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Autor: Bannerman, Elizabeth S.N. / Nicolet, J.

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Institute of Veterinary Bacteriology, University of Berne

Isolation and Identification of Porcine Mycoplasma in Switzerland

Elizabeth S.N. Bannerman and J. Nicolet

There have been extensive studies on the occurrence of mycoplasmas in pigs and their aetiological significance [10, 25, 26]. So far 5 porcine Mycoplasma species associated with diseases in swine have been described. *M. hyopneumoniae* and *M. suipneumoniae* are considered today to be the causative agents of enzootic pneumonia in pigs. The 2 organisms are now known to be serologically identical [8]. M. hyorhinis, a frequent inhabitant of the nasal cavity (25) is often found in Switzerland [2]. Its pathogenic role seems to be limited to a polyserositis-arthritis syndrome in young pigs (up to 12 weeks). The *SEP-Agent* [1] which was at first thought to cause enzootic pneumonia is now known to be identical to M. hyorhinis [4]. Acholeplasma granularum (syn. Mycoplasma granularum [6]) which is a common inhabitant of the nasal cavity is known to cause arthritis of the stifle joints in older pigs [26]. A. granularum arthritis is not accompanied by polyserositis. Another mycoplasma associated with swine arthritis is M. hyoarthrinosa [17]. M. hyogenitalium, isolated from the uterus mucous membrane is believed to be the causative agent of a mastitis-metritis syndrome [18].

In recent years, the identification of Acholeplasma laidlawii (syn. Mycoplasma laidlawii [6]) and avian mycoplasmas like M. iners and M. gallinarum [28] isolated from swine, has revealed the presence of other mycoplasmas in pigs. This made it increasingly necessary to perform a more accurate differentiation in the diagnosis of porcine mycoplasmosis.

With these mycoplasmas and their role as possible pathogens in mind, we decided to examine pathological material presenting known characteristics of the above mentioned diseases. By that means we tried to detect the incidence of mycoplasma infection in pigs in Switzerland, to differentiate the strains involved and as far as possible to discuss their pathogenic role.

Our work was facilitated by the use of a PPLO medium which allows the growth of both M. hyorhinis and M. hyopneumoniae. However, the examination was carried out principally on pigs supplied by the Pig Health Service, among whom enzootic pneumonia is not a very frequent occurrence. This study is therefore not fully representative of the general picture of swine mycoplasmosis in Switzerland, particularly with regard to enzootic pneumonia.

We included in our study the type strains of known mycoplasmas of porcine, avian, ovine, caprine and bovine origin, and we established a serological reference system in order to classify strains which were not of porcine origin.

Materials and Methods

I. Strains

Strain	Species name	Strain designation	Source
Porcine	M. hyopneumoniae	EP 29–37	L'Ecuyer, Canada
	M. suipneumoniae	NCTC 10110	Andrews, London
	M. hyorhinis	F 44	Bakos, Sweden
		SEP 200	Bakos, Sweden
	A. granularum	S 63	Bakos, Sweden
Avian	M. gallisepticum	S 6	Adler, Davis
		X 95-cl-la	Freundt, Aarhus
	M. gallinarum	PG 16	Freundt, Aarhus
	M. iners	PG 30	Freundt, Aarhus
	M. anatis	1340	Freundt, Aarhus
	M. synoviae	WVU 1853	Freundt, Aarhus
Ovine	M. agalactiae	PG 2	Freundt, Aarhus
		M 99	Cottew, Australia
	Brack	2833	Freundt, Aarhus
Caprine	M. mycoides var. capri	PG 3	Leach, London
Bovine	M. mycoides var mycoides	PG 1	Leach, London
	M. agalactiae var bovis	Donetta	Leach, London
	M. bovigenitalium	PG 11	Leach, London
	A. laidlawii	PG 8	Leach, London
	M. bovirhinis	5 M 331	Leach, London
	unnamed	PG 49	Leach, London
	unnamed	PG 50	Leach, London
	unnamed	D 12	Leach, London
	M. arginini	D 1365/68	Nicolet, Berne

II. Medium

The culture medium used for the isolation and propagation of the mycoplasmas was a modification of Olson's biphasic medium with the following composition:

A. Broth

PPLO broth w/o crystal violet (Difco 0554–01)	12 g in 500 ml
Dextrose	1%
Phenol red in aquaeous solution	0.0025%
Adjust to pH 8.0 and autoclave at 1.5 at. for 15 minutes.	
Cool and add the following:	
Yeast extract[1]	2.5%
Horse or pig serum	20%
Thallium acetate	0.25%
Penicillin	1000 I.U./ml
DNA	0.005%

[1] *Yeast extract:* 250 g Fleischmann's active dry yeast for bakers (Standard Brands Inc., New York, USA) in 1 liter distilled water is brought to the boil and then filtered through a Buchner funnel. The pH is adjusted to 8.0. The extract is then sterilized by Seitz filtration. 50 ml aliquots of the sterile yeast extracts are stored at – 20 °C till required.

