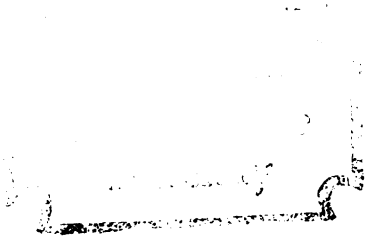

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INDIRECT IMMUNOFLUORESCENT EXAMINATION OF FROZEN LUNG SECTIONS FOR DIAGNOSIS OF MYCOPLASMAL PNEUMONIA IN SWINE WITH MACROSCOPIC AND MICROSCOPIC LESIONS

presented by

HECTOR O. CHACON

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INDIRECT IMMUNOFLUORESCENT EXAMINATION OF FROZEN LUNG SECTIONS FOR DIAGNOSIS OF MYCOPLASMAL PNEUMONIA IN SWINE WITH MACROSCOPIC AND MICROSCOPIC LESIONS

By

Hector O. Chacon

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ABSTRACT

INDIRECT IMMUNOFLUORESCENT EXAMINATION OF FROZEN LUNG SECTIONS FOR DIAGNOSIS OF MYCOPLASMAL PNEUMONIA IN SWINE WITH MACROSCOPIC AND MICROSCOPIC LESIONS

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Thirty pig lungs, selected for presence of macroscopic and/or microscopic lesions suggestive of mycoplasmal pneumonia in swine, were studied. Twenty had macroscopic lesions and all 30 had microscopic lesions. Frozen lung sections, cut to 6 μ thickness, were positive on indirect fluorescent antibody test (IFA) for *Mycoplasma hyopneumoniae* in 26 of the 30 pig lungs.

No bacterial culture or any other laboratory tests were done with the 4 pig lungs IFA negative, and the etiology of the lesions of these was not determined. The specificity of the IFA test for detecting *M. hyopneumoniae* in lung tissue of swine has been established by previous studies, but it is recommended that further studies be done to ascertain the degree of sensitivity of the test.

The results of this study and those of other investigators reviewed in this thesis support the use of the IFA test as a relatively simple, rapid and effective method for detecting *M. hyopneumoniae* in pig lung tissue and as an alternative to culturing the organism for etiologic determination of mycoplasmal pneumonia in swine, suggested on the basis of macroscopic and/or microscopic lesions observed.

Dedicated with love to my wife, Nancy, for her

constant support and understanding

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Rodney Flint Taylor, my major professor. I also wish to thank Drs. Allan L. Trapp, John P. Newman, George A. Padgett, and David J. Ellis for serving on my committee.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Diseases are limiting factors for pig production. Infectious, nutritional, toxic, genetic, and environmental factors play a role in the diseases of pigs. As for the infectious diseases, the etiological agents are bacterial, viral, parasitic, fungal, and mycoplasmal. Such diseases have been studied with microbiologic, clinicopathologic, and epidemiologic methods for purposes of treatment, control, and/or eradication. Swine mycoplasmosis exhibits a varied clinicopathologic picture as well as distinctive epidemiologic behavior. Swine mycoplasmal pneumonia is caused by *Mycoplasma hyopneumoniae*. It may act with one or multiple secondary disease-producing agents, and it affects swine regardless of breed, sex, and age. It is a chronic disease with high morbidity and high mortality in complicated forms. Swine mycoplasmosis is important economically because affected animals usually grow more slowly.

In mycoplasmal pneumonia in swine, there has been an accumulation of knowledge on the pathologic aspects of the disease in the last 50 years. In the last 15 years, there has been etiologic definition. Diagnosis of mycoplasmal pneumonia in swine still remains a complex matter because of "common clinico-pathological features" shared with other swine diseases.

Relatively recently, serologic techniques, epidemiologic observations, and pathologic studies are helping in the diagnosis of swine

pneumonia caused by *M. hyopneumoniae*. The fluorescent antibody staining technique has proven to be a useful supportive diagnostic procedure.

On the basis of the aforementioned concepts, this study was conducted to attempt to demonstrate the effectiveness of the indirect fluorescent antibody test as a supportive diagnostic procedure of mycoplasmal pneumonia of swine, given macroscopic and/or microscopic lesions suggestive of the disease.

