Swine mycoplasmoses

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Summary: Mycoplasma hyopneumoniae is the primary agent of enzootic pneumonia in pigs. The lung lesions, generally observed in young pigs, are characterised by a hyperplasia of the epithelial cells and an increased perivascular and peribronchiolar accumulation of mononuclear cells. Following M. hyopneumoniae infection, immune reactions are observed and resistance is induced in pigs. Laboratory diagnosis is generally performed by an immunofluorescent test and by enzyme-linked immunosorbent assay. Antibiotics are useful but the development of resistance has been described. Vaccination seems to be an effective method of controlling the disease.

M. hyorhinis, generally transmitted by sows to piglets through nasal secretions, exists in a high percentage in the respiratory tract of healthy pigs. But some strains can induce serofibrinous to fibrinopurulent polyserositis and arthritis. M. hyorhinis is isolated from acute and subacute phase lesions and serum antibodies are detectable.

M. hyosynoviae has a special affinity for joint tissue and may cause arthritic disease, leading to economic losses. This mycoplasma is generally located in the tonsils. Piglets are infected by sows after four to six weeks of life. Evidence of disease occurs in animals of between 30 to 40 kg and 100 kg, and bursae and joints are affected. A non-suppurative viscous fluid of a serofibrinous/serosanguineous nature is reported. In chronic cases, the synovial membrane is affected. M. hyosynoviae is isolated from the joints and pharyngeal/tonsillar samples and can induce antibodies in blood and joint fluid. Predisposing factors play an important role.

M. flocculare is widely distributed in swine, in normal and pneumonic lungs and in nasal cavities, but no pathogenic capability has been described. There is great interest in this mycoplasma because of the great similarity to M. hyopneumoniae.


INTRODUCTION

Mollicutes are the smallest autonomously replicating organisms, which differ structurally from other eubacteria in that they lack a cell wall, have a small genome

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and possess a low G + C content of DNA (16). *Mycoplasma hyopneumoniae* is the aetiological agent of enzootic pneumonia (116). This is one of the most common and economically important diseases which occur in swine. Recent studies also suggest a synergistic role for *M. hyopneumoniae* in the initiation of a variety of other bacterial and viral infections which occur under field conditions (83, 116). *M. hyorhinis*, another common mycoplasma of the respiratory tract of pigs, can cause polyserositis and arthritis in piglets. Arthritis in fattening pigs, caused by *M. hyosynoviae*, has been reported from several countries (51, 64, 116, 117). Other swine isolates not known to be pathogenic are present in the respiratory tract: *M. flocculare*, *M. sualvi*, *M. hyopharyngis* and several species of Achroleplasma (116).

**MYCOPLASMA HYOPNEUMONIAE: THE AGENT OF ENZOOTIC PNEUMONIA**

*M. hyopneumoniae* is the primary agent of enzootic pneumonia, one of the most chronic diseases in pig herds, with low mortality but high morbidity. This disease causes economic losses in swine production, through such factors as retarded growth, poor feed conversion and predisposition to bacterial pulmonary infections (37, 116).

**Aetiology**

*M. hyopneumoniae* was isolated simultaneously by Goodwin *et al.* (61) in the United Kingdom (UK) and by Maré and Switzer (94) in the United States of America (USA). It was named *M. suipneumoniae* by the former authors and *M. hyopneumoniae* by the latter. The morphology of *M. hyopneumoniae* cells is round or oval (medium diameter 0.20 µm), and they are bound by a simple plasma membrane about 10 nm thick. Fibrillar nuclear material can be identified in the cytoplasm.

*M. hyopneumoniae* may be cultivated in an artificial medium; however, culture and identification are tedious and time-consuming. The most widely used medium for *M. hyopneumoniae, M. flocculare* and *M. hyorhinis* is described by Friis (44, 50).

The growth of *M. hyopneumoniae* is very slow, compared to that of other porcine mycoplasmas. Glucose is oxidised. *M. hyopneumoniae* produces a slight turbidity and an acid colour shift after 4 to 15 days. For cultures on solid medium, atmospheric air plus 8% carbon dioxide is necessary. Colonies have a size up to approximately 0.5 mm, without a central dark area (Fig. 1).

**Epidemiology**

Under field conditions, grower pigs are the major source of infection with *M. hyopneumoniae*. Clinical mycoplasmal pneumonia is common in young animals but is not generally seen in adults, probably because the latter have suffered from the infection at an earlier age, which protects them against reinfection (62). However, this pattern cannot be the result of age resistance because, when an enzootic pneumonia-free herd becomes infected with *M. hyopneumoniae*, pigs of all ages develop acute pneumonia (140). Goodwin (60) showed that outbreaks had occurred in strictly isolated pneumonia-free herds, apparently because of air-borne transmission, but with decreasing frequency to 3.2 km away. Generally, *M. hyopneumoniae* is transmitted from older to younger pigs. Pigs of various ages seem to be equally susceptible to the
Colonies of Mycoplasma hyopneumoniae on solid medium

disease (82, 116). Surveys conducted in a variety of countries have indicated that typical lesions of enzootic pneumonia occur in 30% to 80% of slaughter pigs (116). In a survey of Minnesota slaughter swine, Pointon et al. (108) demonstrated that 100% of herds and 79.4% of individuals had cranioventral consolidations typical of those caused by M. hyopneumoniae. Feed conversion may be reduced by 14% to 20% and rate of weight gain by 16% to 30% in affected swine (123). In many herds, M. hyopneumoniae is associated with Pasteurella multocida and, in some cases, with Haemophilus parasuis or Actinobacillus pleuropneumoniae.

Clinical signs and lesions

Field conditions

Under field conditions, enzootic pneumonia is characterised by high morbidity and low mortality. Coughing is the principal clinical sign, observed over a few weeks or months, but is not constant. If the infection is complicated by bacteria, mainly Pasteurella multocida, other symptoms, such as laboured breathing and fever, and even death, may occur.

Pneumonia produced by M. hyopneumoniae is a catarrhal bronchopneumonia. The lesions are generally located in the apical and cardiac lobes, the accessory lobe and the cranial portion of the caudal lobes of the lungs. In the chronic phase of the infection, atelectatic lung lesions are observed. Bronchial and mediastinal lymph nodes are often enlarged (116).

Other clinical manifestations connected to infection with M. hyopneumoniae appear to be rare, but some evidence for joint and pericardial lesions has been observed. During investigations into parenteral inoculation of field strains, symptoms of arthritis were often produced in pigs, and were accompanied by lameness and pathological
changes of a serofibrinous nature (91, 92, 93). Apparently, this pathogenic capability varies a good deal between strains. Furthermore, by examination of field cases of arthritis in grower pigs, the isolation of *M. hyopneumoniae* has been a fairly constant, though occasional, finding over several years (N.F. Friis, unpublished findings). No study on the aetiological importance of this has been performed.

Pericarditis of a subacute serofibrinous/fibrinous nature in 100 kg slaughter pigs has been a dilemma during meat inspection. Broad range examinations have been shown to yield *M. hyopneumoniae*, which is by far the dominant microbiological finding by culture (71).

**Experimental infections**

*M. hyopneumoniae* can cause experimentally induced pneumonia in pigs, and this model has been used to examine the pathogenesis of the infection, the clinical, microbiological and serological aspects of the disease; and the efficacy of vaccines and antibiotics in the control of such infection (85).

According to Kobisch and Ross, the strains used in this type of experiment have generally been isolated from outbreaks of enzootic pneumonia. These strains are cultivated in liquid medium, filter-cloned and frozen (−70°C) until the experimental infection. After cultivation, the organisms are harvested before the end of the exponential growth phase. Hysterectomy-derived piglets, housed in experimental units in which the air is forced through absolute filters to prevent any contact with infectious agents, are used.

Piglets from two to 20 weeks of age are infected intratracheally by a sterile needle with 5 ml of broth culture of *M. hyopneumoniae* (10⁹ CCU per ml). The same number of uninfected pigs receive broth medium under the same conditions. Rectal temperatures and clinical signs are recorded daily. Blood samples are taken once a week from the piglets throughout the experiment and serum samples are analysed for antibody response (see ‘Diagnosis’). At random intervals, the piglets are anaesthetised, euthanised by exsanguination, and then necropsied. Post-mortem examinations are conducted on each animal and *M. hyopneumoniae* is detected by culturing from the lung tissue and from mucus in the trachea. Thin sections of fixed and paraffin-embedded lungs are stained with a mixture of haematoxylin and eosin and examined under a light microscope.

