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Mycoplasma hyosynoviae Isolation from the Upper Respiratory Tract and Tonsils of Pigs

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Friis, N. F., P. Ahrens and H. Larsen: ***Mycoplasma hyosynoviae*** **isolation from the upper respiratory tract and tonsils of pigs. Acta vet. scand. 1991, 32, 425–429.** – The occurrence of *Mycoplasma hyosynoviae* at different locations of the upper respiratory tract and tonsils of pigs was investigated in herds with problems of arthritis apparently caused by this microorganism. The isolation of *M. hyosynoviae* was facilitated by the use of a medium selectively suppressing the growth of *Mycoplasma hyorhinis. M. hyosynoviae* was cultured from 106 of 178 tonsils of slaughterhouse pigs from 8 herds but could not be isolated from the mucosa of the nasal cavity or the oral-pharyngeal area of 100 living, 10–20 weeks old pigs in 5 of the herds. The value of the selective principles in the medium appears from the circumstance that 86 of the 106 isolates were obtained despite the presence of *M. hyorhinis.*

It is concluded that the tonsil is a reservoir for *M. hyosynoviae* and is probably the location of choice for an easy demonstration of the presence of this mycoplasma in a pig herd.

arthritis in pigs; mycoplasma hyorhinis.

Introduction

Mycoplasma hyosynoviae is a common microorganism in pigs, sometimes giving rise to a serious arthritis in animals more than 10 weeks old (*Ross & Duncan* 1970, *Ross et al.* 1971, *Burch & Goodwin* 1984). Apparently, the mycoplasma is harboured in the respiratory tract (*Friis* 1971, *Gois & Taylor-Robinson* 1972), mainly the upper part (*Ross & Spear* 1973, *Kawamura et al.* 1982). The organism can be disseminated haematogenously to joints where disease may occur (*Ross & Duncan* 1970). Often many animals within specific age groups are simultaneously affected, probably as a result of stressing factors (*Ross* 1973). The disease may remain enzootically in many herds.

Mycoplasma hyosynoviae is an arginine metabolizing mycoplasma which can be cultivated in a Hayflick's type of medium especially when this is enriched with arginine and bacteriological mucin. Primary isolation from the porcine respiratory tract is often complicated because of overgrowth by *Mycoplasma hyorhinis* and by different bacteria as well. As *M. hyorhinis* also presents a problem for the primary isolation of other mycoplasmas of the porcine respiratory tract, a selective medium has earlier been introduced for isolation of *Mycoplasma hyopneumoniae* and *Mycoplasma flocculare.* In that medium the antibiotic cycloserine is used together with rabbit antiserum against *M. hyorhinis* (*Friis* 1979). Selective isolation of *M. hyosynoviae* on solid medium by suppression of the growth of *M. hyorhinis* has earlier been obtained by *Ogata et al.* (1982) by using strictly anaerobic conditions. *Shimizu* (1983) has used the detergent Tween

80 to obtain selective isolation of *Mycoplasma bovis* from cattle.

The aim of the present work was to establish the frequency of *M. hyosynoviae* at different locations in the upper respiratory tract and tonsils of pigs, and to evaluate the efficiency of a selective medium with cycloserine and another detergent Tween 20 as the active principles (*Friis et al.* 1990).

Materials and methods

The localisation of *M. hyosynoviae* and *M.hyorhinis* in the upper respiratory tract and tonsils was studied in 5 herds with clinical signs of *M. hyosynoviae* induced arthritis (arthritic pain and lameness in age groups more than 10 weeks old). In 2 herds the disease had been confirmed by isolation of *M. hyosynoviae* from diseased joints. From 100, 10–20 weeks old animals swab samples were collected from the nasal cavity (ventral meatus) and from the tonsillary area of the soft palate. One year later when overt clinical symptoms had ceased in 3 of the 5 herds 114 tonsils were collected at slaughterhouses from animals about 6 months old. Additionally, 43 tonsils were collected from animals from 3 more herds with recent outbreaks of enzootic arthritis and with *M. hyosynoviae* recovered from joints in 2 of the herds.

The swabs were collected in medium HCT I, cooled with ice and sent by mail to the Laboratory. The tonsils were cooled with ice or frozen, and mailed.

M. hyosynoviae was cultivated in a modified Hayflick's medium enriched with arginine and bacteriological mucin. For primary isolation selective inhibitors to *M. hyorhinis* and bacteria were added.