LITERATURE REVIEW

History

Name of the Disease

A chronic respiratory disease of swine was reported by Kobe (1933, 1934) in Germany, which was known as "Ferkelgrippe." In Northern Ireland, Lamont (1938) reported a disease in swine with similar clinicopathologic characteristics to those described by Kobe, and he named it pig influenza. Several different names were given over the years by several investigators in different countries to a disease entity having clinicopathologic characteristics of the diseases described by Kobe (1933, 1934) and Lamont (1938). Among these names were: 1) infectious pneumonia of pigs (Pullar, 1948; Gulrajani and Beveridge, 1952), 2) swine enzootic pneumonia (Wesslen and Lannek, 1954; Lannek and Wesslen, 1957); 3) virus pneumonia of pigs (Betts, 1952; Beveridge, 1953; Betts and Beveridge, 1952). Despite the different names given to this "respiratory disease in swine", there was overall agreement on the descriptions of the disease (Switzer and Ross, 1975). In 1952, a possible relationship among the above-mentioned diseases was considered in a review written by Lamont (1952).

Etiologic Identification

In their studies, Kobe (1933) and Lamont (1938) reproduced the disease experimentally by inoculating healthy pigs intranasally with

pneumonic lung suspensions, and they each concluded that a filterable virus was the primary etiologic agent, with the participation of *Haemophilus influenza suis* as a secondary invader (Kobe, 1933, 1934; Lamont, 1938). The latter organism was previously identified by Lewis and Shope in their studies of swine influenza and who gave it its name (Lewis and Shope, 1931).

A clinicopathologic entity was produced by intranasal inoculations of *H. influenza suis* plus one or more other pathogenic microorganisms considered as secondary invaders, such as *Bacillus pyocyaneus* (synonym for *Pseudomonas aeruginosa*). *Bacillus pyocyaneus* and *Salmonella suis* infections also showed clinical similarities with pig influenza (Lamont, 1938). From work done by Betts (1952), Betts and Beveridge (1952), and Beveridge (1953), it was presumed that the etiologic agent of this chronic pig pneumonia was a filterable virus. The presumption that a filterable virus, distinct from swine influenza virus, was the etiologic agent of an "infectious pneumonia of pigs" was also held by Pullar (1948). From their work on experimental transmission of the disease, Gulrajani and Beveridge concluded that "infectious pneumonia of pigs" was caused by a viral agent (Gulrajani and Beveridge, 1951). The assumption that the causative agent of "chronic pneumonia of swine" was a viral agent was reinforced by the fact that pneumonic lung suspensions, passed through bacteria-retaining filters, proved infectious to inoculated healthy swine, and this view persisted until 1957, when Betts and Campbell (1956) demonstrated that enzootic pneumonia of swine could be prevented with administration of tetracyclines. The possibility that the etiologic agent of pig enzootic pneumonia could be a pleuropneumonia-like organism (PPLO) was expressed by Wesslen and Lannek (1954), and pleuropneumonia-like organisms associated with pneumonia in swine were

also reported by Carter and Schroder (1955). Using pneumonic lung suspensions, a cytopathic effect was observed in "pig lung monolayer cell culture" and pleomorphic organisms were seen by light microscope (Goodwin and Whittlestone, 1963). These reported pleomorphic organisms were propagated in pig lung cell culture, which cells had been killed by boiling (Goodwin and Whittlestone, 1964). Pneumonia in swine, produced by the inoculation of *Mycoplasma* cultured in lifeless media, was reported by Goodwin and Whittlestone (1965), and the name *Mycoplasma suipneumoniae* was proposed for the pathogenic microorganism. In the U.S., Mare and Switzer (1965, 1966a,b) grew and propagated the causative agent of a chronic pneumonia in pigs, using artificial media, and then characterized it as a *Mycoplasma* and named it *Mycoplasma hyopneumoniae* (Mare and Switzer, 1965). Results like those reported by Goodwin and Whittlestone (1965) in England and Mare and Switzer (1965) in the U.S. were also reported by L'Ecuyer (1969) in Canada. The results of the work done by Goodwin and Whittlestone in pigs were reported shortly after those of Mare and Switzer. In 1967, *M. hyopneumoniae* and *M. suipneumoniae* organisms were compared by Goodwin et al. (Goodwin, Pomeroy and Whittlestone, 1967), and they concluded that both were the same agent. Since the designation of *M. hyopneumoniae* had chronological priority over *M. suipneumoniae*, the former name has been used to designate the causal agent of a chronic pneumonia in swine. Over the years, *M. hyopneumoniae* isolations in cases of chronic pneumonia in swine have been reported in other countries: Denmark (Friis, 1971, 1975; Meyling, 1971) and Australia (Furlong et al., 1975; Etheridge et al., 1979). Reports of *M. hyopneumoniae* isolations continue to fill the literature. In this thesis chronic pneumonia of swine caused by *M. hyopneumoniae* will be referred to as mycoplasmal pneumonia of swine.

