

***Mycoplasma Hyorhina* in the Etiology of Serositis among Piglets**

By *N. F. Friis* and *A. A. Feenstra*

National Veterinary Laboratory, Copenhagen, Denmark.

Friis, N.F. and A. A. Feenstra: *Mycoplasma hyorhina* in the etiology of serositis among piglets. Acta vet. scand. 1994, 35, 93-98. – In a study on the involvement of *Mycoplasma hyorhina* in serositis of piglets, 26 routine diagnostic animals, 3-7 weeks old, with distinct serofibrinous lesions in the pericardial, pleural and peritoneal cavities were examined. *M. hyorhina* was isolated in 9 cases, non-haemolytic *Escherichia coli* in another 9 cases and in 4 cases both species were found. Neither of the microorganisms were found in the remaining 4 cases.

The presence of *M. hyorhina* in the serous cavities in the absence of non-haemolytic *E. coli* was always accompanied by a diagnosis of other disease conditions, mainly of the respiratory tract. In the cases infected with non-haemolytic *E. coli* complicating problems were absent.

The pathogenicity of *M. hyorhina* was further studied by inoculation of 2 young pigs in which the typical serofibrinous lesions of the serous cavities were produced. It therefore appears that *M. hyorhina* can be regarded as a cause of polyserositis in piglets; under field conditions, however, the synergistic presence of other debilitating syndromes appears necessary for its haematogenous spread from the respiratory tract to the serous cavities.

***Escherichia coli*; joints; mycoplasma hyosynoviae; pericardial; pleura; peritoneal; serosa; swine.**

Introduction

Mycoplasma hyorhina is a very frequently occurring microorganism in swine. Piglets are often infected shortly after birth and the normal habitat is the mucous membranes of the upper respiratory tract and the tonsils. A descending spread to the lungs occurs easily and *M. hyorhina* is regarded as part of the etiologic complex in pneumonias among piglets (Friis 1971, 1974). Sometimes a haematogenous spread takes place with secondary localizations in the serous cavities of the pericardium, the pleura, the peritoneum and in joints. Pronounced pathologic changes may

result and are usually characterized as serofibrinous lesions. They may be found in clinical material (Bannerman & Nicolet 1971, Carter 1954, Carter & Schröder 1955, King 1968) and in experimentally inoculated animals as well (Ennis et al. 1971, Friis 1974, Poland et al. 1971, Roberts et al. 1963).

The importance of *M. hyorhina* as a primary etiological agent in polyserositis and arthritis, alone and in interplay with bacteria, mainly *Escherichia coli*, still remains unsolved. Therefore, an investigation has been performed with the aim of studying the occurrence of *M. hyorhina* in serositis piglets sub-

mitted to this Institute for *post mortem* examination, and to correlate the findings with the bacteriological results.

Materials and methods

Design of the experiment

Piglets 3-7 weeks old were obtained from the routine diagnostic material sent to the National Veterinary Laboratory during 4 years, 1989 through 1992. 40 piglets with various degrees of inflammatory-like changes of the serous cavities were examined. For control, 8 piglets with other diseases were included. The material originated from 44 herds.

The piglets were examined for *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, and bacteria. *Mycoplasma hyopneumoniae* and *Mycoplasma flocculare* were disregarded as they need a cultivation time of 3 weeks. Anamnestic information was most often scarce. About 1/2 of the animals had died and another 1/2 was killed before mailing; no history for the remaining pigs was given. Information about antibiotic treatment was usually missing. The age was exactly stated in some cases; otherwise it was estimated at autopsy.

The pathologic changes of the serous cavities were grouped according to intensity of inflammation as distinct, mild or absent. "Distinct" was defined as evident formation of fibrinous material irrespective of the presence of fluid, in at least 1 of the serous cavities. Among the group of distinct changes, comprising 26 cases, 25 showed acute or subacute lesions of serofibrinous/fibrinous nature. The one animal, 5 weeks old, showed lesions dominated by chronic fibrous changes. Among 14 animals of the group "mild", 6 cases were later diagnosed as *Mulberry Heart* disease. The remaining 8 animals had serofibrinous lesions. The group with normal serous cavities suffered from various diseases of the alimentary tract, joints or skin.

Examination of piglets

Pathologic changes were noted during the autopsy and swabs were carefully collected from the peritoneal, pleural and pericardial cavities, and from the surface of the brain. Aseptic samples were collected from 3 joints, usually right tarsal, stifle and elbow or visually enlarged joints. Likewise, samples were collected from lung, preferably pneumonic areas, from nasal cavity and tonsil. Tonsil and lung were immersed in boiling water for 5-10 sec and ground, the joints were washed in 96% ethanol, opened and swabbed.

Isolation and identification of mycoplasmas and bacteria

The samples were examined for *M. hyorhinis*, *M. hyosynoviae* and bacteria. *M. hyorhinis* was cultivated in a special medium (Friis *et al.* 1991) developed for *M. hyopneumoniae*. In the case of heavily contaminated cultures isolation was reattempted after addition of cycloserine, vancomycin and nalidixic acid to the medium. *M. hyosynoviae* was cultivated in a modified Hayflick-medium enriched with arginine and mucin and made selective against *M. hyorhinis* with cycloserine and Tween 20 (Friis *et al.* 1991). The primary cultures were kept for 1 week. The disc growth inhibition test was used for identification.