B. Agar

Same as for the broth but with PPLO-Agar (Difco 0412–01) and without thallium acetate and penicillin.

Tubing: 10 cc size tubes are filled with 2.5 ml agar (B), allowed to set and then 2.5 ml broth (A) added.

III. Methods of Isolation

1. *Pericarditis*

0.5 ml of the pericardial fluid was taken sterile and inoculated into the biphasic medium. To isolate any bacteria that might be present, a serum thioglycolate medium and blood agar and chocolate agar plates were inoculated. The blood agar plate was streaked with a strain of Staphylococcus albus to enable the growth of haemophilic bacteria.

2. *Arthritis*

The joints were opened sterile and the exudate was taken with a sterile Pasteur pipette. In cases where the joints were fibrosed, strands of fibrine were directly inoculated into the medium. The exudate or the fibrine was treated as above.

3. *Pneumonia*

2–6 g tissue from the apical lobe was cut, chopped and put in a centrifuging glass. 10 ml phosphate buffered saline (PBS) pH 7.5 was added to 6 g tissue. This mixture was homogenized with an Ultraturrax (Ultraturrax-Polytron, Kinematisches Hochfrequenzgerät, Typ PT 20 manufactured by Kinematica GmbH, Luzern, Switzerland) under cooling in an ice bath. To ensure that the mixture remained cool the Ultraturrax was switched on and off at 20-seconds intervals. The homogenized mixture was made up to a 20% suspension with more PBS and then centrifuged at 1800 rpm for 10 minutes. 0.5 ml of the supernatant was inoculated into a biphasic medium. Blood agar plate + Staphylococcus, chocolate agar plate and McConkey + mannitol for the propagation of Bordetella [27] were inoculated.

4. *Mastitis-metritis syndrome*

Udder: a 20% suspension was prepared. Blood agar and bromthymol blue lactose agar plates as well as a biphasic medium were inoculated.

Uterus: The biphasic medium was inoculated with a sterile spatula. Blood agar plate and brothymol blue lactose plate were also inoculated.

All the cultures were incubated aerobically at 37°C. After 3–4 days of incubation, the biphasic medium cultures were plated on PPLO agar and 0.5 ml passed into a second biphasic medium. The passaging was carried out 3 times at 3 day intervals, and if the medium was still not turbid a final plating on agar was oned to confirm the absence of mycoplasma in the culture. The negative tubes were incubated for another 3 weeks and then checked for growth. Positive cultures were frozen at – 20°C in Kahn tubes.

The PPLO agar plate cultures were hermetically sealed with Scotch tape and incubated at 37°C.

The plates for the routine bacteriology were also incubated aerobically at 37°C for 3 days with daily checks.

IV. Microscopy

Impression smears of the pneumonic lungs were fixed in methanol and stained in a 4% solution of Giemsa-Fluka (Buchs SG) in phosphate buffer, pH 7.5 for 2 hours [13]. The stained smears were then rinsed in distilled water and rapidly dipped in acetone to clear the slides of any stained deposit and non-specific background.

V. Biochemical Test

Reactions in glucose, trehalose, saccharose, lactose und mannitol were tested. Each medium consisted of:

Bacto phenol red broth (Difco 0092–02)	1.6 g in 100 ml distilled water
Sugar	1%
PPLO serum fraction (Difco 0441–63)	2%
Thallium acetate	1:1000
Penicillin	2000 I.U./ml

VI. Serology

a) Preparation of antigens for the gel diffusion test

The isolated mycoplasma strains which had been stored at – 20° C were subcultured in the biphasic medium till dense outgrowth and then it was passaged into a 25 ml PPLO broth from which penicillin and thallium acetate were omitted, and normal rabbit was substituted for horse or pig serum. This culture was incubated on a shaker at 37° C. A dense outgrowth was usually obtained after 3 days. The culture was then centrifuged for 20 minutes at 15.000 rpm and 4° C. The supernatant was discarded and the sediment washed 3 times in PBS, pH 7.5. The washed sediment was resuspended in 2 ml distilled water and the suspension frozen and thawed 10 times in solid carbon dioxide + ethanol before use as antigen. The antigen stock was kept at – 20° C. All reference strains were cultured in 300 ml broth containing rabbit serum. After washing, the sediments were resuspended in 5 ml distilled water and used as antigens after they had been sonicated at maximum power for 1 minute with a Branson Sonifier, Model S 110 (Branson Instruments Inc., Stamford, Connecticut, USA).

b) Preparation of antisera

Antisera were prepared from all the reference strains used in this study. The strains were grown in 300 ml broth (rabbit serum), sedimented and washed as described above. The washed sediments were resuspended in 3 ml PBS, pH 7.5 and then emulsified with equal amounts of Freund's complete adjuvant. The technique for the immunization of the rabbits was that of Morton [19]. 0.1 ml of the antigen-adjuvant mixture was injected into each foot pad and 0.7 ml of it was injected intra-musculary into each leg. After 3 weeks the rabbits were given a series of intravenous injections (2×1 ml, 2×2 ml, 4 ml and 6 ml) every other day with antigens without adjuvant. The rabbits were bled 1 week after the last injection. The serum was sterilized by filtration and stored at – 20° C.