The Organism

Nomenclature

Mycoplasma sp. are placed in one order, Mycoplasmatales, one family, Mycoplasmataceae, and one genus, *Mycoplasma*, all in the class Mollicutes (Buxton and Fraser, 1971). These pleomorphic organisms are distinguished as species on the basis of serologic reactions, and about 25 to 30 different *Mycoplasma* sp. have been recognized.

Growth Requirements

The *Mycoplasma* sp. are fastidious organisms. For growth, complex media containing meat infusion broth, Bacto-brain heart infusion, Bacto-PPLO without crystal violet, peptones, sodium chloride, yeast extract, some other chemicals, and porcine and/or horse serum are needed. The optimum temperature for growth is 37 C.

Isolation

Isolation from lung in solid media. In experimentally infected pigs, direct isolation of *M. hyopneumoniae* colonies from bacteriologic lung culture on solid media has been positive in a few cases, but for practical purposes of routine diagnosis this technique is not considered satisfactory (Whittlestone, 1973).

Isolation in tissue culture. Isolates of pleomorphic organisms were first obtained by tissue culture methods. A cytopathic effect has been observed in both pig lung monolayer cell culture and pig testicle cell culture (Wesslen and Lannek, 1954; Goodwin and Whittlestone, 1963).

Isolation in liquid media. The first reported lifeless media to be used for bacteriologic culture of *M. hyopneumoniae* were: 1) a tissue

culture feeding fluid and 2) broth with dead pig lung cell culture (Goodwin and Whittlestone, 1965; Mare and Switzer, 1965). Following the successful results of the isolation attempts above reported, other media have been developed for bacteriologic culture of *M. hyopneumoniae* or other *Mycoplasma* sp. of porcine origin, and details and methods for preparation of these media have been described elsewhere (Switzer, 1972; Friis, 1971, 1975; Goodwin, 1976).

Identification

The *Mycoplasma* sp. organisms are isolated in broth media. This isolate is then grown in colony form on a filter membrane and can then be identified by means of an indirect immunofluorescent test. The isolation and identification process requires about 2 weeks (Armstrong, 1980). Other means for identification of isolates are metabolism inhibition and growth inhibition (Whittlestone, 1973).

Morphologic Features

Usually, *M. hyopneumoniae* colonies may be detected when subcultured on solid media after 3 days of incubation, having a diameter of 20 to 100 μm and reaching 0.5 mm in diameter after 7 to 10 days of incubation. The colonies commonly are convex with a granular surface and with no observable central portion burrowing into the media (Whittlestone, 1973). The morphological appearance varies from a) coccoid to b) ring-like triangles to c) rod-like or irregular shape (Whittlestone, 1973). The variety of morphologic structures which have been observed and described for *M. hyopneumoniae* seem also to be influenced by the method of growth and preparation used, as below.

Lung tissue. The organism manifests itself morphologically as rings, cocci, triangles, and other less common forms. The observed size is about 0.5 μm in diameter for ring-like organisms, about 0.2 to 0.5 μm in diameter for coccoid-like organisms, and about 1.0 μm in diameter for triangular organisms. The cilial region of the bronchial epithelial cells, as well as between the cilia, were the sites where the organism was found most easily (Whittlestone, 1973).

Broth culture. Circular colonies, mainly coccoid-like, reaching an average diameter of 10 to 25 μm, have been seen growing over the surface of glass coverslips, according to Goodwin and Whittlestone (1966, cited by Whittlestone, 1973).

Tissue culture. Coccoid- and ring-like forms were usually seen in tissue culture, frequently in large groups and at times extracellularly located (Goodwin and Whittlestone, 1963).

Solid media. On agar medium, many species of *Mycoplasma* have a morphologic appearance which is referred to as "fried egg" colonies, but *M. hyopneumoniae* usually do not form "fried egg" colonies.