Examination for bacteria was performed by inoculation of the samples onto 5% calf blood agar (Colombia blood agar base, Oxoid) in 3 different dilutions and onto Drigalski agar (lactose-sucrose-bromthymol blue). Aerobic incubation at 37°C was used. The plates were read after 24 and 48 h incubation. The bacteriological diagnosis was based on standard laboratory identification procedures.

Experimental infection

Three strains of *M. hyorhinis* isolated from joints or pericardium of 3 piglets were filtered

Table 1. Isolation of *Mycoplasma hyorhinis* and non-haemolytic *Escherichia coli* from 40 routine autopsy cases of serositis-like changes in piglets together with 8 controls from a total of 44 herds.

Serositis	Number of animals	M hyorhinis without non-haem E coli	M hyorhinis together with non-haem E coli	Non-haem E coli without M hyorhinis	Neither M hyorhinis nor non-haem E coli
distinct	26	9* (4.8)	4 (4.5)	9 (3.6)	4 (4.3)
mild	14	1 (6.0)	0	4 (4.5)	9 (3.9)
absent	8	0	0	1 (5.0)	7 (5.3)

non-haem. = non-haemolytic

* number of piglets; () average age in weeks.

through 0.45 μm membrane and cloned once from solid medium. A pool of the 3 strains was inoculated intraperitoneally and intranasally (spray and dropwise) into 2 SPF pigs, 3 months old. 15 ml of a suspension containing 10^9 ccu of mycoplasma in Hanks' balanced salt solution was inoculated ip into each pig. 1 ml of culture, approx. 10^8 to 10^9 ccu was applied dropwise into each nostril of the pigs which were also aerosolized for 5 min. Autopsy with reisolation attempts was done 1 month later.

Results

Forty piglets with pathological changes in the serous cavities were examined microbiologically. Among the 26 piglets (Table 1) with distinct serositis *M. hyorhinis* was found in 9. Non-haemolytic *E. coli* was found in another 9 cases, while 4 cases contained both microorganisms and 4 contained neither of them.

Of these 4 cases containing neither *M. hyorhinis* nor non-haemolytic *E. coli* together with 9 like animals of group "mild", most were found sterile (not in table) but *Haemophilus parasuis*, *Streptococcus suis* and less significant findings were also noted.

In the 13 animals with *M. hyorhinis* present in distinct serositic lesions, these changes were found in all the pericardial and pleural cavities and in 10 of the peritoneal cavities; *M.*

hyorhinis was isolated in 11, 13 and 8 of the locations, respectively. Titers $\geq 10^6$ were often noted. A calculation of the mean age showed that animals with *M. hyorhinis* in serositic lesions were 1 week older than animals with non-haemolytic *E. coli*; however, the difference is uncertain because the age had to be estimated in some cases.

The infections with *M. hyorhinis* and *M. hyosynoviae* at various locations of the animals are described in Table 2. It appears that 8 of the 13 animals infected with *M. hyorhinis* in the serous cavities also showed joint infections. Furthermore, the respiratory tract was found infected with this mycoplasma in 40 of the 48 examined animals. The tonsils were infected in 19 and the brain in 15 cases. *M. hyosynoviae* was found only in tonsils.

Among the 9 piglets with *M. hyorhinis* infection but without non-haemolytic *E. coli* in the serous cavities, lesions of the respiratory tract, mainly pneumonia were regularly noted. All except 1 were found infected with additional pathogenic bacteria: *Actinobacillus pleuropneumoniae* (2 cases), *Streptococcus suis* (2 cases), *Pasteurella multocida* (2 cases), *Streptococcus* and *Pasteurella* (1 case), *Bordetella bronchiseptica* (1 case). These bacteria were found in very low numbers in the serous cavities in 3 animals (2 *Streptococcus*, 1 *Actinobacillus*), thus *M. hyorhinis* was present at

Table 2. Isolation of mycoplasmas from various locations in piglets in relation to the presence/absence of *Mycoplasma hyorhinis* in serous cavities.

	Number of animals	M. hyorhinis					M. hyosynoviae	
		Total	Serosa	Joint	Resp tract	Tonsil	Brain	Tonsil
Serositis, distinct								
M. hyorhinis found in serous cavities without non-haem. E. coli	9	9	9	5	9	4	5	2
M. hyorhinis found in serous cavities together with non-haem. E. coli	4	4	4	3	4	1	1	1
M. hyorhinis found in serous cavities, total	13	13	13	8	13	5	6	3
Non-haem. E. coli found in serous cavities without M. hyorhinis								
Neither M. hyorhinis nor non-haem. E. coli found in serous cavities	4	4	0	0	4	2	1	1
M. hyorhinis not found in serous cavities, total	13	10	0	0	10	5	5	1
Serositis, mild	14	11	1	0	10	6	2	1
Serositis, absent	8	7	0	0	7	3	2	0

non-haem. = non-haemolytic; resp. tract = respiratory tract.

this site in totally pure culture in 6 animals. The 9 piglets represented 9 herds of which most had anamnestic information on different clinical problems, such as pneumonia, diarrhoea, unthriftiness, skin itch, impetigo and influenza. Among the 9 piglets with pure culture of non-haemolytic *E. coli* in the serous cavities, the bacterium could be isolated from almost any site examined, but no other infections or clinical evidence were found.