c) Agar gel precipitation (Ouchterlony)

The micro modification method of Wadsworth [30] was used. Tests were performed on glass slides with 0.6 mm thick layer of agar gel consisting of 1% w/v of Noble Agar Difco in 0.01 ml PBS pH 7.0. A 6 mm thick plastic template with 7 wells was used. The wells were 3 mm in diameter, with a distance of 2.5 mm separating them from one another. After filling the wells with the reagents, the plates were kept in a humidified chamber at room temperature for 48 hours. The precipitation patterns were recorded under illumination.

d) Growth Inhibition Test

The method of Clyde [3] with filter paper discs soaked in antiserum was used. The diameter of inhibition was recorded after 48 to 72 hours.

e) Metabolic Inhibition Test

The Method of Taylor-Robinson (29) was used.

Results

I. Isolation

A total of 206 specimens from pigs of different ages and body weights were examined. 10 of these specimens were clinically healthy and were examined as control for the pneumonia cases. Frequency of isolation fluctuated from organ to organ as shown in Table 1. Bacteria isolated from the specimens and their frequency of isolation together with mycoplasmas is summarized in Table 2.

Table 1 Isolation of mycoplasmas

Organ or specimen	Number examined	Number isolated (%)	Negative cultures (%)	Contaminated cultures (%)
Lungs	60	40 (66.7)	6 (10)	14 (23.3)
Pericardial fluids	93	27 (29)	29 (31.2)	37 (39.9)
Synovial fluids	26	9 (34.6)	17 (65.4)	0
Vagina	6	1	2	3
Udder	5	1	1	3
Uterus	4	1	0	3
Milk	2	1	1	0
Total Number with clinical changes	196	80 (40.8)	56 (28.6)	60 (30.6)
Control lungs	10	2	8	0
Total	206	82 (39.8)	64 (31.1)	60 (29.1)

Table 2 Bacteria isolated with M. hyorhinis

	Lung	Pericardial fluid	Synovial fluid
H. parahaemolyticus	9	4	0
H. parasuis	4	0	0
H. suis	1	0	0
Past. multocida	8[1]	3	0
Bord. bronchiseptica	1	0	0
E. coli	5	3	0
alpha Sc.	4[2]	3	0
Aeromonas sp.	1	0	0
Ery. insidiosa	0	1	1
Total	33	14	1

[1] A. granularum and M. hyorhinis found in one case.
[2] A. granularum found in one case.

II. Serological Identification

1. *Agar gel precipitation test*

a) Reference System

In preliminary tests unabsorbed of the porcine strains were tested against the different constituents of medium used for the cultures. There

were no reactions between the sera and the media. Tests were also carried out with porcine, ovine, avian, caprine, and bovine antisera against antigens of the porcine strains. The results are summarized in Table 3.

b) Diagnostic Strains

82 antigens were tested against antisera of the porcine strains. Antiserum to A. granularum absorbed with PG 8 and D 1365/68 as well as the unabsorbed serum were used. 71 of the isolates gave precipitation lines with both F44 and SEP200; 6 with unabsorbed A. granularum antiserum but 2 of these failed to react with the absorbed serum. The 2 isolates, one from vagina and the other from the uterus finally gave precipitation lines with Acholeplasma laidlawii (PG 8). One isolate which exhibited 2 morphologically different colony cultures reacted with both M. hyorhinis and A. granularum.

Isolate S 614 (synovial fluid did not precipitate any of the porcine, caprine, avian, ovine and bovine strain antisera.

Table 3 Ouchterlony – Reference System

Unabsorbed antiserum against	M. hyorhinis F 44	M. hyorhinis SEP 200	A. granularum S 63	M. hyopneumoniae EP 29–37	M. suipneumoniae NCTC 10110	A. laidlawii PG 8	PG 49	M. arginini D 1365/68
F 44	3–5	3–5	0	0	0	0	0	0
SEP 200	3–5	3–5	0	0	0	0	0	0
S 63	0	0	5	0	0	3	1	2
EP 29–37	0	0	0	2–4	2–4	0	0	0
NCTC 10110	0	0	0	2–4	2–4	0	0	0
S 6	0	0	0	0	0	nd	nd	nd
X 95-cl-la	0	0	0	0	0	nd	nd	nd
PG 16	0	0	0	0	0	nd	nd	nd
PG 30	0	0	0	0	0	nd	nd	nd
1340	0	0	0	0	0	nd	nd	nd
WVU 1853	0	0	0	0	0	nd	nd	nd
PG 2	0	0	0	0	0	nd	nd	nd
M 99	0	0	0	0	0	nd	nd	nd
2833	0	0	0	0	0	nd	nd	nd
PG 3	0	0	0	0	0	nd	nd	nd
PG 1	0	0	0	0	0	nd	nd	nd
Donetta	0	0	0	0	0	nd	nd	nd
PG 11	0	0	0	0	0	nd	nd	nd
PG 8	0	0	3	0	0	nd	nd	nd
PG 49	0	0	1	0	0	nd	nd	nd
PG 50	0	0	0	0	0	nd	nd	nd
5 M 331	0	0	0	0	0	nd	nd	nd
D 12	0	0	0	0	0	nd	nd	nd
D 1365/68	0	0	2	0	0	nd	nd	nd