Structurally, *Mycoplasma* sp. lack a cell wall, as do the L-forms of bacteria. However, there are basic and substantial differences, such as: 1) L-form bacteria, as well as occurring naturally, can also be induced by laboratory methods; 2) most L-form bacteria revert to their parent bacteria; 3) L-form bacteria have the nutritional and biochemical properties of their progenitors. The pleomorphism observed in *Mycoplasma* sp. is due to a great extent to the shape imposed by the physical environment and facilitated by the absence of a cell wall (Madden et al., 1967).

Epidemiology

Survival of the Organism

In pneumonic lung tissue, *M. hyopneumoniae* remains infective at -30 C for up to 20 months (Whittlestone, 1958), at 4 C for 4 days, at 20 C for 1 day, and at 37 C for 4 hours (Whittlestone, 1958).

Host Range

Swine are the only animals known to be susceptible to *M. hyopneumoniae* infection. Experimental transmission attempts to laboratory animals such as ferrets and mice (Goodwin and Whittlestone, 1963, 1964) and mice (Wesslen and Lannek, 1954) have been unsuccessful.

Transmission of the Disease

The disease may be transmitted over short distances by aerosol from infected to susceptible swine; in the natural environment the organism appears to be short-lived (Switzer and Farrington, 1977). Pneumonia is also produced when cultures of *M. hyopneumoniae* are inoculated into healthy swine. Lung suspensions from these swine inoculated into healthy pigs also produce pneumonia (Pattison, 1956).

Contributory Factors

Consideration has been given to a multiple factor disease criterion, in which other factors in addition to the infective agent play an important role. Bacterial agents, parasites, dust, humidity, or irritant gases may be associated with mycoplasmal pneumonia in swine. These factors disrupt the lung's normal functioning, lowering the natural resistance to infection (Jericho, 1968), and contribute to the severity of the disease and characteristics of gross pathology (Schofield, 1956). *Mycoplasma hyopneumoniae* itself is known to be immunosuppressive (Adegboye, 1978).

Geographical Distribution

The disease is considered to be of worldwide distribution, based on descriptions of the clinical signs, macroscopic and histologic lesions, as well as isolation of the *M. hyopneumoniae* agent and epidemiologic observations of the disease.

Clinical Picture

The clinical course of mycoplasmal pneumonia of swine is chronic and relatively mild in uncomplicated cases. Swine grow slowly and cough with no increased body temperature and show dyspnea on rare occasions. Progressive involvement with respiratory signs can be recognized throughout all age groups in large infected herds which are intensely managed. Adult animals can be severely affected. Although there is a high morbidity rate, the case fatality rate is generally low (Switzer and Ross, 1975; Switzer and Farrington, 1977). Complicated forms of the disease are frequently observed if the swine are exposed to contributory pathogenic factors, and in such situations the mortality rate can be higher.

Pathology

Macroscopic Lesions

At necropsy of swine which died of mycoplasmal pneumonia, the most common macroscopic lesions found in the lungs are described as circumscribed, plum-colored, grayish, consolidated areas. Primarily affected are the anteroventral parts of the anterior lung lobes (the right cardiac, the right apical, the left cardiac, the left apical, and the intermediate lung lobes), all of which show about the same incidence of lesions with a symmetrical tendency. The resulting pneumonia is catarrhal in type and of lobular distribution. If a pneumonic lung is

incised, the cut surface appears smooth, homogeneous, and wet, and a clear or mucoid exudate can be expressed by gently squeezing the central bronchiole. The exudate can be yellowish or opaque, suggesting a secondary bacterial infection (Schofield, 1956; Switzer and Ross, 1975; Jericho, 1968). Irregularly distributed emphysematous lobules, atelectatic areas and grayish consolidation, necrosis and abscessation caused by secondary bacterial infection provide a colorful aspect to the macroscopic lesions.

Descriptions of macroscopic lung lesions similar to those mentioned above were made by Lamont (1938), who also mentioned enlarged pulmonary lymph nodes, fibrinous pleuritis, pericarditis, and epicarditis. This author affirmed that on the basis of macroscopic lung lesions, a diagnosis of swine influenza, as the disease was then known, could be made. Throughout the years, the lung lesions of both field and experimental cases have been observed and described by workers in different countries. These workers are basically in agreement in their descriptions of macroscopic appearance of the lesions as enlarged, edematous pulmonary lymph nodes, grayish consolidation, and pulmonary edema (Kobe, 1934; Pullar, 1948; Pattison, 1956; Schofield, 1956; Switzer and Ross, 1975), with complete healing if recovery occurred.