Experimental infection

The inoculated pigs showed distinct serofibrinous and fibrinous lesions in the cavities of the pericardium, pleura and peritoneum at autopsy. *M. hyorhinis* was recovered from the pericardial samples of both animals and from the pleural and peritoneal cavities of 1 animal.

The titres varied from 10^3 to 10^5 . Concomitant bacteria could not be isolated. The joints showed no obvious enlargement.

Discussion

In a microbiological study of polyserositis in 3-7 weeks old piglets, 26 cases with distinct serofibrinous lesions of the pericardial, pleural and peritoneal cavities were examined and 50% (13 animals) were found infected at these sites with *M. hyorhinis*; 15% (4 animals) of these cases harboured non-haemolytic *E. coli* which was also found in another 35% (9 animals). Thus, at least 1 of the 2 microorganisms could be isolated from 85% of the material. From these results it can be concluded that both microorganisms may be involved in the etiology of polyserositis of pig-

lets. As the 2 organisms appear independently of each other in most cases, they probably represent 2 different disease entities. Among 14 animals with mild changes of the serous cavities only 1 harboured *M. hyorhinis* at these sites.

In the piglets infected in serous cavities with *M. hyorhinis*, lesions of the respiratory tract were usually evident. As various other porcine pathogens were also regularly isolated, it appears likely that the haematogenous spread of *M. hyorhinis* accompanied by development of serositis to some degree depends on the presence of such stressing conditions. On the contrary, the 9 distinct serositic cases caused by non-haemolytic *E. coli* were all without concomitant syndromes or agents. As 13 out of 14 cases of *M. hyorhinis* in serous lesions were evaluated as "distinct", this infection seems apt to result in heavy pathologic changes. The presence of *M. hyorhinis* in fibrinous lesions of the serous cavities is a well known phenomenon, thus *Bannerman & Nicolet* (1971) isolated it from 27 (29%) of 93 cases of pericarditis.

The experimental reproduction of serositis in pigs inoculated with *M. hyorhinis* also suggests that this mycoplasma has a pathogenic capability. A number of other workers has likewise been able to induce serositis. Thus *Poland et al.* (1971) induced serositis in 4 of 9 gnotobiotic piglets and arthritis was found in 7 of them. In an experiment with 24 piglets inoculated intraperitoneally, *Roberts et al.* (1963) produced serositis in most animals killed after 6-10 and 15 days. However, pericarditis was found in only 2 of the animals.

It seems reasonable to conclude that *M. hyorhinis* has been etiologically responsible for about one third of the examined 26 clinical cases of distinct serositis in piglets. Reduced resistance resulting from other pathologic conditions appears to be a necessary trigger

mechanism for the haematogenous spread accompanied by development of lesions in the serous cavities and joints.

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Sammendrag

Den ætiologiske betydning af Mycoplasma hyorhinis ved serositis hos smågrise.

Ved en undersøgelse over betydningen af *M. hyorhinis* for serositis hos smågrise undersøgte 26 dyr, 3-7 uger gamle, med tydelige serofibrinøse betændelsesforandringer i de serøse kropshuler. *M. hyorhinis*

blev påvist i 9 tilfælde uden samtidig forekomst af non-hæmolytiske *Escherichia coli*. I 4 tilfælde fandtes *M. hyorhinis* sammen med denne bakterie, der tillige blev fundet som monoinfektion hos yderligere 9 dyr. Hos 4 dyr fandtes ingen af de 2 mikroorganismer.

Tilstedeværelsen af *M. hyorhinis* i de serøse kropshuler uden non-hæmolytiske *E. coli* var altid ledsaget af andre sygdomme, hovedsageligt i luftvejene. Ved infektioner med non-hæmolytiske *E. coli* var ledsagesyndromer ikke til stede.

Patogeniteten af *M. hyorhinis* blev yderligere studeret ved podning af 2 unge grise, hos hvilke typiske serofibrinøse læsioner i de serøse huler kunne reproducere.

Det kan derfor konkluderes, at *M. hyorhinis* må anses for at være ansvarlig for visse tilfælde af serositis hos smågrise. Imidlertid synes tilstedeværelse af andre resistensnedsættende sygdomsforhold nødvendig for den hæmatogene spredning af *M. hyorhinis* ledsaget af sekundær lokalisering i de serøse huler.

(Received July 14, 1993; accepted December 16, 1993).

Reprints may be requested from: N.F. Friis, National Veterinary Laboratory, Bulowsvej 27, DK-1790 Copenhagen V, Denmark.