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Mycoplasma Hyosynoviae in Joints with Arthritis in Abattoir Baconers

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Friis, N.F., K.K. Hansen, A.L. Schirmer and S. Aabo: *Mycoplasma hyosynoviae* in joints with arthritis in abattoir baconers. Acta vet. scand. 1992, 33, 205-210. The occurrence of *Mycoplasma hyosynoviae* in synovial fluid of baconers with chronic arthritis was studied at an abattoir. Cultural examination of synovial fluid samples from diseased tarsal joints of 50 animals from 42 herds yielded *M. hyosynoviae* in 10 cases from 8 herds. Streptococci were found in 6 cases from 6 other herds. *M. hyosynoviae* antigen was found in 1 of 47 of the samples, and antibody to the mycoplasma was found in 14 of 40 of the samples by ELISA test. The presence of *M. hyosynoviae* in a joint was usually accompanied by the corresponding antibody. In joints with streptococcal infection antibody to *M. hyosynoviae* could not be found.

swine.

Introduction

Chronic arthritis is a fairly common finding at meat inspection in baconers at abattoirs. The larger joints of the legs, mainly stifle and elbow *(Cross & Edwards* 1981) are affected with various non-suppurative lesions (*Bond* 1976, *Johnston et al.* 1987, *Meijer et al.* 1975, *Ross et al.* 1977, *Turner* 1982) which apparently reflects the later phases of an infection. The lesions are usually characterized by an increased amount of a viscous and brownish synovial fluid sometimes accompanied by a hypertrophic thickening of the joint capsule and erosion of the cartilage.

Several microorganisms have been isolated and the bacterium, *Erysipelothrix rhusiopathiae* appears to be the most frequent finding and is often ascribed the etiologic role for the lesions (*Bond* 1976, *Johnston et al.* 1987, *Meijer et al.* 1975, *Turner* 1982). Other bacteria may be found as well. *Mycoplasma hyosynoviae* is also known to cause arthritis that may persist to the time of slaughter (*Ross & Duncan* 1970, *Ross et al.* 1971, 1977). *M. hyosynoviae* is a well-known arthritogenic pathogen among swine in this country (unpublished) and it is often isolated from inflamed joints in herds with enzootically occurring arthritis among animals in the age groups from 30 to 100 kg. Also other microorganisms may be recovered from inflamed joints of younger pigs; thus *E. rhusiopathiae* and various streptococci are often found *(Jorsal,* personal communication).

The aim of the present work was to investigate the occurrence of *M. hyosynoviae* in chronic arthritis, and to compare cultivation to demonstration of antigen and antibody by an immunodiagnostic method, the enzyme-linked immunosorbent assay (ELISA). Cultivation for bacteria was performed for comparison.

Materials and Methods

Arthritic samples

A sample of synovial fluid from inflamed tarsal joints was aspirated aseptically from 50 slaughterhouse pigs with a body weight of 100 kg and representing 42 herds. Diseased joints were revealed from enlargement and brownish discoloration of regional lymph nodes in combination with distension of joint capsules. Most samples of synovial fluid appeared turbid and yellowish-brownish discolored.

The samples were sent by ordinary mail to the Laboratory (24 h) from November through February and were immediately processed for microbiological examination. The samples were kept at -25°C for later immunodiagnostic examination.

Isolation of mycoplasmas and bacteria

Two different media (*Friis et al.* 1991) were used for cultivation of mycoplasmas: A Hayflick's type of medium enriched with arginine and mucin was used for *M. hyosynoviae,* while for other species a medium originally intended for *Mycoplasma hyopneumoniae* was used. Isolated mycoplasmas were identified by the disc growth inhibition test (DGI) *(Friis* 1974) with antiserum for reference strain M60, serologically indistinguisable from the type strain S16 of *M. hyosynoviae.*

Examination for bacteria was performed by inoculation of the synovial fluid onto 5% calf-blood agar (Columbia blood agar base, Oxoid) in 3 different dilutions and by aerobic incubation at 37°C. The plates were read after 24 and 48 h incubation. The bacteriological diagnosis was based on ordinary cultural and biochemical characteristics. Lancefield's serological grouping of streptococci was performed as a capillary precipitation test.

Immunodiagnostic examinations

Antisera. Rabbit antiserum to *M. hyosynoviae* reference strain M60, *Mycoplasma hyorhinis* type strain BTS-7 and *Mycoplasma bovirhinis* type strain PG43 was produced (*Friis & Jensen* 1984) with organisms harvested from cultures in rabbit broth *(Friis* 1977). For cultivation of *M. hyosynoviae* the broth was enriched with arginine and mucin (*Friis et al.* 1991). Crude IgG was produced by precipitation with $(NH_4)_2SO_4$ followed by purification on a DEAE—Sephadex A-50 column as described by *Harboe & Ingild* (1983). Forty mg crude IgG dissolved in 10 ml carbonate buffer pH 9.6 was biotinylated using 54 mg biotin-amidocaproate N-hydroxysuccinimide ester (Sigma B-2643) in 500 µl dimethylsulphoxide (DMSO, Sigma D5879) mainly as described by *Guesdon et al.* (1979).