Figures indicate number of precipitation lines nd = not done

Isolates SM (milk), SG 693, SG 731 and SG 732 (lungs) reacted with the antisera against M. hyopneumoniae. They gave one precipitation line only whereas M. hyopneumoniae gives 2–4 lines with its homologue as shown in Table 4. These strains together with S 614 were classified as unidentified and subjected to further test (Table 9).

Table 4 Ouchterlony – Diagnostic Strains

	M. hyorhinis % of isolates	A. granularum	A. laidlawii	unidentified
Lungs	36 (90%)	2[1]	0	3
Pericardial fluid	27 (100%)	0	0	0
Synovial fluid	7	1	0	1
Vagina	0	0	1	0
Udder	1	0	0	0
Uterus	0	0	1	0
Milk	0	0	0	1
Normal lung	2	0	0	0

[1] A. granularum isolated together with M. hyorhinis in the same lung.

2. *Growth Inhibition Test (GIT)*

a) Reference System

The results are tabulated in Table 5. The tests were not carried out on the M. hyopneumoniae strains although antisera of these strains were used.

Table 5 GIT – Reference System

Antiserum against	F 44	Strains SEP 200	S 63
M. hyorhinis F 44	18.8	–	–
SEP 200	17.6	17.6	–
A. granularum	–	–	19.1
M. hyopneumoniae			
EP 29–37	–	–	–
NCTC 10110	–	–	–

Figures indicate inhibition zone in millimeters – = no inhibition

b) Diagnostic Strains

With the GIT the isolates which had been differentiated with the Ouchterlony test as M. hyorhinis strains were found to vary in their sensitivity to antisera. The results are summarized in Table 6.

Table 6 GIT – M. hyorhinis Strains

	Antisera against	
	F 44	SEP 200
26 isolates	+	+
23 isolates	+	–
10 isolates	–	+
13 isolates	–	–

+ = sensitivity to antiserum
– = resistance to antiserum

3. *Differentiation of unidentified strains*

a) Morphology

S 614 showed a granular growth in liquid medium. The granules were relatively coarser than those of A. granularum. The strain did not ferment glucose and formed a lipid film both on agar and in liquid medium. SM exhibited a homogenous turbidity in the liquid medium. No granules were discernible. On agar the SM colonies were small; showing correspondingly small central nipples.

SG 693, SG 731 and SG 732 exhibited only a very slight turbidity which could be seen only by direct trans-illumination. The turbidity could be enhanced by slightly shaking the culture tube as the strains tended to form very minute granules or fibers that settle on top of the agar in the biphasic medium. These three strains grew poorly on agar and only under micro-aerophilic conditions. They exhibited no central nipple.

b) Biochemical Reactions

The reaction of the strains are shown in Table 7. The reactions of the porcine reference strains are given for comparison.

Table 7 Biochemical Reactions

Strains	Treha-lose	Saccha-rose	Lactose	Mannitol	Glucose
M. hyorhinis F 44	+	+	+	+	+
SEP 200	+	+	+	+	+
A. granularum	+	–	–	+	+
M. hyopneumoniae EP 29–37	+	–	–	–	+
M. suipneumoniae NCTC 10110	+	–	–	–	+
SM	+	+	–	–	+
S 614	–	–	–	–	–
SG 693	–	–	–	–	–
SG 731	–	–	–	–	–
SG 732	–	–	–	–	–

+ = fermentative
– = not fermentative

c) Serological Reactions

c) i Ouchterlony

Strain SM gave 1 line of precipitation with PG 49 and with PG 8. After the SM antiserum has been absorbed with these strains it reacted with the M. hyopneumoniae antigens giving 1–2 lines of precipitation. The 3 SG strains also reacted with M. hyopneumoniae giving 1 line of precipitation.

S 614 did not precipitate any of the porcine, avian, ovine, caprine and bovine reference strains.

c) ii Metabolic Inhibition Test (MIT)

The results for glucose fermentation are given in Table 8. S 614 does not ferment glucose but liberates ammonia from arginine. The MIT for S 614 was thus performed with arginine and a titre of 1:6400 was obtained.