Medium HS: 3,500 ml water, 75 g Bacto PPLO broth w/o CV (Difco), autoclaving at 120 C for 2 min, 180 ml yeast extract (made from 100 g YSC-2 (Sigma) in 750 ml water), 10 ml of 0.6 % phenol red solution, 1.0 g ampicillin, 325 ml horse serum, 325 ml pig serum, 27.5 ml arginine/mucin solution (8 % L-arginine (Merck) and 0.8 % Bacto mucin bacteriological (Difco)), pH 7.0. For details see *Friis* (1975a, 1975b).

Medium HCT I: 100 ml HS, 30 mg D-Cycloserine (Fluka AG) 0.085 ml Tween 20 (Merck), pH 7.0.

Medium HCT II: 100 ml HS, 40 mg D-Cycloserine, 0.1 ml Tween 20, pH 7.0.

M. hyorhinis was cultivated in a medium intended for primary isolation of *M. hyopneumoniae* and *M. flocculare;* in certain cases supplemented with more antibiotics.

Medium NHS: 1,400 ml water, 2,500 ml Hanks' balanced salt solution, 15 g Bacto brain heart infusion (Difco), 16 g Bacto PPLO broth w/o CV (Difco), autoclaving at 120 C for 2 min, 180 ml yeast extract (see above), 10 ml of 0.6 % phenol red solution, 800 mg bacitracin, 800 mg methicillin, 510 ml horse serum, 510 ml pig serum, pH 7.35. For details, see *Friis* (1975a).

Medium NHS-A: 100 ml NHS, 15 mg cycloserine, 20 mg vancomycin, 0.5 mg nalidixic acid, pH 7.35.

Solid media were prepared with Agar-Agar (Oxoid) at 0.8 %. The swab samples were cultivated in media HCT I and NHS, if necessary also in NHS-A. The tonsils in HCT II and NHS-A. Subcultures were made in HS and in NHS.

The swabs and a 10 % suspension of ground tonsillar tissue (a pool of the right and left tonsil, surface-decontaminated by dipping 5 sec in boiling water before grinding) were incubated at 37 C as a 10-fold dilution (*Friis* 1975a) to 10^{-5} in liquid media. Cultures for *M. hyosynoviae* were kept stationary in a rack while those for *M. hyorhinis* were rolled in a drum. Subcultures were performed only from tubes with colorshift. The primary cultures were evaluated finally after 1 week.

Isolated mycoplasmas were identified by the disc growth inhibition test DGI (*Friis* 1974) using rabbit hyperimmune antisera; for *M. hyorhinis* was used a pool of antisera against the type strain BTS-7 (NCTC 10130) and reference strains GDL (NCTC 10121) and M99; for *M. hyosynoviae* an antiserum against reference strain M60 was used. This strain is serologically indistinguishable from the type strain S16 (NCTC 10167).

Results

M. hyosynoviae could not be isolated from any of the swabs sampled from the nasal cavity or from the oral-pharyngeal area of 20 pigs in each of 5 herds with enzootic arthritis (Table 1). From the tonsils, however, *M. hyosynoviae* was isolated from 106 out of 178 examined animals. In some of the herds, II, VI and VIII, most tonsils were found to harbour *M. hyosynoviae,* while in others, III and IV, only few tonsils were found infected. In the 3 herds with a recent outbreak of disease, VI, VII, VIII, half or more of the tonsils were found infected with *M. hyosynoviae.* By comparison of the final dilution (not in table) of the two mycoplasma species it appeared that recovery of 86 strains of *M. hyosynoviae* was obtained despite the presence of *M. hyorhinis* to the same or a higher concentration. Thus, only 20 strains were isolated from tubes free from *M. hyorhinis. M. hyorhinis* was recovered from all 3 locations examined and usually from the majority of samples. The growth of this species was distinctly retarded in medium supplemented with several antibiotics (NHS-A) as compared to ordinary medium (NHS).

Discussion

In the present work it has not been possible to demonstrate *M. hyosynoviae* in mucous

Table 1. Occurrence of *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis* on the mucous membranes of the upper respiratory tract and in tonsils of pigs in herds affected with enzootic arthritis.