To detect *M. hyopneumoniae*, frozen thin sections of lungs are treated with antibodies against *M. hyopneumoniae* which have been labelled with fluorescein isothiocyanate (FITC) (78) (see ‘Diagnosis’).

Samples of the trachea and bronchi are collected from some pigs and prepared as previously described for scanning and transmission electron microscopy (12) (see ‘Pathogenesis’).

**Symptoms**

Age has no influence on the expression of the clinical signs. The body temperature of the control piglets is 39.5°C. In infected pigs, a slight hyperthermia (40°C to 40.3°C) may be noticed one week after challenge. Coughing begins two weeks after infection, peaks after five weeks, then gradually declines. Twelve weeks post infection, no clinical symptoms are noticeable. A second challenge, 14 weeks after the first one, does not induce any rectal temperature or coughing.
**Post-mortem findings**

No lesions are observed in the negative control group which received the broth medium.

Macroscopic lesions typical of mycoplasmal pneumonia are generally observed from 1 to 11 weeks post inoculation in the infected group. These lesions are located in the apical and cardiac lobes, the anterior part of the diaphragmatic lobes and in the intermediate lobe of the lung (Fig. 2).

![Macroscopic Mycoplasma hyopneumoniae pneumonia located in apical and cardiac lobes](image)

In the acute stage of the disease, catarrhal pneumonia is observed with exudate in the airways. The bronchial and mediastinal lymph nodes are often enlarged. In the chronic stage of the disease, recovering lesions, consisting of fissures of collapsed alveoli adjoining areas of alveolar emphysema, are generally observed nine weeks after infection.

**Histopathology**

Pulmonary tissue modifications observed in the lungs of pigs in the acute phase of infection consist of hyperplasia of the epithelial cells and an increased perivascular and peribronchiolar accumulation of mononuclear cells (Fig. 3). Lymphocytes and plasmocytes may both be detected, but the latter are more abundant. As the disease progresses, characteristic perivascular and peribronchiolar nodules, often compressing the lumen of the bronchioles, can be observed in recovering lesions. Samples of trachea collected from the infected piglets show very mild lesions of tracheitis,
Hyperplasia of the epithelial cells and increased perivascular and peribronchiolar accumulation of mononuclear cells consisting of epithelial hyperplasia and infiltration of the lamina propria by small numbers of lymphoplasmatic cells. The mucosal glands are normal. The lumen of the bronchi contains an exudate of mucoid material and polymorphous inflammatory cells with many neutrophils and some macrophages. In control pigs, the lung tissue does not show any lesions.

Pathogenesis

In vitro

In order to study the colonisation process of *M. hyopneumoniae* and to evaluate its effect on the epithelium of the porcine upper respiratory tract, an organ culture model, using tracheal rings of gnotobiotic piglets, is very useful (69). Good ciliary activity is preserved in non-infected rings for at least nine days, whereas infected rings retain their activity for the first few days but lose it completely after nine days. These results are in agreement with those of Debey and Ross (27). Histopathological observations showed marked differences in the appearance of the epithelium between non-infected and infected rings. The latter show a slight necrosis of the mucosa after 24 h to 30 h incubation with *M. hyopneumoniae*. After 48 h, a clear increase in epithelial necrosis and desquamation is observed. After five or more days of contact, the epithelium is completely ulcerated and mycoplasmas are seen associated with the remaining cells. A total of 48 h after inoculation, observation by electron microscopy shows that the mucosal surface is less ciliated and mycoplasmas are associated with the remaining cilia. It seems that receptors for *M. hyopneumoniae* are located exclusively at the apex of the cilia.
In the control pigs, normal tracheal and bronchial surfaces, observed by scanning electron microscopy, are composed of ciliated epithelial cells mixed with microvilli (Fig. 4). In pigs killed during the first or second week after challenge, colonisation of epithelial cells by *M. hyopneumoniae* is noticeable. Two to eight weeks after inoculation, loss of cilia is evident and, in some cases, ciliated cells are seen to be exfoliating (Fig. 5). Scanning and transmission electron micrographs show that the mycoplasmas are predominantly found closely associated with the tops of cilia (Fig. 6). At 8 to 11 weeks post-inoculation, the trachea and bronchi of pigs with recovering macroscopic lesions appear to be heterogeneous. After this period, the trachea and bronchi of pigs appear normal.

**Fig. 4**

Scanning electron microscopy: tracheal surface of a non-infected pig (ciliated epithelial cells and microvilli)

In these experimental conditions, a capsule layer covering the cells, reported by Tajima *et al.* (135) and Young *et al.* (143), was not observed. Nevertheless, filaments stainable by ruthenium red are occasionally seen radiating from the cells. These filaments may represent capsular material. It seems that *M. hyopneumoniae* capsular polysaccharide plays a role in the interaction of mycoplasmas and their host cells (143).

Results obtained from *in vitro* and *in vivo* experiments suggest that the earliest response is the attachment of mycoplasmas to the ciliated cells. This ability of mycoplasmas to adsorb to host cells is a prerequisite for pathogenicity. However, the mechanism used by mycoplasmas to colonise tissue is not well understood and the relationship between this phenomenon and the cilia-stopping effect is not known. Zhang *et al.* (144) demonstrated that a 97 kDa protein is an adhesin of *M. hyopneumoniae* with a glycolipid receptor. Recently, Chen *et al.* (22) have
indicated that an adhesin of P 114 is implicated in the colonisation of *M. hyopneumoniae*. This protein contains two subunits, P 28.5 and P 57. The latter is a glycoprotein. A characterisation of adherence of *M. hyopneumoniae* may lead to a
better understanding of the pathogenesis. Collier et al. (24) suggested the possibility that nutritional deprivation of host cells by mycoplasma injuries, caused by a metabolic competition between host and parasite, may create a functional impediment. The authors suggested that *M. hyopneumoniae* has the ability to break down the terminal bars, opening the intercellular spaces and disrupting tissue architecture. The exfoliation observed after *M. hyopneumoniae* infection could have been induced by a similar process. The attachment of mycoplasmas to the ciliated cells is followed by a cytopathic effect and exfoliation of epithelial cells. Simultaneously, cellular reactions occur, consisting mainly of increased peribronchiolar and perivascular mononuclear cell accumulation (12). But little information is known about this phenomenon.

**Immunological and pathological reactions**

Immunoblot analysis of the sera of piglets experimentally infected with *M. hyopneumoniae* allows identification of the major immunogenic proteins. Young and Ross (142) have found five major immunogenic proteins of *M. hyopneumoniae*, with molecular weights of 110, 64, 50, 41 and 36 kDa. These results are in accordance with the observations of Strasser et al. (133). Le Potier et al. (89) have found six immunogenic components of *M. hyopneumoniae*, evident four weeks after infection. These antigens ranged in molecular weight from 36 kDa to 138 kDa, the major component being 40 kDa, and persisted for at least 20 weeks. These results differ from those of Mori et al. (98), who reported that protein bands with molecular weights from 38 kDa to 96 kDa are detected after *M. hyopneumoniae* infection.

Some proteins are possessed in common with those of *M. flocculare*, especially 74 kDa and 46 kDa. As described by Frey et al. (37), protein P36, an L lactate dehydrogenase (LDH) of *M. hyopneumoniae*, belongs to the predominant immunogenic proteins in pigs which are naturally or experimentally infected.

Enzyme-linked immunosorbent assay (ELISA) with Tween 20-extracted antigen gives information on the humoral immunoglobulin G (IgG) antibody response (101). Feld et al. (34) have developed a blocking ELISA by using a monoclonal antibody specific to an epitope of the 74 kDa antigen, a protein common to the three main porcine mycoplasmas, according to Bölkske et al. (15). The antigenic reaction against *M. hyopneumoniae* LDH has been shown to be species-specific (37). Using LDH as an antigen for an ELISA, these authors show that pigs which are experimentally or naturally infected with *M. hyopneumoniae* raise antibodies against LDH in two steps. An early, relatively weak anti-LDH response is detected between five and ten weeks post-infection, when clinical signs and lung lesions occur. A second, strong rise in anti-LDH antibodies is observed from the 12th week after infection, at a time when the signs of disease and the infectious agent disappear. The high anti-LDH titres persist until 21 weeks post-infection.

