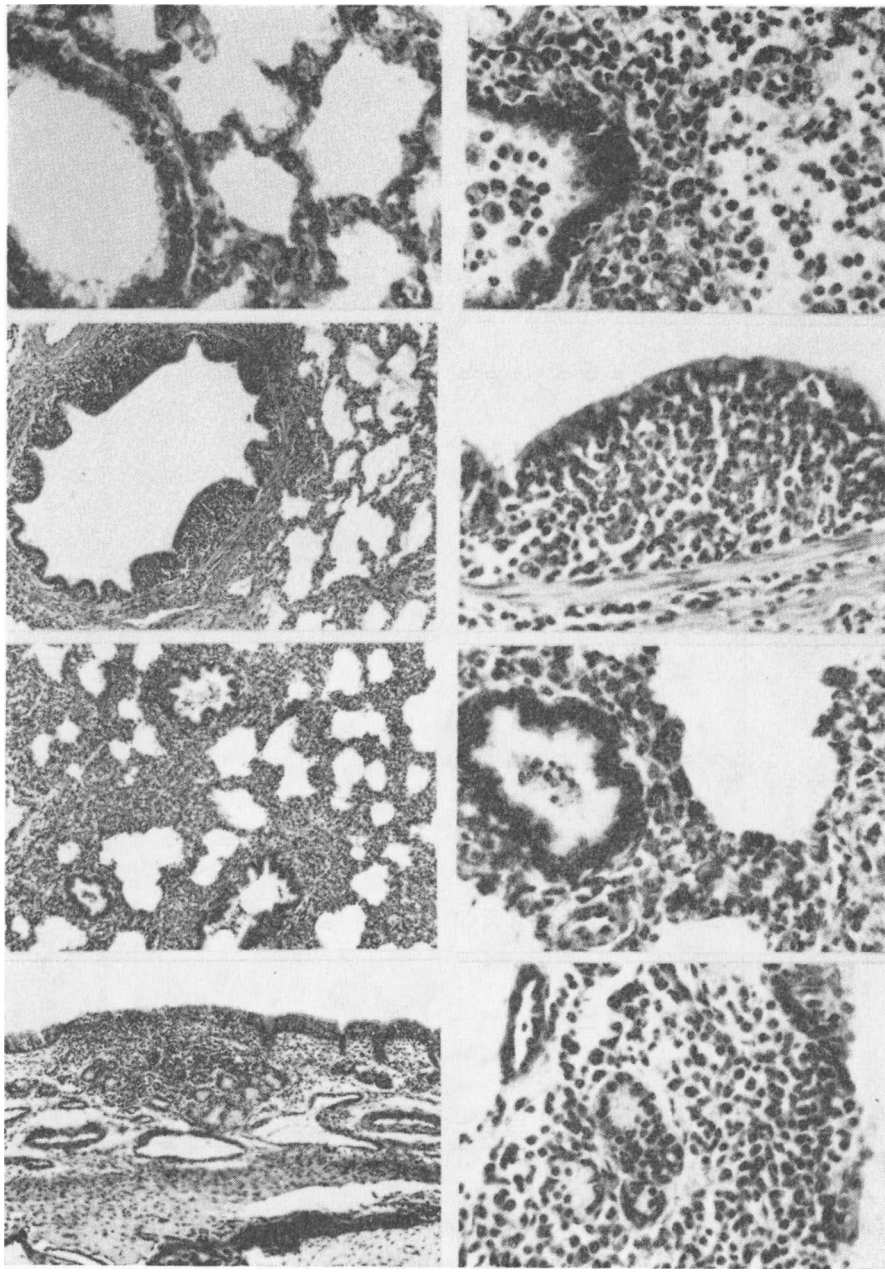

Table 1. Results of inoculation experiments with M. suipneumoniae and M. flocculare on gnotobiotic piglets.

Exp.	Strain	Pig	Infection days of age	Necropsy days p.i.	Lesions					Reisolation**				
					nasal cavity		lung		other sites	nasal cavity	lung			cerebrum
					g	h*	g	h	g	et	r c	l a	l d	
I	My817 M. suip.	144	9	15	0	0	0	+	0	0	8	6	7	0
		145		21	0	0	+	+	0	0	7	7	7	0
		146		27	0	0	+	+	0	0	8	5	5	0
II	Mp267 M. suip.	178	11	21	0	0	+	+	0	0	4	5	5	0
		180		23	0	0	+	+	0	0	7	6	6	0
		181		23	0	0	+	+	0	0	7	6	7	0
III	Mp270 M. suip.	188	8	23	0	0	+	+	0	5	7	6	7	0
		189		23	0	0	+	+	0	6	6	(ra:6)	8	2
		191		27	0	0	+	+	0	0	7	7	7	0
IV	My811 M. floc.	158	8	15	0	+	0	0	0	6	6	5	6	0
		160		22	0	+	0	+	0	7	6	6	7	0
		162		33	0	+	0	+	0	7	4	5	6	0
V	Mp326 M. floc.	190	8	24	0	+	+	+	0	7	(ra:5)	(lc:6)	7	0
		193		26	0	+	0	+	0	7	6	5	5	1
		194		26	0	+	0	+	0	6	5	5	5	0
VI	Mp334 M. floc.	192	8	25	0	+	0	+	0	6	5	4	4	0
		195		28	0	+	+	+	0	7	5	3	6	1
		196		28	0	+	0	+	0	7	7	1	5	2

g = gross, h = histologic, et = ethmoturbinates, r = right, l = left, c = cardiac lobe, a = apical lobe, d = diaphragmatic lobe.