Herd	Swab samples*							Tonsils**			
	Nasal cavity			Soft palate			Clin.				Clin.
	No.	Mhs.	Mhr.	No.	Mhs.	Mhr.	dis.	No.	Mhs.	Mhr.	dis.
I	20	0	16	20	0	6	+	17	10	12	+
II	20	0	2	20	0	20	+	55	45	49	0
III	20	0	20	20	0	10	+	20	3	16	+
IV	20	0	19	20	0	17	+	20	2	19	0
V	20	0	15	20	0	8	+	20	14	18	0
VI								16	16	16	+
VII								25	11	23	+
VIII								5	5	4	+
Total	100	0	72	100	0	61		178	106	157	

* = animals 10–20 weeks old; cultivation in media HCT I and NHS or NHS-A.
** = from animals at slaughterhouses; cultivation in media HCT II and NHS-A.
Clin. dis. = enzootic arthritis at the moment of sampling.
No. = number of samples.
Mhs, Mhr = *Mycoplasma hyosynoviae, Mycoplasma hyorhinis.*
Tonsils from herds I through V were collected 1 year after the swabs.
Tonsils from herds VI through VIII were collected a few months after start of disease.

samples from the upper respiratory tract of pigs in 5 herds with clinical evidence of enzootic arhritis. However, others have earlier succeeded in the recovery of *M. hyosynoviae* from nasal and pharyngeal mucosa of pigs of different age groups above 6 weeks (*Kawamura et al.* 1982, *Ross & Duncan* 1970, Ross & Spear 1973) and using selective suppression of *M. hyorhinis* (*Ogata et al.* 1982). *M. hyosynoviae* was recovered from tonsils of baconers from all 8 examined herds, but the rate of isolation varied (Table 1). Thus, all 3 herds (VI, VII, VIII) having had a recent outbreak of arthritis showed a high rate of isolation of *M. hyosynoviae,* but in the 5 herds with an earlier start of the problems, the rate seemed to vary and to be independent of the actual health state. Although *M. hyosynoviae* may be recovered from about 10 % of pneumonic lungs (*Friis* 1971, *Kawamura et al.* 1982) it seems reasonable to conclude that the tonsil is the dominant reservoir for this mycoplasma as has been found previously by *Ross & Spear* (1973). Therefore, an examination of tonsils from slaughterhouse pigs appears to be a useful method for demonstration of the presence of *M. hyosynoviae* in a pig herd. *M. hyorhinis* could very frequently be recovered from all examined parts of the upper respiratory tract, but the antibiotics in medium NHS-A appeared close to critical amounts as judged from the retardation of its growth.

A reliable estimate of the frequency of *M. hyosynoviae* in the mucosa of the respiratory tract and especially in the tonsils, requires a medium with good selective properties acting against the very rapidly propagating, almost ubiquitous species, *M. hyorhinis,* and also against the doggedly resistant bacterial flora present in the tonsils. With the combined use of the 2 selectively suppressing compounds, cycloserine and Tween 20, in medium HCT II, 1 of the prerequisites was fulfilled. An almost complete neutralisation of *M. hyorhinis* was obtained with little influence on *M. hyosynoviae.* The combined antibacterial effect of ampicillin and the high amount of cycloserine could control the bacterial growth in most cases despite the absence of the traditionally used thallium acetate.

The high sensitivity of *M. hyorhinis* to the detergent Tween 20 remains unexplained. However, a very great number of such compounds have been used for years for their special ability to dissolve membranes of microorganisms thus bringing otherwise insoluble proteins and lipids into solution (*Helenius & Simons* 1975). Therefore it may be conjectured that the noted difference between the 2 species is connected with an apparent abundance of integral membrane proteins tightly associated with lipids in *M. hyorhinis* (*Brincher et al.* 1988).

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Sammendrag

Påvisning af Mycoplasma hyosynoviae i de øvre luftveje hos svin.

Forekomsten af *Mycoplasma hyosynoviae* på forskellige lokaliteter i de øvre luftveje hos svin blev undersøgt i besætninger med enzootisk optrædende ledbetændelse. Der anvendtes et substrat, som specifikt er udviklet med henblik på undertrykkelse af den nærmest allestedsnærværende og hurtigt voksende *Mycoplasma hyorhinis.*

Det har ikke været muligt at påvise *M. hyosynoviae* ved dyrkninger fra slimhinden i næsehulen eller i mund-svælg området af 100 ungsvin i 5 besætninger. Derimod påvistes *M. hyosynoviae* meget hyppigt i tonsiller af slagtesvin fra de samme besætninger og blev således fundet i 106 ud af 178 prøver fra dyr i 8 besætninger. Værdien af det nyligt udviklede selektive princip fremgik af, at 86 af isolaterne blev opnået til trods for tilstedeværelse af *M. hyorhinis.*

Det kan konkluderes, at tonsillen er et naturligt reservoir for *M. hyosynoviae* og at tonsiller af slagtedyr er et egnet organ til påvisning af denne mykoplasma på besætningsniveau.

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