A blocking ELISA has also been developed by using a monoclonal antibody specific to an epitope of the 40 kDa protein (89). This ELISA is used to test sera from pigs infected experimentally with *M. hyopneumoniae*. All the animals are seropositive three to four weeks after infection. Peak responses are observed 10 to 20 weeks after infection and then decline very slightly, but are detectable 20 weeks post-infection. These results reflect the progress of infection with *M. hyopneumoniae*: disease evolution, clinical symptoms and lung lesions (82).

Similar observations have been made when pigs are experimentally infected during the fattening period (16 weeks of age): the disease can be established independently
of the age of the animals. When pigs are infected twice, at two weeks and at 16 weeks of age, ELISA titres clearly increase after the booster infection, then slowly decline. These animals show only recovering lesions characterised by lymphoid nodules, induced by the first infection. Resistance is induced in pigs after *M. hyopneumoniae* infection. The association of the pathomechanism of *M. hyopneumoniae* and the immune reactions implicated in the disease could explain the resistance of pigs after a second challenge. These observations suggest that cell-mediated immunity is very important in the pathogenesis of the disease. Immune responses of pigs inoculated with *M. hyopneumoniae* have been analysed. Examination of lung sections revealed that cells producing IgM, IgA and IgG were present. Resolution of the pneumonia appeared to correlate with the development of sensitisation of the blood lymphocytes and marked increases in immunoglobulins, particularly IgG, in lung washings (96). A significant increase of antigen-specific plasma cells in the lymph nodes and lung tissue, as well as of antibodies in tracheobronchiolar secretions, was demonstrated two weeks after infection by Sutter et al. (134). This specific reaction was followed by a marked increase of immunoglobulin-containing cells in lymph nodes and lung tissue. According to Sutter et al. (134), lung lesions and the immune response after *M. hyopneumoniae* could be the result of auto-immune reactions. Taylor and Howard (136) demonstrated that, in mice infected with *M. pulmonis*, an intact immune system was necessary to induce lung lesions. These observations could explain the resistance observed in twice-infected pigs.

**Diagnosis**

Pigs infected with *M. hyopneumoniae* alone do not develop severe clinical signs: a weak elevation of body temperature, inconstant coughing over eight to ten weeks, weak retarded growth, low mortality and high morbidity.

But, in many cases, *M. hyopneumoniae* is associated with *Pasteurella multocida* or *Actinobacillus pleuropneumoniae*. This association induces severe clinical signs and retarded growth (80).

Lesions of mycoplasmal pneumonia, described in the previous section, are characteristic and differ from those induced by other agents. *M. hyopneumoniae* is isolated from tracheal mucus and from lung tissue (44, 50). However, culture identification is tedious and time-consuming. Usually, detection of *M. hyopneumoniae* in the pig lung is performed by an immunofluorescent test, using polyclonal antibodies (5, 78). Frozen thin sections of lung are treated with anti-*M. hyopneumoniae* antibodies labelled with fluorescein isothiocyanate (Fig. 7). A positive immunofluorescent test is generally associated with extensive pneumonia or, in some cases, with recovering lesions (85). A good correlation exists between these results and the isolation of *M. hyopneumoniae*, which is generally not isolated from recovering lesions.

However, the immunofluorescent test is ambiguous, since previous studies have revealed that *M. hyopneumoniae* shares antigenic determinants with two other porcine mycoplasmas: *M. flocculare* and *M. hyorhinis* (7, 15). Identification of *M. hyopneumoniae* deoxyribonucleic acid (DNA), using specific DNA probes, has been proposed as a suitable method for detection of mycoplasmal pneumonia (3, 4, 127). Abiven et al. (2) have constructed a partial genomic library of *M. hyopneumoniae* in plasmid pBR 322, using *Hind III* chromosomal fragments. One selected recombinant plasmid, designated 1141 (1-65 kbp), has been evaluated for
specificity against genomic DNA from numerous mycoplasmas and other bacterial species isolated from the respiratory tract of pigs. The $^{32}$P labelled I 141 can specifically detect down to 400 pg of *M. hyopneumoniae* genomic DNA.

The results obtained with hybridisation, compared with an immunofluorescent test using clinical tracheobronchial specimens from piglets experimentally infected with *M. hyopneumoniae*, show that there is 63% agreement between the two tests.

More recently, Blanchard et al. (13) have used polymerase chain reaction (PCR) to detect *M. hyopneumoniae* in tracheobronchiolar washings collected from experimentally infected piglets. I 141 probe (accession number u 02537) primers have been chosen to produce an amplified fragment of 1.561 bp. All the *M. hyopneumoniae* strains tested can be detected by the PCR test. DNA from other mycoplasmal and bacterial species isolated from the respiratory tract of piglets gives negative results. The detection limit is estimated to be 500 fg of purified DNA, corresponding to $4.10^2$ organisms. The sensitivity of PCR reaction is also evaluated. On micro-organisms in culture, the limit of sensitivity is $2.5 \times 10^3$ organisms. These authors show that the PCR tests performed either on DNA or on crude extract samples are identical in 133/143 cases analysed. Moreover, a good correlation is observed between the immunofluorescence test and PCR in 121/143 cases. Very recently, Verdin et al. (139) used a nested PCR to detect *M. hyopneumoniae* under field conditions. The amplified product, corresponding to a specific *M. hyopneumoniae* DNA fragment of 1.561 bp, is used in a two-step (nested) PCR, directly in tracheobronchiolar washings from living pigs. These authors have designed a set of primers for nested PCR of *M. hyopneumoniae*, which allows the amplification of a DNA fragment 706 bp long. In this study, 43 piglets, belonging to three herds, were individually followed from birth to slaughter. Blood samples and tracheobronchiolar washings were collected at two,
four and six months of age for serological and PCR analysis. At slaughter, each lung was macroscopically examined for pneumonia, and the immunofluorescent test was used to detect *M. hyopneumoniae*. Comparing the results of both tests indicates that, in the early stage of infection, whereas the serological assays using a blocking ELISA (89) gave negative results, nested PCR tests showed positive results (42%). Post-mortem observations also correlated well with PCR results. According to these authors, the nested PCR is the most sensitive assay for detecting *M. hyopneumoniae* in living pigs under field conditions and can be used in current diagnosis.

An accurate diagnosis of *M. hyopneumoniae* infection is essential to prevent it from spreading. Pigs infected with *M. hyopneumoniae* develop antibodies which are detectable by serological techniques. Different tests have been evaluated, including agglutination, indirect haemagglutination, complement fixation and ELISA (116). But non-specific reactions occur as a result of *M. flocculare* or *M. hyorhinis* infections (15). A more specific ELISA, using Tween 20 extracted antigen, was developed by Nicolet et al. (101) and there was no cross-reactivity between *M. hyopneumoniae* and *M. flocculare* using this ELISA (133). A purer antigen appears to be essential to improve the specificity of the test. Another approach was to enhance the specificity through the antibody (34, 89), as described in the previous section.

Sørensen et al. (125) used an ELISA for the surveillance of *M. hyopneumoniae* infection in Danish pig herds, for which ‘herd predictive values’ had been calculated. In specific pathogen-free (SPF) herds giving positive results by ELISA, 42% were subsequently found to be infected, while 100% of herds giving negative results were uninfected. Among naturally infected animals, reaction in the colostrum was more frequent than in the serum, and this difference was most pronounced if the colostrum samples were obtained shortly before or after farrowing. These authors indicate that the time required for seroconversion following natural exposure to *M. hyopneumoniae* differs according to the management system. Antibodies were first detected from three weeks until five weeks after exposure.

Levonen (90) used an ELISA to detect enzootic pneumonia in pig herds and showed that sow colostrum is a suitable sample for diagnosis. If enough samples are collected from a herd, antibody assay in colostrum can demonstrate which herds are free from enzootic pneumonia.