* upper and lower turbinates. ** expressed as reciprocal of log. endpoint titer of mycoplasmas recovered from 10-fold dilutions of 10 % ground tissue suspensions.

Experiment IV has been reported on previously (*Friis* 1973).

thelium. In the animals of Exp. VI a few of the larger nodules were found to infiltrate the superficial part of the glandular layer. Apart herefrom this layer, and the osteoblastic layer as well, was unaffected. As far as the lower turbinates were concerned the lesions appeared to increase in severity outward along the turns of both folds.

Microbiologic examination

A few non-hemolytic streptococci were cultivated from the nasal mucosa of Pigs 180 and 181, both of Exp. II. Apart herefrom neither bacteria nor viruses were recovered. The reisolations of mycoplasmas are surveyed in Table 1. Their identity was proved by g.i. It appears that M. suip., and M. floc. as well, could always be found in the lungs, while in the nasal cavities only M. floc. was consistently present. Only 1 strain of M. suip. (Exp. III) seemed able to colonise the nasal cavity, and this only in 2 of the 3 animals. The brain tissue of a few animals contained mycoplasmas at low titers.

DISCUSSION

Three Danish field strains of M. suip. were introduced into gnotobiotic piglets by a "natural" route of infection. With each strain the experiment comprised 3 piglets. Macro- and microscopic lung lesions typical of enzootic pneumonia were found in all animals except 1 (144, Exp. I) in which only microscopic lesions had developed, and which had probably been killed too early after infection. This demonstration of a pneumonogenic capability in M. suip. confirms results obtained by other workers (e.g. *Hodges et al.* 1969, *Huhn* 1971).

In the experiments with 3 Danish field strains of M. floc., likewise introduced each into 3 gnotobiotic piglets, gross lesions of the lungs were found in just 2 of the 9 piglets. In both animals the lesions were small (Diagram 1). In Pig 195 of Exp. VI they were located right at the tips of the cardiac lobes, and were hardly visible. By histologic examination they were found to be of a purely atelectatic nature. In the other case (Pig 190 of Exp. V) the consolidated areas, though mostly atelectatic, showed indisputable signs of inflammation. Although minor histologic lesions were found in the lungs of 8 of the animals there can hardly be any doubt that the mycoplasmas belonging to the species M. floc-

culare are far less pneumonogenic than those of the species M. suipneumoniae. It other words, it seems unlikely that M. floc. should play a primary etiologic role in the enzootic pneumonia complex, such as is the case with M. suip.

While no gross lesions were noted in the nasal cavities of any of the animals, histologic alterations were consistently found in the nasal mucosa of pigs infected with M. floc. Seemingly, processes were going on as follows: Diffuse cellular infiltrations would appear in the lamina propria. By focal multiplication of the infiltrating cells nodules might be found, and the epithelium covering these nodules be damaged and finally burst, leaving a small ulcer. In an earlier experiment (*Friis* 1973) with 1 of the strains (My811, Exp. IV) performed with secondary SPF-pigs, such mucosal lesions could be found 2 months after infection. It would appear, therefore, that the lesions induced by M. floc. in the porcine nasal mucosa are apt to take a chronic course.

M. suip. was rarely present in the nasal cavity, and in no case was it found to have induced histologic alterations.

The consistency with which M. suip. was present in pulmonary tissue, held together with its irregular occurrence in the nasal cavities and almost complete absence elsewhere, lends support to the view that the lungs are the sole natural habitat of M. suip. As regards M. floc., which was always present in both the lungs and nasal cavities, it appears that the whole of the respiratory tract should be regarded as its natural habitat.

Apart from the brain, M. suip. and M. floc. do not seem to occur in tissues outside the respiratory tract, and, as has also been found with M. hyorhinis and M. hyosynoviae (author's unpublished data), their presence in the brain seems to be dependent on their presence in the nasal cavity (cf. Table 1). Thus, the invasion of the brain being likely to take place via the olfactory nerves, M. suip. and M. floc. seem little apt to metastasise hematogenously.

At present it is difficult to evaluate the importance of the histologic lesions observed in the porcine nasal cavity after infection with M. floc. However, it is felt that M. floc. should not be disregarded in work on the etiology of chronic infectious rhinitis syndromes.

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SAMMENDRAG

Mykoplasma suipneumoniae og Mykoplasma flocculare i sammenlignende patogenitetsstudier.

Tre nyisolerede stammer af Mykoplasma suipneumoniae hver podet på 3 gnotobiotiske grise fremkaldte i 8 af dyrene sådanne makro- og mikroskopiske lungeforandringer, som er typiske for enzootisk

pneumoni. I tilsvarende forsøg resulterede podning med 3 stammer af M. flocculare kun i opståen af lette, typiske makroskopiske lungeforandringer hos een af 9 grise. Derfor konkluderes, at M. flocculare er ikke af primær etiologisk betydning ved svinets enzootiske pneumoni.

Hyppigheden, med hvilken reisolationsforsøg lykkedes fra næsehuler, lunger samt andre lokaliteter, antydede, at lungerne er det eneste naturlige habitat for M. suipneumoniae; i modsætning til M. flocculare, for hvilken både lunger og næsehule må anses for at være det naturlige habitat.

Ingen af de 2 mykoplasma-arter syntes at spredes via blodbanerne. M. flocculare var i modsætning til M. suipneumoniae i stand til at fremkalde mikroskopiske forandringer i næsehulens slimhinde.

(Received March 8, 1974).

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