Antigens. Antigens of the 3 mycoplasmas were harvested by surface washing with PBS of 3 days old cultures on solid pig-serum-free Hayflick's medium enriched (above) with arginine/mucin and 15 % horse serum. The cell suspensions were washed 4 x in PBS and the final pellet dissolved in 5 ml distilled water and freeze-thawed 5 cycles. After centrifugation at 5,000 x g (average) for 20 min, PBS was added to the supernatant to 25 ml which was stored at –25°C as antigen for ELISA.

ELISA for detection of antigen. Wells of Nunc-Immuno-Plates, maxisorp (Nunc, Roskilde, Denmark) were coated with 0.2 ml IgG (1:1,000 of original serum volume in 0.1 M carbonate buffer pH 9.6) to *M. hyosynoviae* or *M. hyorhinis* and incubated overnight at + 4°C. The plate was tapped dry and washed 4 x in a hypertonic diluent (PBS-T) consisting of NaCl 0.5 M, Na_2HPO_4 0.01 M,

KH_2PO_4 0.003 M, 0.05% Tween 20 (Merck) and pH adjusted to 7.4 with K_2HPO_4 1.0 M. Synovial fluid was added in dilutions 1/1, 1/3, 1/10, 1/30, 1/100 in PBS-T in a volume of 0.1 ml (all later volumes being 0.1 ml) incubated for 1 h at 37°C and washed as above, and biotinylated IgG for *M. hyosynoviae* added (diluted 1:10,000 in PBS-T) and incubated for 1 h at 37°C. After washing, horseradish peroxydase-conjugated avidin (Dako, Herlev, Denmark) diluted 1:7,500 in gelatine buffer (2.5 g gelatine in 500 ml PBS-T, 10 min in 37°C water bath) was added and left for 1 h at room temperature and washed with PBS-T and also once in citric acid buffer (0.035 M citric acid and 0.066 M Na_2HPO_4, pH 5.0). Enzyme substrate (8 mg 1.2-orthophenyl-diamine dihydrochloride, 12 ml citric acid buffer, 5 µl H_2O_2) was added and color development stopped with 0.5 M H_2SO_4 after 10 min. Spectrophotometrical absorption was read at 490 nm using 650 nm as reference. Values for *M. hyosynoviae* were regarded as positive, if they were at least 100 % above both control figures as obtained from the wells coated with IgG to *M. hyorhinis* and from synovia-free wells and values 50-100 % above as dubious. A positive reaction was obtained for broth cultures of *M. hyosynoviae* M60 in dilution 1/300, corresponding to approx. 10^5 organisms per ml.

ELISA for detection of antibody. Wells were coated with 0.2 ml antigen of *M. hyosynoviae* and *M. bovirhinis* (approx. 300 ng/well) diluted in carbonate buffer (above) kept overnight at + 4°C and washed. Synovial fluid, 0.1 ml of dilutions 1/10, 1/20, 1/40, 1/80, 1/160 was added and incubated 1 h at 37°C; 1/40, 1/80 and 1/160 made by diluting 1/20 in PBS-T with 5% SPF pig serum. Washing and reacting with peroxydase-conjugated, rabbit anti-pig immunoglobulin, diluted 1:1,000, for 1 h at room temperature. Addition of enzyme substrate and reading of the plates as above. Figures of at least 100% above control values as obtained from the wells with IgG to *M. bovirhinis* and from synovia-free wells, were noted as positive, and 50-100% above as dubious. The rabbit hyperimmune antiserum was found positive for *M. hyosynoviae* in dilution 1:40,000.

Results

M. hyosynoviae was isolated from 10 samples from 8 farms (Table 1) by cultivation of 50

Table 1. Isolation of mycoplasmas and bacteria from arthritic tarsal synovial samples of 50 abattoir baconers and examination of the samples for *Mycoplasma hyosynoviae* antigen and antibody by ELISA.

	Synovial samples examined	Results		
Cultivation	50	10 M. hyosynoviae isolates 6 Streptococci* isolates		
		Negative	Dubious	Positive
ELISA**				
Antigen	47	45	1	1
Antibody	40	17	9	14***

* 3 Group L and 3 Group C.

** Figures indicate number of samples.

*** Titres estimated to 1:10(3), 1:20(7), 1:40(2), 1:80(1), 1:160(1).

Table 2. Antigen and antibody of *Mycoplasma hyosynoviae* demonstrated by ELISA in 16 arthritic tarsal synovial samples from which mycoplasmas or streptococci were isolated.