More recently Rautiainen et al. (109) indicated that the ELISA test is a very good tool for regular monitoring of herds for *M. hyopneumoniae* infection. Colostrum samples are easy to collect and store, which can be done by the owner.

**Treatment**

The absence of a cell wall has restricted the selection of antibiotics available for use in *Mollicutes*. These organisms are insensitive to ß-lactam antibiotics and generally resistant to polymyxins, rifampin and the sulfonamides. The most useful antibiotics to treat mycoplasma infection are the tetracyclines, the macrolides and some fluoroquinolones. The aminoglycosides and chloramphenicol possess less inhibitory activity (10).

Some authors have reported the *in vitro* activity of antimicrobial agents against *M. hyopneumoniae* (66, 141). Ter Laak et al. (137) showed that spiramycin, tylosin, kitasamycin, spectinomycin, tiamulin, lincomycin and clindamycin are effective against all strains of *M. hyopneumoniae* tested and that quinolones are highly effective.
The activity of quinolones was also demonstrated in vivo (81). Hannan and Ripley (67) showed the in vitro development of resistance in \textit{M. hyopneumoniae} to SDZ PMD 296, tiamulin, tylosin and oxytetracycline. Control of enzootic pneumonia can be undertaken by antibiotherapy, using a continuous or a pulse-dosing system (20). In this way, Le Grand and Kobisch (87, 88) showed that pulse medication (200 ppm of tiamulin and 600 ppm of chlortetracycline) in a herd which is chronically infected with \textit{M. hyopneumoniae} induces a significant reduction of pneumonia, but does not reduce the presence of the micro-organism. These results confirm those of Kavanagh (73).

Control and prevention

Early methods for the prevention of enzootic pneumonia have been reviewed (116). Effective control depends on an optimal environment (air quality, ventilation, temperature, etc.). Methods including the periodic examination of lungs from slaughter, clinical inspections and serological diagnosis, etc. are necessary.

Total eradication of enzootic pneumonia from swine herds has been achieved by the separate breeding of adult sows in isolation. This method is based on the fact that \textit{M. hyopneumoniae} is eliminated from its habitat, the lower respiratory tract, in adult sows. No animals under the age of one year are present in a herd for one month. This method has been tried with success in Denmark.

To eradicate or control \textit{M. hyopneumoniae}, repopulation of infected herds with enzootic pneumonia-free animals seems to be the most effective practice, but this is difficult to achieve because of the high cost (141). Vaccination seems to be an alternative to control the disease.

Vaccines which differ in their method of preparation show varying degrees of efficacy in swine (122). Killed whole cell vaccines have been introduced to control \textit{M. hyopneumoniae} infection (26, 87, 110). Generally, active immunisation of the piglets is employed: 2 ml doses of vaccine intramuscularly at 7 and 21 days of age. However, passive immunisation is an alternative, through the vaccination of pregnant sows (79, 80). In these cases, the authors used purified membrane proteins of \textit{M. hyopneumoniae}. Recently, subunit membrane vaccines have also been described (28, 29).

Combined infection with \textit{M. hyopneumoniae}, \textit{Pasteurella multocida} and \textit{Actinobacillus pleuropneumoniae} is very common and is responsible for major economic losses in pig herds world-wide. It has been proved that the use of \textit{M. hyopneumoniae} vaccine may help to control the mycoplasmal infection (initiating agent) and to prevent the exacerbation of \textit{P. multocida} (84, 87) and \textit{A. pleuropneumoniae} (95).

\textbf{MYCOPLASMA HYORHINIS POLYSEROsisTIS AND ARTHRITIS}

\textit{M. hyorhinis} is a very frequently occurring micro-organism in swine. According to Friis and Feenstra (52), the normal habitat is the mucous membranes of the upper respiratory tract and the tonsils. Cole \textit{et al.} (23) mention that \textit{M. hyorhinis} resides in a high percentage of normal nasal cavities in adult pigs and growing pigs. A descending spread to the lungs occurs easily and \textit{M. hyorhinis} is regarded as part of
the aetiological complex in pneumonias among piglets (39). *M. hyorhinis* is frequently present in cases of polyserositis and it has also been recovered from the conjunctiva of swine (45).

This micro-organism is also a very common contaminant in cell culture lines.

**Aetiology**

The media and methods for isolation of *M. hyorhinis* have been described by Friis *et al.* (44, 50). For isolation, three to five days are necessary but established cultures grow in one to two days (122). *M. hyorhinis* ferments glucose and does not utilise arginine or hydrolyse urea. Colonies of *M. hyorhinis* develop in two to five days, measuring 0.5 mm to 1 mm in diameter, and have the typical ‘fried egg’ morphology.

**Epidemiology**

*M. hyorhinis* infections are transmitted to young piglets shortly after birth by sows or older pigs. The micro-organism can be isolated from the nasal or sinus secretions of approximately 10% of sows (120) and from the nasal secretions of about 30% to 40% of weaning pigs (116). Kobisch *et al.* (77) show that *M. hyorhinis* can be isolated from pneumatic lung tissue of SPF piglets (20%) or of conventional piglets (66%). Many infected pigs show no clinical signs (116). *M. hyorhinis* often spreads haematogenously from the respiratory tract and may then be isolated from piglets of three to seven weeks old, with distinct serofibrinous lesions in the pericardial, pleural and peritoneal cavities (52). *M. hyorhinis* has also been implicated in arthritis in fattening pigs (75). In Japan, Morita *et al.* (99) followed the pathology of pigs (from one day to one year) affected with otitis. *M. hyorhinis* was revealed on the luminal surface of the eustachian epithelium.

**Clinical signs and lesions**

**Field conditions**

According to Ross (116), outbreaks of polyserositis generally occur in swine of three to ten weeks of age, but occasionally the disease occurs in young adults. The evidence of illness occurs three to ten days after exposure or following stress. Ross reports that acute signs consist of a moderate elevation in temperature, lameness, moderate inappetence, difficulty in moving and swollen joints (116). Laboured breathing, stretching movements and lying in sternal recumbency characterise the main clinical signs (116). Two to three or even six months later, the lameness and joint swelling may become less severe.

**Experimental infections**

There are different opinions as to the role of *M. hyorhinis* in swine diseases, probably because this organism varies in virulence, depending on the strain. Kobisch (76) used five field strains of *M. hyorhinis* isolated from pigs (lungs and joints) and infected two-week-old SPF piglets intranasally (five for each strain and five control pigs). Pigs were sacrificed from three to 14 weeks post-infection. Clinical signs appeared in four groups one week post-infection: hyperthermia (41°C), joint swelling, lameness and growth delay. In these groups, *M. hyorhinis* caused polyserositis and
arthritic. In the acute stage, affected pigs showed serofibrinous pericarditis, pleuritis and peritonitis. Synovial membranes were hypertrophied, synovial fluid was serosanguineous, and the amount of fluid increased. The chronic stage of lesions consisted of organised fibrous adhesions, and articular erosion was observed (Fig. 8). Neither pneumonic lesions nor rhinitis were noted. Only one piglet inoculated with the fifth strain developed pleural adhesions. The results suggest that these strains of *M. hyorhinis* express differences in virulence as described by Gois and Kuksa (57). Microscopic changes were characterised by fibrinous inflammation of the serosal membrane and by mononuclear cell infiltration. *M. hyorhinis* was isolated from nasal secretions, lungs, joints, lymph nodes and from pleural, peritoneal and pericardial exudate. Bacteriological examinations were negative in control pigs.

![Image](image_url)

**FIG. 8**

Chronic stage of infection with *Mycoplasma hyorhinis* in a pig (articular erosion is observed)

The experimental reproduction of serositis in pigs has been also obtained by other workers (52).

**Diagnosis**

Gross lesions of serofibrinous to fibrinopurulent polyserositis and arthritis in three to ten week-old pigs are suggestive of *M. hyorhinis* disease (122). However, similar lesions can be caused by *Haemophilus parasuis*, *Streptococcus suis*, etc. *M. hyorhinis* can be isolated from acute and subacute phase lesions. Serum antibodies from infected pigs are detected by different techniques, such as complement fixation test, indirect haemagglutination, etc. After experimental infection, antibodies can be detected six weeks post-infection (76).