Table 8 MIT – Glucose Fermentation

Antiserum	F 44	S 63	Strain NCTC 10110	EP 29–37	SM
M. hyorhinis F 44	1:6400	–	–	–	–
M. granularum S 63	–	1:1280	–	–	–
M. suipneumoniae NCTC 10110	–	–	1:640	1:640	–
M. hyopneumoniae EP 29–37	–	–	1:640	1:640	–
SM	–	–	1:20	1:20	1:1280
SG 731	–	–	1:80	1:80	–

Discussion

Using the medium described, mycoplasmas were isolated from 40.8% of the 196 specimens examined. All the isolates could be propagated in this medium. It must be noted that among other things, the specimens included 93 pericardial fluids, of which as much as 39.8% were heavily contaminated with bacteria. It was also possible to isolate a very fastidious mycoplasma strain from 3 of 5 lungs with typical enzootic pneumoniae (EP).

We found that the differentiation of the porcine strains could be achieved quite satisfactorily with the agar gel test because unlike the bovine strains [29] there were not many cross-reactions. The GIT was however not adequate since M. hyorhinis strains vary in their sensitivity to antisera. This difference in sensitivity has also been observed by Dinter and Taylor-Robinson [5]. We consider therefore that the best tests for differentiating porcine mycoplasma strains are the MIT and the Ouchterlony-reaction.

Mycoplasma hyorhinis was isolated from 60% of the pneumonic lungs from farms in Switzerland. In comparison, Bakos and Dinter [1] isolated M. hyorhinis from 46.1% of the lungs originating from EP farms in Sweden. In America, L'Ecuyer et al. [15] recovered this mycoplasma species from 51.1% of 86 pneumonic lungs and 6.6% out of normal lungs. In Finland,

Schulman [24] recovered M. hyorhinis from 50% of pneumonic lungs and 18% of normal lungs. Out of 10 healthy and histologically normal lungs examined, M. hyorhinis was recovered from 2. Thus the occurrence of M. hyorhinis in pneumonic and normal lungs in Switzerland seems to be comparable with those in Sweden, Finland and the USA. The presence of A. granularum in pneumonic lungs is not frequent, although this mycoplasma is often encountered in nasal cavities [23]. We found it in 2 instances (3.3%). As is stated in the introduction, we limited ourselves to the isolation of mycoplasmas other than M. hyopneumoniae. However, on a farm with a history of enzootic pneumonia we isolated a fastidious mycoplasma from 3 of 5 lungs examined. This mycoplasma showed similar cultural behaviour to M. hyopneumonia and exhibited some serological cross-reactions with M. hyopneumoniae but not a full identity [14]. Further investigations will be necessary to determine the nature and pathogenicity of this agent. It is possible that the agent of enzootic pneumonia does not necessarily belong to a homogenous group. We did not principally examine nasal cavities as such but there is strong evidence that mycoplasmas do frequently occur in the nasal cavities of pigs in Switzerland [2]. Mycoplasmas were isolated from 29% of the 93 pericardial fluids examined. All were identified as M. hyorhinis. 18 of these pericardial fluids were taken from pigs which had pneumonia as well. Mycoplasmas were recovered from 4 of these fluids while 13 of the lungs had mycoplasma. M. hyorhinis was isolated from the pericardial fluids of two 18-week old pigs. Switzer is of the opinion that though M. hyorhinis polyserositis mainly affects young pigs, this condition can sometimes occur in older pigs which are under some stress [25]. It must be emphasized that about 40% of the pericardial fluids examined were contaminated. We attribute this contamination to improper handling of material at the necropsy.

Mycoplasmas were recovered from 9 of the 26 synovial fluids. M. hyorhinis was isolated 7 times, and in all these cases there was evidence of a polyserositis which seems to support the theory that M. hyorhinis is one of the aetiological agents of this disease. However, in another arthritis-polyserositis case, A. granularum was isolated from the synovial fluid. This seems to contradict the Switzer [24] theory that A. granularum arthritis is not accompanied by polyserositis. A mycoplasma which could not be identified with the known porcine species [14] was isolated from the synovial fluid of a 24-week old pig suffering from polyarthritis. This isolate [S 614) liberates ammonia from arginine and gives with its homologous serum a titre of 1:6400 in the MIT. It does not ferment glucose and other sugars (Table 7) and is characterized by the formation of a lipid film. It is possible that S 614 is similar to a new species, M. suidaniae, isolated by Friis from lungs of pigs [7].

[1] M. hyosynoviae recently described by Ross and Karmon (J. Bact. *103*, 707–713, 1970) seems also to show the same properties.

2 isolations were made from the metritis cases. The mycoplasmas from both cases were found to be serologically identical to Acholeplasma laidlawii (PG 8), a known saprophytic species. M. hyorhinis was isolated once from the udder of a sow. The udder did not show any histological changes, thus this mycoplasma seems in this case to have no pathogenic significance. The strain *SM* was isolated from the milk of a sow showing typical clinical manifestations of the mastitis-metritis syndrome described by Moore et al. [16]. The sow recovered spectacularly after treatment with tetracyclines. Serologically this strain also seems to represent a new species, although it does cross-react to some extent with M. hyopneumoniae. Unfortunately it was not possible to obtain from Dr. Moore any strain or antiserum of Mycoplasma hyogenitalium for comparison. It was not possible to assess how much mycoplasmas are involved in the aetiology of the mastitis-metritis syndrome as only few specimens of this condition were examined. It is likely, however, that mycoplasmas may be involved in this disease.