In experimental cases of mycoplasmal pneumonia of swine, appearance of macroscopic lung lesions postinoculation, as reported by Pattison (1956), has been as follows: at 11 days, "greyish-pink consolidated areas at the tip of the right and left apical lobes and over about one-third of the left cardiac lobe"; at 18 days, progressive consolidation; at 33 days, "consolidation of the entire right apical and left cardiac lobes and patchy consolidation on the anterior border of the diaphragmatic lobe."

From reports by Whittlestone (1973), macroscopic lung lesions could sometimes be detected at 7 to 10 days postinoculation and practically all infected pigs by 6 weeks postinoculation had moderately extensive pneumonic gross lesions.

Macroscopic lung lesions were observed by L'Ecuyer and Boulanger (1970) in only 2 of 10 pigs killed between 7 and 21 days postinoculation, and those 2 were at 12 and 20 days postinoculation, respectively. Of the 12 remaining pigs, killed between the 25th and 49th postinoculation days, all except 1 showed from slight to extensive gross lesions.

Other workers (Livingston et al., 1972) have reported that gross lesions became evident at the 14th day postinoculation and developed maximum severity between the 21st and 28th days postinoculation and then regressed.

Macroscopic lung lesions were observed (Etheridge and Lloyd, 1980) in experimentally infected pigs 4 to 6 weeks postinoculation, and the same authors observed macroscopic lung lesions at 4 to 10 weeks post-exposure in pigs infected by contact with experimentally infected pigs.

In uncomplicated field cases, as well as in experimental cases of the disease, the macroscopic lung lesions which appear similar persist for long periods of time. Market weight swine are those which show the higher incidence, according to reports in the literature (Goodwin and Whittlestone, 1960; Schofield, 1956; Switzer and Ross, 1975; Switzer and Farrington, 1977). The incidence of chronic lesions observed is less in slaughter sows than in butcher-weight swine, which has been interpreted as an age-clearing condition (Switzer and Ross, 1975). Macroscopic lung lesions similar to those described for mycoplasmal pneumonia in swine can also be observed in other respiratory diseases

(Pattison, 1956; Whittlestone, 1957; Goodwin and Whittlestone, 1960; Switzer and Ross, 1975).

Histopathology

In natural cases of the disease, histopathologic descriptions have been made as follows (Pattison, 1956): slight edema and cellularity increase in the interalveolar spaces, alveolar lumens filled with a few large mononuclear cells and with increased edema and cellularity, and extensive lymphoid hyperplasia, mainly of peribronchiolar and perivascular distribution. The dense accumulations of mononuclear inflammatory cells obliterate the alveolar tissue. Substantial amounts of cellular debris fill the bronchi and bronchiolar lumens. Hyperplastic bronchiolar epithelium, constricted lumens of bronchi, and bronchiolitis in some cases were seen. Lymphoid hyperplasia as well as collapse of lobular tissue were also observed. The above histologic changes described were those corresponding to consolidated lung tissue (Pattison, 1956). Plasma cells, a few histiocytes, and neutrophils have also been observed in the alveolar lumens (Schofield, 1956). Peribronchiolar and perivascular accumulations of inflammatory mononuclear cells, as described above for the natural cases of the disease, can also be caused by other pathogenic agents, such as bacteria, parasites, virus, dust, humidity, or any particulate matter (Jericho, 1968, 1977; Switzer and Ross, 1975; Whittlestone, 1973).

In field cases of the disease, histologic lung lesions which have been observed include slight peribronchiolar accumulations of lymphocytes as well as interalveolar tissue infiltrated by macrophages throughout the lung lobes. These have been regarded as nonspecific histologic lesions and frequently have been reproduced by experimental inoculations

of fluids (Pattison, 1956; Jericho, 1968; Whittlestone, 1973; Switzer and Ross, 1975) and are also induced by dust, irritant gases, or any other particulate foreign matter (Jericho, 1968; Switzer and Ross, 1975).