Synovial fluid antibody levels have been observed to be higher than corresponding serum titres (23).
Immunological mechanisms may be involved in joint damage, as evidenced by the persistence of *M. hyorhinis* antigens in joint tissues a long time after cultures have turned negative. Further evidence is provided by the ability of an intradermal injection of killed *M. hyorhinis* to induce a flare of arthritis 18 months after the initial induction of disease, and by the absolute lymphocytosis and increased gamma globulin levels seen in the later stages of disease (23). After experimental intraperitoneal inoculation, microscopic lesions have been reported in brain tissue, in the absence of clinical signs (23). These results indicate that *M. hyorhinis* can gain access to the brain.

**Treatment and prevention**

*M. hyorhinis* is sensitive to the antibiotics described in the section on *M. hyopneumoniae*. According to Ross (116), antibiotic therapy for swine clinically ill from *M. hyorhinis* infection is not very satisfactory. The inflammatory response seems either to prevent antibiotic penetration or to be self-perpetuating. It seems that stress and different diseases may predispose animals to disease caused by *M. hyorhinis*.

**MYCOPLASMA HYOSYNVOIAE**

*M. hyosynoviae* is a porcine mycoplasma species with a special affinity for joint tissue. It may be the cause of serious and troublesome arthritic diseases, with high rates of affected animals in certain herds and great economic losses for the owner.

*M. hyosynoviae* was first described as a separate species of *Mycoplasma* by Ross and Karmon (118). Moreover, the participation of *M. hyosynoviae* in non-complicated swine arthritis was also recognised (117). It was found simultaneously in the upper and lower airways of swine in Denmark (38, 40) and in airways and joints in England (56, 112). Since those early days, this micro-organism has been demonstrated in arthritic joints and in the respiratory tract, including the tonsils, of swine in many countries around the world: in Oceania (54), in Korea (106), in Japan (74, 105), in various European countries (9, 14, 18, 19, 50, 51, 55, 58, 59, 100, 121, 138), and in the USA (14, 72, 86, 124, 145).

*M. hyosynoviae* is a rapidly propagating species, in so far as it is cultivated in traditional media especially enriched with mucinous material from swine. Its growth on solid medium shows the characteristic ‘fried egg’ morphology.

**Epidemiology**

*M. hyosynoviae* appears to be world-wide in distribution and is assumed to be present in by far the majority of swine herds in countries with an industrialised scale of swine production. Thus, its occurrence appears to concur with the conditions known to favour the other swine mycoplasma species, *M. hyopneumoniae* and *M. hyorhinis*.

Swine are the natural and only host for *M. hyosynoviae*. This micro-organism is localised mainly in the *tonsilla palatina* (50), which tends to remain infected (117) throughout the life of the host. Piglets may become infected from their mothers (120) after approximately four to six weeks of life but seldom during their first four weeks, when they appear somehow naturally resistant; a situation unknown with other porcine mycoplasmas. After such piglets have been weaned and mixed with other young swine, the infection gradually spreads among the animals, sometimes reaching
infection levels approaching 100% (63, 65, 103, 104). In the recently infected animal, the mycoplasma finds its hiding place in the tonsils, from where infections of the pharyngeal and, to a lesser degree, the nasal mucosal surfaces are sustained (63, 65).

Pathology

From the tonsillar reservoir, the infection may descend through the respiratory tract to pneumonic lungs (54, 56, 58), where an occurrence of approximately 10% has been noted (40). In infected animals, the mycoplasma may spread haematogenously (64), showing great affinity for joint synovial membranes, while other organs are seldom affected, apart from during a short period following generalisation (64, 71, 119).

Evidence of disease occurs in animals of between 30 kg and 40 kg in weight and 100 kg or more (103, 117). High morbidity rates of between 10% and 50% can be noted, especially among animals of 50 kg to 60 kg in grower units, but breeding boars and gilts of 100 kg or more may also be affected. Symptoms appear fairly acute, i.e. difficulty in moving, stiffness, lameness, arched backs and an inability to get up and reach the trough. The large joints in one or more of the legs, especially the hind legs, are principally affected, but only in hock joints are the swellings readily visible (Fig. 9). By palpation, lesions of other joints may be located and the animal reacts to pain. In addition to lesions in joints, the bursae are often affected. It is possible to locate subcutaneous enlarged bursae, mainly on the outer side of the hock joint, which have a typical brownish content. These bursae are usually non-communicating to the joint. Moderate inappetence and slight fever may complete the acute situation. In many animals, both under natural and experimental conditions (64, 119), the disease may last for seven to ten days, but in some animals a protracted course, with even a chronic relapse, may occur.

![Fig. 9](image.png)

Joints of two pigs, 3.5 months old, infected with *Mycoplasma hyosynoviae*, one month post-infection. Note the several swellings around the hock joint.
Macroscopic examination of the affected joints reveals a yellow or brownish non-suppurative viscous fluid, often of increased volume and of a serofibrinous/serosanguineous nature. In more chronic cases, the joint capsule may be distended from the great increase in brownish fluid (Fig. 10). The synovial membrane may appear oedematous, hyperaemic or discoloured brownish, although changes of the articular cartilage are rare. Histopathological changes (63) are dominated by synovitic changes involving hyperplasia of the synovial lining, moderate villous hypertrophy, oedema and, later, mononuclear cell infiltrations with slight fibrosis of the capsule.

![Joint of a pig of about 60 kg, infected with *Mycoplasma hyosynoviae*. Field case, knee opened. Note brownish-coloured fluid](image)

Reproduction of the disease has been achieved in a number of trials (54, 64, 119, 121, 145). It has been possible to reproduce clinical signs of arthritis or at least joint infection fairly consistently. An incubation period of approximately seven days is reported, with the clinical symptoms lasting from one to three or four weeks or longer.

Suppression and even masking of such symptoms by antibiotics used as feed additives is a reality today. Indeed, in many countries and herds this application of antibiotics is a general occurrence, making an exact evaluation of disease conditions somewhat problematic.

Predisposing factors (103, 115, 120) appear to be of great importance for the development of the disease as a herd problem. Although *M. hyosynoviae* should be regarded as being present in most herds, it is obvious that relatively few herds actually experience the arthritis as a problem. Often farmers are unaware of the disease, which nevertheless can be diagnosed at slaughterhouse inspection (51), with isolation of the agent from affected joints. In groups of pigs, the infection may persist for weeks or months before arthritis occurs (65). Thus, triggering mechanisms appear to be responsible for the outbreak of disease. Several factors have been highlighted in this
connection, including genetic background, early weaning, moving of animals to new units or even houses, deep bedding, too much free running space, floor types, etc. However, more research is needed to clarify such hypotheses. A well-known releasing factor is the moving of animals to new surroundings, as the disease often begins one to three weeks after a transfer.

**Diagnosis of agent**

Isolation (44, 50, 51) of *M. hyosynoviae* by culture with subsequent serological identification is an easily performed, highly sensitive and definitive procedure, which takes between four and eight days (see incorporated recipe). As growth is often inhibited by grounded tissue, the primary set-up must comprise 10-fold dilutions to at least $10^{-4}$ of swab samples or grounded tissue suspension, with enough material kept at $-25°C$ for possible repetition. *M. hyorhinis* and most bacteria are suppressed by using selective media. Moreover, by repeating the process for overgrown cultures after some weeks, using the frozen material, problems with bacteria, especially in pharyngeal swab samples, are overcome. Despite the readiness of *M. hyosynoviae* to grow in primary cultures, isolation results from affected joints are often very disappointing. Indeed, it seems that the agent is often present in concentrations below demonstration level during the subacute and chronic phases, when both the amount of joint fluid and the antibody content are steadily increasing, parallel with a decrease in the number of mycoplasmas. For collection of pharyngeal samples, it is essential to use a special scraping tool instead of cotton swabs to obtain mucus with mycoplasma content (102).