The bacteriological findings seem to suggest that in some cases there is a multiple aetiology. With few exceptions the bacteriological examination shows that the bacteria isolated could not have been the primary pathogens in the cases involved. Bacteria was not isolated in 38 of the mycoplasma positive specimens. Haemophilus parahaemolyticus, which has been identified by Nicolet et al. [20] as a primary pathogen of pleuropneumonia of pigs was isolated 13 times simultaneously with mycoplasmas. This bacteria occurred concurrently with P. multocida in 4 of the lungs where M. hyorhinis was isolated. We assume that in the cases of pneumonia, M. hyorhinis might have acted as some predisposing factor, since it has been observed that H. parahaemolyticus acts as a primary pathogen only under certain conditions such as climatic changes, unhygienic conditions in the pig pens and other stress factors. It is possible that M. hyorhinis plays an important role in the pathogenesis of Haemophilus pleuropneumonia. Other haemophilic strains isolated from the respiratory tract are H. parasuis (4 times) and H. suis (once). H. suis has been shown to cause Glässer's disease [9] but it has not as yet been shown to cause pneumonia in pigs. We thus assume that H. suis or H. parasuis act synergistically with M. hyorhinis to cause enzootic-like pneumonia in pigs. This sort of synergistic action may also be true for Pasteurella multocida, which was isolated several times together with mycoplasmas from pneumonia, pericarditis and arthritis cases. This view has also been expressed in a paper by Little [16], though P. multocida was found by Roberts et al. to give no additive effect [22].

We isolated P. multocida and A. granularum from one lung. It has been stated that A. granularum though a common inhabitant of the nasal cavity can occasionally be recovered from pigs with pneumonia [25]. It is however possible that this mycoplasma can under certain conditions cause pneumonia. Jericho [11] succeeded in producing pneumonia in hysterec-

tomy produced colostrum deprived [H.P.C.D.) pigs with aerosols of A. granularum.

In addition to H. parahaemolyticus, only 2 other bacteria known to be pathogenic for pigs were found together with mycoplasma. They are Bordetella bronchiseptica and Erysipelothrix insidiosa (Table 2). Histological examinations were carried out primarily on the lungs and we observed that only about half of the pneumonia cases could be classified as EP-like. Purulent bronchitis and bronchopneumonia occurred in a considerable number of the lungs from which M. hyorhinis was isolated. Schulman [24] isolated M. hyorhinis from 43% of purulent bronchopneumonia cases. The question arises, then, as to whether mycoplasmas are able under certain conditions to provoke purulent bronchopneumonia.

The histological and bacteriological findings seem to indicate some sort of multiple complex aetiology of the diseases associated with mycoplasma. The pneumoniae are a typical example of this sort of complex aetiology, and our results seem to support Jericho's theory of multiple complex aetiology for such endemic pneumoniae. Cultures were not set up to isolate viruses and it is possible that in some severe cases viruses or other more fastidious strains of mycoplasma could have taken part in the infections. The experiment of Kasza et al. [12] with M. hyopneumoniae and swine adenovirus support this view.

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Summary

This study deals with the isolation and differentiation of 83 strains of mycoplasma from 206 specimens of pigs affected with pneumonia, pericarditis, arthritis, mastitis and metritis.

With the gel precipitation test, 73 (87.9%) of the strains were found to be M. hyorhinis, 3 (3.6%) A. granularum and 2 (2.4%) as A. laidlawii. 5 (6%) of the strains probably belong to 3 new species.

Zusammenfassung

Wir berichten über die Isolierung und Charakterisierung von Mykoplasmastämmen aus 196 Untersuchungen an Schweinen, die an Pneumonien, Perikarditiden, Arthritiden, Mastitiden und Metritiden erkrankten. Mycoplasma hyorhinis wurde am häufigsten isoliert und war in 60% der Pneumonien, 29% der Perikarditiden, in 7 von 26 arthritischen Gelenken und in einer Milchprobe (jedoch ohne histopathologische Veränderung des Euters) zu finden. Acholeplasma granularum wurde nur dreimal isoliert (2 Lungen mit Pneumonien und 1 Arthritisfall). Zwei Stämme von Acholeplasma laidlawii wurden bei Metritisfällen isoliert.