Sample number	M. hyosynoviae*	Antigen**	Antibody**	Sample number	Streptococci	Antigen	Antibody
48	1	0	d				
10	1	nd	nd	13	Gr L	0	0
4	2	0	d	14	Gr L	0	0
24	2	0	20	17	Gr L	0	nd
26	2	0	0	27	Gr C	0	0
49	2	0	40	31	Gr C	0	0
46	2	0	nd	41	Gr C	0	nd
50	2	3	nd				
39	3	0	160				
9	4	0	nd				

* Figures indicate endpoint for growth as reciprocal of log 10 dilution.
** Positive reaction indicated as reciprocal of dilution.
d = Dubious reaction for antibody to M. hyosynoviae by ELISA.
nd = Not done.
0 = Negative.

arthritic joint fluids of pigs collected at a slaughterhouse. Moreover, 6 isolates of bacteria were obtained from pigs from 6 other farms; all of them identified as streptococci, 3 Group C and 3 Group L.

By ELISA, antigen of *M. hyosynoviae* was demonstrated in 1 of 47 examined samples (Table 1). This sample was also positive by culture (Table 2). Another sample reacted dubious at the antigen examination and it was found positive in the antibody test. Antibody to *M. hyosynoviae* was found in 14 of 40 examined samples. A further 9 samples were recorded as dubious.

M. hyosynoviae was isolated in 6 cases among the 40 samples examined in the antibody test (Table 2). Three of these were serologically positive, 2 were dubious and 1 was negative. Streptococci were isolated in 4 cases, all without any reaction for antibody to *M. hyosynoviae* in the synovial fluid.

Discussion

M. hyosynoviae was cultivated from 20% of examined arthritic lesions of pigs from an abattoir, generally in low numbers. Antigen was found by ELISA in 2% and antibody in 35%. As 17 (43%) of 40 samples examined by all 3 procedures reacted positive for *M. hyosynoviae* in at least 1 test with a further 7 (17%) reacting dubious, it appears reasonable to suggest that *M. hyosynoviae* has been involved in the etiology of the examined cases of chronic arthritis. Further, the results are in agreement with the chronic nature of the lesions as antibody is by far the most frequent of the 3 parameters. Among the 16 negative samples streptococci were isolated in 4 cases probably representing other etiologies of the lesions.

The very low number (1 positive, 1 dubious) of samples reacting for antigen of *M. hyosynoviae* may be surprising. However, the low number of organisms obtained by cultivation

($\leq 10^4$) are all below the detection limit (10^5) for demonstration by ELISA in broth cultures (see Materials and Methods). Further, the chronic nature of the lesions may imply that many epitopes are already occupied by specific antibodies resulting in false negative tests.

The values for antibody in the joint fluids, as estimated by the ELISA test, are low as compared to a value of 40,000 for the rabbit antiserum. The immunoglobulins may at least partially have been produced locally in the joint capsule, as excessive accumulations of lymphoid and plasma cells have earlier been found in experimental studies with *M. hyosynoviae* (*Ross & Duncan* 1970). In experimental work with *Erysipelothrix rhusiopathiae*, *Timoney & Yarkoni* (1976) tentatively suggest a capability for local production of antibody.

In some studies on the etiology of the arthritis in abattoir baconers, authors have mainly focussed upon *E. rhusiopathiae* (*Bond* 1976, *Johnston et al.* 1987, *Meijer et al.* 1975, *Turner* 1982) regarding it as the prime cause as it is often found in about half of examined lesions, contrary to few isolations of *M. hyosynoviae* (*Johnston et al.* 1987, *Ross et al.* 1977). During the present investigation an almost opposite situation was revealed as *E. rhusiopathiae* was actually not isolated although it is a fairly common cause of arthritis in younger pigs. However, according to *N.C. Nielsen* (personal communication) a reasonable explanation for this failure may be the fact that tarsal joints of animals earlier infected with *E. rhusiopathiae* usually show anchylosis and periarticular exostosis without increased synovium in 100 kg baconers, having therefore escaped the synovial sampling. Even if present such synovium does usually not produce *E. rhusiopathiae* anymore when cultured.

It therefore appears that different microorganisms may be etiologically involved in the chronic arthritis found among baconers.

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Sammendrag

Mycoplasma hyosynoviae's ætiologiske betydning ved artritis hos slagterisvin.

Betydningen af *M. hyosynoviae* for udviklingen af de kroniske ledbetændelser, som optræder hos baconsvin på et slagteri er blevet undersøgt. Ved dyrkning fra 50 haseled af dyr fra 42 besætninger fandtes *M. hyosynoviae* i 10 tilfælde fra 8 besætninger, medens streptokokker fandtes i yderligere 6 tilfælde fra 6 andre besætninger. Med ELISA proceduren fandtes antigen af *M. hyosynoviae* i 1 af 47 af prøverne og korresponderende antistof i 14 af 40 af prøverne. Tilstedeværelse af *M. hyosynoviae* i led var sædvanligvis ledsaget af antistof mod mykoplasmen; dette var ikke tilfældet i prøver med fund af bakterier. Det må anses for sandsynligt, at *M. hyosynoviae* er årsag til mange af disse kroniske ledbetændelser, medens bakterier formodentlig er ansvarlige for de øvrige.

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