For serological identification of isolates, the disc growth inhibition test (DGI) and the immunofluorescent test on agar-grown colonies are very reliable, but of course hyperimmune sera from rabbit or swine are needed. The DGI test is performed by culture of the mycoplasma on solid medium with dried antibody-soaked filter paper discs in an atmosphere consisting of $N_2$ and 5% to 10% $CO_2$. Especially beneficial for the test is the fact that all strains are easily inhibited, probably indicating that distinct subgroups within the species hardly exist, and that little variation in antigenic composition occurs. However, it must be pointed out that comparisons of strains are needed.

Immunohistochemical demonstration of the mycoplasma can be performed by immunofluorescence. Either the direct method, using FITC-conjugated swine antiserum, or an indirect method, using rabbit antiserum and an anti-rabbit FITC conjugate, may be employed (114). Alternatively, the immunoperoxidase procedure can be tried, but much more research and experience are needed in this area.

PCR appears to be a promising technique (4), which enables the detection of the mycoplasma in culture and in tissue. Apparently, all field strains can be recognised by the primers used, but this technique is less sensitive in direct examinations than the culture method.

**Serodiagnosis**

The demonstration of antibodies in blood samples and joint fluid has been performed by conventional methods over the years (33, 51, 120, 145; N.F. Friis *et al.*, unpublished findings). These experiments have clearly shown that *M. hyosynoviae* can provoke the development of antibodies in blood and in joint fluid. In summarising the
experiments, it appears that piglets readily become passive carriers of maternal antibodies (easily demonstrable during their first two months of life) and that the antibodies are gradually reduced, becoming virtually undemonstrable at three to four months of age. During the last one to two months before slaughter, many pigs gradually build up their own actively induced antibodies based on natural infection. It thus appears that the distinct clinical symptoms of arthritis coincide with a low level of antibodies in the blood.

An attempt to construct a monoclonal-based, highly sensitive, competitive ELISA procedure has been presented by Pedersen et al. (107) and appears to be promising for future use.

A traditional, polyclonal antibody ELISA, described by Friis et al. (51) and further improved, is here given in brief. The test is performed with a hypertonic ELISA buffer and every measurement is performed with an antigen of *M. hyosynoviae* and a nonsense antigen (*M. bovirhinis*) for reference. Antigen is harvested from culture on solid medium and washed 4 times by centrifugation in phosphate-buffered saline (PBS). The final pellet is suspended in 5-10 ml of distilled water, freeze-thawed for 5 cycles, filled to 25 ml with PBS and, without centrifugation, is stored at -80°C in small batches and later titrated. A plate is coated with antigen and, next morning, washed with buffer and blocked at room temperature for 30 min, with 2% horse serum in buffer. This is again washed and test serum added, while the peroxydase conjugate (1:5,000) is absorbed with horse serum, added to reach 1%, in movement at 37°C for 15 min. Wash, add the peroxydase conjugate and wash again. Add the enzyme substrate and stop the reaction with 0.1 M H\textsubscript{2}SO\textsubscript{4}, and take an optical density (OD) reading. By using positive sera, the optimal dilution of *M. hyosynoviae* antigen can be estimated. By using negative sera, the concentration of the nonsense antigen can be adjusted to give the same OD value as obtained for the *M. hyosynoviae* antigen.

**Prevention of the disease**

Several antibiotics which have a low minimal inhibitory concentration (MIC) value *in vitro* for *M. hyosynoviae* are available. They are as follows: tiamulin, lincomycin, fluoroquinolones, tetracyclines and macrolides (25, 53, 66, 137). Such drugs offer the potential for effective treatment. When used to cure sick animals in therapeutic work, or when added to feedstuffs as a prophylactic, a reasonably good effect has been obtained, provided very early treatment is administered (14, 18, 117).

There are few data on vaccination programmes, but Feenstra et al. (33; unpublished findings) reported a distinct antibody response, however, with no or unsatisfactory protection in field and experimental trials.

The total eradication of mycoplasma infection in herds by separate breeding and farrowing of adult sows under cover of antibiotics is hardly possible for *M. hyosynoviae*, because this mycoplasma probably remains forever in the sows, hidden in the tonsils, thus constituting a source of infection for the piglets. This situation differs from that of *M. hyopneumoniae*, which is immunologically eliminated from its only natural habitat, the lower respiratory tract, thus probably leaving the adult animals free of the mycoplasma.

As discussed earlier, several predisposing factors are assumed to play an important role in the development of the disease. Great attention should be paid to these circumstances and, if possible, changes in management should be instituted.
MYCOPLASMA FLOCCULARE

*M. flocculare* is a porcine mycoplasma species, the habitat of which is the respiratory tract. Although no pathogenic capability has been ascribed to it so far, this micro-organism has attracted considerable scientific and diagnostic interest because of its great similarity to *M. hyopneumoniae*, the agent of enzootic pneumonia.

*M. flocculare* was first described as a separate species of *Mycoplasma* by Friis (41) and Meyling and Friis (97), and finally confirmed by Rose *et al.* (113). Since then, this micro-organism has been isolated from the upper respiratory tract of pigs and from both normal and pneumonic lungs in several countries in Europe (9, 31, 32, 132). *M. flocculare* has also been demonstrated in North America (6, 8, 11, 128).

*M. flocculare* is a fastidious and slowly propagating species, which, however, may be reliably cultivated in Friis medium. Swine are the only natural host and the mycoplasma has never been isolated from other mammalian species.

Epidemiology

*M. flocculare* has been demonstrated to be widely distributed among swine in several countries and, indeed, is probably present in most if not all swine-raising countries. Its normal habitat is the respiratory tract (31, 32, 41), where it can be found in both normal and pneumonic lungs, and also in the nasal cavity. Piglets are infected by their mothers and, after weaning, the mycoplasma gradually spreads among the animals, consolidating its presence on the mucous epithelium in the nasal cavity. *M. flocculare* may be present in almost any pig in larger herds (126). This mycoplasma usually colonises the non-pneumonic lung by descending spread through the trachea. In the lung it is demonstrable during routine examination (48). However, the presence of *M. flocculare* at this site appears to be of restricted duration (7). In addition, the pneumonic lung may be infected but the presence of *M. flocculare* is often masked by the high titre of *M. hyopneumoniae*. It is reasonable to assume that *M. flocculare* tends to remain in the nasal cavity, where it can be found in adult animals, throughout the life of the host.

In addition to the respiratory tract, the conjunctival sac might also constitute a possible locus for *M. flocculare* (38). As well as its biological occurrence, *M. flocculare* can also be cultured from air samples, collected on filter pads from the aisles of many swine buildings, by using equipment with a filtration capacity of one thousand litres of air per min (N.F. Friis, unpublished findings).

Cultural characteristics

*M. flocculare* possesses some characteristic growth features which distinguish this mycoplasma from *M. hyopneumoniae* (41, 50, 68). Thus, its growth in liquid medium is slower and its metabolism of glucose more intense, with pH reduction down to 6.0 (70, 113, 130). Moreover, *M. flocculare* is able to grow at a lower temperature of 30°C, a feature probably related to its affinity for the upper respiratory tract. The morphology in liquid medium is dominated by pleomorphism and mycelial growth, in contrast to *M. hyopneumoniae*; this may be seen by phase-contrast microscopy and confirmed by electron microscopy (68). Even with the naked eye, these particles are just visible as faint flocks. It is from this that the species designation, *flocculare*, is derived. On solid medium, colonies of heterologous size develop and, as is the case for *M. hyopneumoniae*, the central dark area is missing.
Pathology

*M. flocculare* is restricted to the porcine respiratory tract and, apart from some isolations from the conjunctival sac, has never been found at other sites in swine herds.

*M. flocculare* has often been isolated from the apparently normal lungs of field animals, as well as from those of animals with pneumonic lesions. However, bearing in mind experimental studies, all conclusions indicate that *M. flocculare* should be regarded as a non-pathogenic micro-organism for the porcine lung (1, 8, 11, 32, 42, 43, 133). As far as the nasal cavity is concerned, no indication of the participation of *M. flocculare* in atrophic rhinitis or other lesions has ever been observed.

Histological changes, however, can be found in experimentally infected SPF and gnotobiotic pigs (42, 43). These consist of lympho-histiocytic cell proliferations in the lamina propria and damage to the covering epithelial layer in the nasal cavity and also in the lung.