Da unser Untersuchungsmaterial hauptsächlich aus dem Schweinegesundheitsdienst stammt, haben wir uns nicht auf die Isolierung des Erregers der enzootischen

Pneumonie konzentriert. Jedoch in einem reinfizierten Bestand isolierten wir Stämme, morphologisch mit H. hyopneumoniae nah verwandt, biochemisch und serologisch jedoch verschieden. Es stellt sich die Frage, ob die enzootische Pneumonie nicht auch von anderen Spezies verursacht werden kann. In einem Fall von Mastitis-Metritissyndrom isolierten wir einen Stamm, der sich nicht identifizieren ließ, aber gemeinsame Antigene mit M. hyopneumoniae besitzt. Endlich isolierten wir einen Stamm aus einem Arthritisfall, der serologisch auch nicht identifizierbar war, zeigte jedoch die Eigenschaft, Arginin zu spalten.

M. hyorhinis war häufig mit einer Begleitflora (H. parahaemolyticus, H. parasuis, P. multocida, B. bronchiseptica usw.) zu finden. Diese Beobachtung erweckt die Idee, besonders bei Pneumoniefällen, einer multiplen Ätiologie. Wir diskutieren diesen Aspekt der Ätiologie der Pneumonien beim Schwein.

Résumé

On rapporte les résultats d'une enquête sur la présence de mycoplasmes chez des porcs souffrant de pneumonie, de péricardite, d'arthrite, de mastite et métrite. Nous avons pu isoler 80 souches de mycoplasmes sur 196 analyses, soit dans le 40,8% des cas. Mycoplasma hyorhinis se rencontre très fréquemment, on le retrouve dans 60% des pneumonies, 29% des péricardites, dans 7 sur 26 arthrites et dans un lait de truie sans toutefois que la mamelle ne montre des lésions histopathologiques. Acholeplasma granularum est plus rare, puisque seules 3 souches ont été isolées (2 cas de pneumonie et une arthrite). Il en est de même pour Acholeplasma laidlawii que nous avons isolé dans deux cas de métrite. Nous n'avons pas tenu compte dans cette étude de l'agent de la pneumonie enzootique, une affection relativement rare dans le service sanitaire porcin. Toutefois, dans une exploitation réinfectée, nous avons isolé des souches dont les propriétés morphologiques se rapprochent de M. hyopneumoniae, mais s'en distinguent biochimiquement et sérologiquement, ce qui pose le problème de l'hétérogénéité possible des agents de la pneumonie enzootique. Nous avons également isolé d'un cas de métrite-mastite, une souche de mycoplasme non identifiable. Une souche isolée d'un cas d'arthrite s'est révélée capable de scinder l'arginine et s'avère sérologiquement différentes des souches connues.

M. hyorhinis est souvent accompagné d'une flore bactérienne (H. parahaemolyticus, H. parasuis, P. multocida, B. bronchiseptica, etc.) ce qui suggère, surtout dans les cas de pneumonie, que nous sommes en présence d'une étiologie multiple. Nous discutons du reste cet aspect du problème de la pneumonie du porc.

Riassunto

Diamo relazione sull'isolamento e la caratterizzazione dei ceppi di micoplasmi in 196 ricerche su suini, affetti da polmonite, pericardite, artrite, mastite e metrite. Mycoplasma hyorhinis fu il più frequentemente isolato e fu individuato nel 60% delle polmoniti, 29% delle pericarditi, in 7 su 26 affezioni articolari, in 1 latte (tuttavia senza alterazione patologica della mammella). Acholeplasma granularum venne isolato solo tre volte (due polmoni con polmite e un caso d'artrite). Due ceppi di Acholeplasma laidlawii vennero isolati in casi di metrite.

Siccome il nostro materiale proviene per lo più dal servizio sanitario porcino, non ci siamo concentrati sull'isolazione dell'agente. Tuttavia in un effettivo reinfettato isolammo due ceppi parenti stretti morfologicamente di M. pyopneumoniae, ma diversi dal lato biochimico e sierologico. Si pone la domanda se la polmonite enzootica non possa esser causata anche da altre speci. Nel caso di una sindrome mastite-metrite isolammo un ceppo che non fu possibile identificare, ma che aveva antigeni comuni con M. Hyopneumoniae. Infine isolammo un ceppo da un caso di

artrite, sierologicamente anche non identificabile, ma che aveva tuttavia la capacità di scomporre l'arginina.

M. Hyorhinis era apesso accompagnato da un flora (H. parahaemolyticus, H. parasuis, P. multocida, B. bronchispetica, ecc.). Questa constatazione fa sorgere l'idea, specie nei casi di polmonite, di una causa multipla. Discutiamo questo aspetto dell'eziologia della polmonite nel suino.