Diagnosis of agent

Isolation of *M. flocculare* by culture may be tedious, but the procedure has no actual problems, provided that the recipes given here are followed exactly (44, 46, 50). However, *M. flocculare* will be outgrown by both *M. hyopneumoniae* and *M. hyorhinis*, if present. *M. hyorhinis* can be suppressed by the combined use of 0.3-0.4 mg/ml of cycloserine and 5% specific rabbit antiserum, the latter preferably raised against three different strains and pooled. *M. hyopneumoniae* may be suppressed by using 2% specific rabbit antiserum (C.H. Armstrong, personal communication). For isolation from the nasal cavity, further use of bacteria-inhibiting antibiotics, such as vancomycin and nalidixid acid, may be necessary. Storage of the samples at −25°C for two to four months, for repetition of the isolation trial, is also beneficial. Isolates may be identified by the DGI test on solid medium after three to four passages in broth. Alternatively, immunofluorescent staining of the organisms can be performed, as described by Armstrong and Friis (6). Direct visualisation of *M. flocculare* in tissue should be possible by immunohistochemical procedures. Using immunofluorescence (8, 47), reasonable staining was achieved for some lungs, but in many cases Armstrong and Friis failed to obtain distinct staining, despite the presence of very high numbers of culturable organisms.

Identification of *M. flocculare* in liquid media, employing chromosomal and ribosomal genomic analyses, has been performed mainly by focusing upon the separation of this mycoplasma from the closely related *M. hyopneumoniae*. Distinct differences between the two species have been noted from chromosomal restriction endonuclease analysis and hybridisation (21, 36, 129), whereas comparisons of the 16S/23S ribosomal ribonucleic acid (rRNA) operons have revealed a striking homology (131), thus confirming their close phylogenetic relationship. Furthermore, direct demonstration of *M. flocculare* has been achieved by PCR, even from lung tissue, by Stemke (128).

Serodiagnosis

Attempts to introduce serological procedures to demonstrate antibodies against *M. flocculare*, and also against *M. hyopneumoniae*, have been hampered by a very close antigenic relationship between the two species, as demonstrated by gel
electrophoresis, immuno-electrophoresis, immunoblotting, etc. (15, 35, 49, 111). The detection of antibodies against *M. flocculare* in swine serum samples has been conducted using various modifications of traditional ELISA systems (1, 7, 11, 35, 132).

It has been possible to detect antibodies in experimentally infected animals, but seroconversion occurred as late as some weeks after inoculation and reached only low titres. However, the antibodies seemed to persist for several months and the very prominent cross-reactions noted in antigenic comparisons appear to be of minor, if any, importance for serodiagnostic work.

**Preventing the infection**

The non-pathogenic status generally agreed upon for *M. flocculare* makes considerations about therapeutic treatment and preventive measures seem superfluous. However, for experimental studies of other infections, perhaps principally in regard to histopathologic lesions, *M. flocculare*-free animals might be desirable, and should be obtained by hysterectomy or by very early weaning. In the case of antibiotic treatment, the same compounds which are effective against *M. hyopneumoniae* appear to be useful (137).

**MYCOPLASMA HYOPHARYNGIS**

*M. hyopharyngis* is an arginine-metabolising porcine mycoplasma, which was first described by Erickson et al. (30) in the USA. Several strains from two herds were isolated from nasal and pharyngeal swab samples. DGI and immunofluorescent tests showed these samples to form a homologous group which differed from other mycoplasmas. Later, Bradbury et al. (17) isolated several strains from the arthritic joints of pigs in the UK, showing them to belong to this species. Several strains were also isolated in a herd in Denmark (N.F. Friis et al., unpublished findings) from pharyngeal scrapings of sty-mates. These strains were similarly identified.

At present, little is known about this species; neither its distribution and frequency, nor its pathogenic capabilities. Apparently, requirements for culture are somewhat similar to those of *M. hyosynoviae*. Nevertheless the propagation of *M. hyopharyngis*, especially in broth passages, appears difficult.

**CULTIVATION OF PORCINE MYCOPLASMAS**

A medium for *M. hyopneumoniae*, *M. flocculare* and *M. hyorhinis*, essentially as described by Friis et al. (44, 46, 50)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1,400 ml</td>
</tr>
<tr>
<td>Hanks' balanced salt solution</td>
<td>2,500 ml</td>
</tr>
<tr>
<td>Bacto brain heart infusion</td>
<td>15 g</td>
</tr>
<tr>
<td>Bacto pleuropneumonia-like organisms (PPLO)</td>
<td></td>
</tr>
<tr>
<td>broth without crystal violet</td>
<td>16 g</td>
</tr>
</tbody>
</table>
Dispense among 7 one-litre infusion bottles and autoclave at 120°C for one min, or at 110°C for 20 min. Pool again.

Add the following:
- Yeast extract 180 ml
- Bacitracin 800 mg
- Meticillin 800 mg
- Phenol red solution 10 ml

Dispense in volumes of 320 ml as basal broth and store at -25°C (will keep for at least one year).

The final medium is prepared from 320 ml basal broth by the addition of 40 ml horse serum and 40 ml swine serum (20% total serum). For primary isolation, 25% serum is beneficial for *M. hyopneumoniae* and *M. flocculare*, and 15% for *M. hyorhinis*, with the pH adjusted to 7.3. For improved antibacterial effects, add 0.15 mg/ml of cycloserine and, if necessary, a further 0.2 mg/ml of vancomycin and 0.005 mg/ml of nalidixic acid.

Selective suppression of *M. hyorhinis*, which is in danger of overgrowth, may be obtained with up to 0.3-0.4 mg/ml of cycloserine, together with rabbit anti-*M. hyorhinis* serum to 5%.

**Medium for *M. hyosynoviae* and *M. hyopharyngis*, a modified Hayflick’s recipe**

- Water 3,500 ml
- Bacto PPLO broth without crystal violet 75 g

Dispense among 6 one-litre infusion bottles and autoclave at 120°C for one min or at 110°C for 20 min. Pool again and add:
- Yeast extract 180 ml
- Phenol red solution 10 ml
- Ampicillin 1.0 g

Dispense in volumes of 340 ml as basal broth and store at -25°C (will keep for at least one year).

The final medium is prepared from 340 ml basal broth by the addition of:
- Horse serum 30 ml
- Swine serum 30 ml
- Arginine/mucin solution 2.5 ml

Adjust pH to 7.0. For improved antibacterial effects add 0.15 mg/ml of cycloserine.

Selective suppression of *M. hyorhinis* and bacteria may be achieved as follows. For tissue-containing cultivations, take 400 ml of the final medium and add cycloserine to 0.4 mg/ml (= 160 mg) and 2.0 ml of Tween 20 stock solution. For swab samples, take 400 ml of the final medium and add cycloserine only to 0.3 mg/ml (= 120 mg) and 1.8 ml of Tween 20 stock solution.

**Solid medium**

Hanks’ balanced salt solution with 23 ml of diethylaminoethyl (DEAE)-Dextran, plus the addition of 1.8 g agar (Oxoid L28 purified agar).
Autoclave at 120°C for one min, cool to 100°C and mix with 200 ml of medium preheated to 56°C.

**Transport medium**

Basal broth
  (the Hayflick’s type containing ampicillin) 340 ml
Horse serum 60 ml
Bacitracin 80 mg
Cycloserine 80 mg
Vancomycin 40 mg

Adjust pH to 7.1-7.2. Dispense as 1.8 ml in tubes; may be kept for 2 years at –25°C.

**Yeast extract**

Yeast extract may be prepared from two different recipes, either from commercial dry yeast or from fresh baker’s yeast. Either of these two extracts may be used.

Use 100 g dry yeast to 750 ml water. Heat to 37°C and keep for 20 min, then heat to 90°C and keep for 5 min; cooling and centrifuging at about 3,000 × g for 15 min. Dispense in 180 ml volumes and heat to 115°C for one min. Store at –25°C for up to one year. Note: YSC-2 dry yeast must be kept at a temperature of no more than +4°C, and for no more than 2 years from the factory date.