References

[1] Bakos K. und Dinter Z.: Das SEP-Agens. XVII. Welttierärztl. Kongr., Hannover. 549–553 (1963). – [2] Bertschinger H.U. und Nicod B.: Untersuchungen über die Nasenflora bei Schweinen: Vergleich zwischen SPF-Herden und schwedisch sanierten Herden. Schweiz. Arch. Thke. *112*, 493–499 (1970). – [3] Clyde W.A.: Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immun. *92*, 958–965 (1964). – [4] Dinter Z., Danielsson D. and Bakos K.: Differentiation of porcine mycoplasma strains. J. gen. Microbiol. *41*, 77–84 (1965). – [5] Dinter Z. and Taylor-Robinson D.: Susceptibility and resistance of various strains of Mycoplasma hyorhinis to antisera, polymyxins and low pH values. J. gen. Microbiol. *57*, 263–272 (1969). – [6] Edward D.G. ff. and Freundt E.A.: Amended Nomenclature for strains related to Mycoplasma laidlawii. J. gen. Microbiol. *62*, 1–2 (1970). – [7] Friis N.F.: A new porcine mycoplasma species: Mycoplasma suidaniae. Acta vet. scand. *11*, 487–490 (1970). – [8] Goodwin R.F.W., Pomeroy A.P. and Whittlestone P.: Characterisation of Mycoplasma suipneumoniae: a mycoplasma causing enzootic pneumonia of pigs. J. Hyg., Camb. *65*, 85–96 (1967). – [9] Hjärre A.: Enzootic virus pneumonia and Glässer's disease of swine. Adv. vet. Sci. *4*, 235–263 (1958). – [10] Hodges R.T.: The role of mycoplasma in some diseases of pigs. Br. vet. J. *125*, 340–343 (1969). – [11] Jericho K.W.F.: Pathogenesis of pneumonia in pigs. Vet. Rec. *82*, 507–517 (1968). – [12] Kasza L., Hodges R.T., Betts A.O. and Trexler P.G.: Pneumoniae in gnotobiotic pigs produced by simultaneous inoculation of swine adenovirus and Mycoplasma hyopneumoniae. Vet. Rec. *84*, 262–267 (1969). – [13] Keller H. und Bertschinger H.U.: Nachweis der pleomorphen Organismen (PO) in der Diagnostik der enzootischen Pneumonie der Schweine. Berl. Münch. Tierärztl. Wsch. *6*, 101–107 (1968). – [14] Leach R.H.: Personal communication. – [15] L'Ecuyer C., Switzer W.P. and Roberts D.: Microbiologic survey of pneumonic and normal lungs. – [16] Little T.W.A.: Haemophilus infection in pigs. Vet. Rec. *87*, 399–402 (1970). – [17] Moore R.W., Redmond H.E. and Livingston C.W.: Pathologic and serologic characteristics of a mycoplasma causing arthritis in swine. Am. vet. Res. *27*, 1649–1656 (1966). – [18] id.: Mycoplasma as the etiology of a metritis-mastitis syndrome in sows. Vet. Med./Small Animal Clinician. 883–887 (1966). – [19] Morton H.E. and Roberts R.J.: Production of anti-mycoplasma (PPLO) antibodies in rabbits. Proc. Soc. exp. Biol. Med. *125*, 538–543 (1957). – [20] Nicolet J., König H. und Scholl E.: Zur Haemophilus-Pleuropneumonie beim Schwein. II. Eine kontagiöse Krankheit von wirtschaftlicher Bedeutung. Schweiz. Arch. Thkde. *3*, 166–174 (1969). – [21] Nicolet J. et de Meuron P.A.: Isolement et caractérisation de mycoplasmes dans le syndrome de la pneumoentérite du veau. Zbl. Vet. Med. *17*, 1031–1042 (1970). – [22] Roberts E.D., Switzer W.P. and L'Ecuyer C.: Influence of Pasteurella multocida and Mycoplasma hyorhinis (PPLO) on the histopathology of field cases of swine pneumonia. Cornell Vet. *52*, 306–327 (1962). – [23] Ross R.F., Switzer W.P. and Maré C.J.: Incidence of certain micro-organisms in Iowa swine. Vet. Med. *58*, 562–565 (1963). – [24] Schulman A.: On occurrence of Mycoplasma hyorhinis in the respiratory organs of pigs with special reference to enzootic pneumonia. Int. Pig Vet. Soc., Proc. 1st Congr., Cambridge. No. 151 (1969). – [25] Switzer W.P.: Swine Mycoplasma. J. Am. Vet. Med. Assoc. *151*, 1656–1661 (1967). – [26] id.: Mycoplasmosis. In Diseases of Swine. Ed. Howard W. Dunne. (1964). – [27] id.: Atrophic rhinitis today. J. Am. Vet. Med. Assoc. *146*, 348–351 (1965). – [28] Taylor-Robinson D. and Dinter Z.: Unexpected serotypes of mycoplasmas isolated from pigs. – [29] Taylor-Robinson D., Purcell R.H., Wong D.C. and Chanock R.M.: A colour test for the measurement of antibody to certain mycoplasma based upon the inhibition of acid production. J. Hyg., Camb. *64*, 91–104 (1966). – [30] Wadsworth C.: A slide micro-technique for the analysis of immuneprecipitates in gel. Int. Arch. Allergy appl. Immun. *10*, 355–359 (1957).