Fresh baker’s yeast: add 250 g yeast to 500 ml water, which has been preheated to 40°C. Mix well and add another 500 ml water. Adjust pH to 4.6 and heat to 85°C for 5 to 10 min, cooling and centrifuging at about 3,000 × g for 15 min, or pass through a filter paper. Adjust pH to 7.2 and conduct sterile filtration through 0.45 µm membrane. Dispense in 180 ml volumes and store at –25°C for up to one year.

**Hanks’ balanced salt solution**

*Stock A*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>80.0 g</td>
</tr>
<tr>
<td>KCl</td>
<td>4.0 g</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1.0 g</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>1.0 g</td>
</tr>
</tbody>
</table>

Dissolve in 400 ml water, add 1.4 g of CaCl₂ (anhydrous) and water up to 500 ml.

*Stock B*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂HPO₄·12H₂O</td>
<td>1.5 g</td>
</tr>
</tbody>
</table>

Dissolve in 400 ml water, then add more water.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0.6 g</td>
</tr>
</tbody>
</table>

Fill up with water to 500 ml.

Both stock solutions are heated to 90°C and kept for 5-10 min.

For use: 25 ml stock A, 400 ml of water, 25 ml stock B, add more water to 500 ml.
Hanks’ balanced salt solution with DEAE Dextran (0.1%)

Mix 180 ml water, 10 ml stock A, 10 ml stock B and 200 mg DEAE-Dextran. Autoclave. The final concentration in solid medium is 0.01%.

Phenol red solution (0.6%)

Phenol red solution (2.5 g) is added to 75 ml of 0.1 M NaOH. Dissolve and fill with water to 400 ml, leaving at +4°C until the next day. Filter, adjust pH to 7.0, autoclave and keep at +4°C.

Arginine/mucin solution

| Water | 100 ml |
| L-arginine | 8.0 g |
| Mucin (bacteriological) | 0.8 g |

Autoclave and store at −25°C in 2.5 ml volumes. The final concentration in the medium is 0.05% and 0.005%.

Tween 20 stock solution (20.6%)

10.3 ml Tween 20 (at 37°C) is added to water and filled to 50 ml (aseptic). Store at −25°C in volumes of 2.0 ml and 1.8 ml for no more than six months. The final concentration in the medium is 0.1% and 0.085%.

Animal serum

Horse and swine serum is collected aseptically or filter (0.2 µm) sterilised. Dispense in volumes of 200 ml in bottles with cotton stoppers and leave at +4°C for 2 months (for horse serum) and one month (for swine serum). Examine for sterility. Store for up to one year at −25°C in closed infusion bottles. Use preferably SPF swine serum.

Water

The degree of refinement should be as follows: reagent grade, distilled, demineralised, permiate. These are all equally good.

Autoclaving procedures

Heat to 120°C for one min or to 110°C for 20 min. In the case of yeast extract, use 115°C for one min.

Never heat to 120°C for 20 min.

Storage in frost

It is recommended to store items at −25°C and not at −18°C.

Tubes for standard culture

Disposable 10 ml glass tubes washed in 1% HCl and sterilised are very suitable. These should be closed with a soft rubber stopper.

Alternative swine infusion medium

| Chopped swine meat and heart | 250 g |
| Water | 2,000 ml |
Mix well and heat to 90°C for 20 min, centrifuging at about 3,000 × g, or pass through a filter. Add 20 g Bacto peptone and 10 g NaCl. An amount of 100 ml Hanks’ balanced salt solution is added per 400 ml infusion. Autoclave. The final medium is prepared by adding yeast extract, antibiotics, phenol red and arginine/mucin. Swine serum is added to 6.0% to 10% for M. hyosynoviae, to 10% to 15% for M. hyorhinis and to 20% for M. hyopneumoniae and M. flocculare. Adjust pH as required.

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MYCOPLASMOSES PORCINES. – M. Kobisch et N.F. Friis.

Résumé : Mycoplasma hyopneumoniae est l’agent étiologique primaire de la pneumonie enzootique du porc. Les lésions pulmonaires, généralement observées chez le porcelet, se caractérisent par une hyperplasie des cellules épithéliales et une accumulation de cellules mononucléées au niveau péribronchillaire et pérvaseulaire. L’infection à M. hyopneumoniae est suivie de réactions immunitaires qui confèrent à l’animal une résistance lors d’un nouveau contact avec l’agent infectieux. Le diagnostic individuel de laboratoire s’effectue par immunofluorescence et par un test sérologique, à l’échelle du troupeau. Le traitement antibiotique est utile mais on a pu noter le développement d’une antibiorésistance. La vaccination semble une méthode efficace de lutte contre la maladie.

M. hyorhinis, généralement transmis des truies aux porcelets, par le biais des sécrétions nasales, est présent chez un grand nombre de porcs sains. Certaines souches peuvent provoquer une polysérite sérofibrineuse ou fibrinopurulente et des arthrites. On isole M. hyorhinis de lésions en phase aiguë et subaiguë ; les anticorps sériques sont détectables.

M. hyosynoviae a une affinité particulière pour les articulations et peut provoquer des arthrites aux conséquences économiques graves. Ce mycoplasme est généralement localisé dans les amygdales. La transmission se fait de la truie au porcelet, après quatre à six semaines. Les signes cliniques apparaissent chez les animaux pesant de 30-40 kg à 100 kg ; les lésions observées sont des bursites et des arthrites. On signale la présence d’un liquide visqueux non suppuratif, sérofibrineuse/sérohémorragique. Dans les cas chroniques, la membrane synoviale est atteinte. M. hyosynoviae est isolé des articulations et de la muqueuse pharyngée ; il peut induire l’apparition d’anticorps dans le sang et dans le liquide synovial. Certains facteurs prédisposants jouent un rôle important.

M. flocculare est largement répandu chez les porcins, se localisant dans les poumons normaux et pneumoniques et dans les cavités nasales, mais aucun pouvoir pathogène n’a été décrit. Ce mycoplasme suscite un grand intérêt en raison de sa forte similitude avec M. hyopneumoniae.


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Resumen: Mycoplasma hyopneumoniae es el principal agente causante de neumonía enzoótica en los cerdos. Las lesiones pulmonares, que se observan por lo general en cerdos jóvenes, están caracterizadas por una hiperplasia de las células epiteliales y un aumento de la acumulación perivascular y peribronquial de las células mononucleares. Tras una infección por M. hyopneumoniae se observa la presencia de reacciones inmunes y la inducción de resistencia en los cerdos. El diagnóstico de laboratorio suele realizarse mediante pruebas de inmunofluorescencia y ensayos inmunoenzimáticos. Los antibióticos resultan útiles en el tratamiento de la infección, aunque se han descrito casos de resistencia. La vacunación, por otra parte, parece un método efectivo para controlar la enfermedad.

M. hyorhinis, por lo común transmitido a los lechones a través de las secreciones nasales de la madre, está presente en un elevado porcentaje de cerdos sanos. Algunas cepas pueden sin embargo inducir el paso de artritis y poliserositis serofibrinosa o fibrinopurulenta. M. hyorhinis puede ser aislado a partir de las lesiones que se producen en las fases aguda y subaguda de la enfermedad. Es posible también detectar la presencia de anticuerpos en el suero.

M. hyosynoviae posee una especial afinidad por el tejido articular, por lo que puede causar artritis y dar lugar con ello a pérdidas económicas. Este micoplasma suele localizarse en las amígdalas. La infección de los lechones por vía materna se produce tras cuatro a seis semanas de vida. La enfermedad, que afecta las bursas sinoviales y articulaciones, se manifiesta en animales que pesan de 30-40 kg a 100 kg. Se ha descrito la secreción de un líquido viscoso no supurativo de naturaleza serofibrinosa o serosanguínea. En los casos crónicos, la membrana sinovial se ve también afectada. M. hyosynoviae puede ser aislado en las muestras de tejido articular y faríngeo, y es capaz de inducir la proliferación de anticuerpos en sangre y líquido articular. Por otra parte, ciertos factores de predisposición desempeñan también un importante papel.

M. flocculare existe de forma abundante tanto en pulmones normales y neumónicos como en las cavidades nasales de los cerdos, aunque no se ha observado ningún tipo de poder patógeno. El gran interés de este micoplasma radica en su notable semejanza con M. hyopneumoniae.


REFERENCES