In pigs experimentally infected with *M. hyopneumoniae* organisms, the early histologic lung lesions observed have been as follows: 1) At 7 days postinoculation, there was peribronchiolar and perivascular lymphocytic accumulation and increased cellularity in the interalveolar spaces. Neutrophils were rare and there was a very slight bronchiolar exudate (Pattison, 1956). 2) At 11 days postinoculation, there was collapse of lobules and increased accumulation of lymphocytes. The bronchiolar epithelium had a hyperplastic appearance. There were neutrophils, small round cells, and exudate in some bronchioles (Pattison, 1956). 3) At 33 days postinoculation there were extensive lymphoid hyperplasia, accumulation of mononuclear cells in the alveolar walls, and a few large mononuclear cells with slight edema in the alveolar spaces. Progressive lymphoid hyperplasia related to bronchi and bronchioles led to obliteration of alveolar tissue and dense accumulations of lymphocytes in the peribronchial and peribronchiolar spaces. The lumens of the bronchioles often appeared very constricted. It was also seen that lymphoid hyperplasia could occur in the absence of alveolar involvement. The bronchiolar epithelium had a hyperplastic appearance (Pattison, 1956). 4) From 17 to 24 days postinoculation, progressive lymphoreticular hyperplasia, perivascular accumulations of mononuclear inflammatory cells, and development of alveolar cell pneumonia occurred (Whittlestone, 1973). 5) From 69 to 269 days postinoculation, the bronchiolar tree appeared surrounded by lymphoreticular nodes and the ciliate bronchiolar epithelium was regenerated (Whittlestone, 1973).

As for the presence of *M. hyopneumoniae* organisms associated with the surface of the bronchiolar epithelium, it has been reported in large numbers in the period from 7 to 42 days postinoculation (Livingston, Stair, Underdahl, and Mebus, 1972; Whittlestone, 1973; Kobisch, Tillon, and Vannier, 1978), and low numbers have been detected up to 262 days (37 weeks) postinoculation in experimentally infected swine (Whittlestone, 1973). As for the lung pathologic examination, it has been observed that in field conditions many pigs do not develop macroscopic lesions but will show microscopic lesions, and swine with macroscopic and histologic lesions have been observed coming from areas which are considered "swine lung normal areas" (Switzer and Ross, 1975).

Serologic Procedures

As an aid for the diagnosis of mycoplasmal pneumonia in swine, serologic procedures which have been used include the following.

Complement Fixation Test (CFT)

In pigs experimentally infected by inoculation of *M. hyopneumoniae*, serum antibodies have been detected by the CFT at 3 to 6 weeks postexposure (Switzer and Ross, 1975; Slavik and Switzer, 1979; Etheridge, 1980), and such antibodies persisted for up to 15 weeks postexposure (Slavik and Switzer, 1979) and also for up to 35 weeks postexposure, as reported by Etheridge (1980).

In swine infected by contact, CFT serum antibodies have been detected at 8 to 15 weeks postexposure, while Etheridge (1980) had detected CFT serum antibodies at 4 to 40 days postcontact, and such antibodies persisted for up to 35 weeks postcontact.

In field conditions, swine infected with a virulent strain of *M. hyopneumoniae* exhibited clinical signs of pneumonia and showed positive

titers to the CFT, while in separate observations pigs infected with a less virulent strain of *M. hyopneumoniae* usually were CFT negative. In both instances, the swine were 8 to 10 weeks of age. It has also been observed that swine infected with mild strains of *M. hyopneumoniae* usually became serologically positive by about 16 weeks of age (Switzer and Farrington, 1977).

In normal conventionally infected herds, a peak incidence of seropositive animals (60 to 65%) in the 6 month to 1 year age range has been reported, and as the breeding stock gets older, the incidence of seropositive animals declines to an expected 20 to 25% in animals over 30 months of age. But seronegative animals may become seropositive during periods of stress (Switzer and Ross, 1975; Switzer and Farrington, 1979). The presence of CFT serum antibodies has been correlated with observations of gross and microscopic lesions. The CFT titers are still present when the gross lesions have disappeared. When the CFT titer converts to negative, microscopic lesions can still be detected (Switzer and Farrington, 1977; McKean, Andrews, and Farrington, 1979).

Latex Agglutination Tube Test

With this test, in experimentally infected swine (by inoculation with *M. hyopneumoniae*), serum antibodies have been detected as early as 2 to 3 weeks postinoculation and for up to 48 weeks postinoculation. The antibody titers persist even after the microscopic lung lesions disappear (Slavik and Switzer, 1979; Switzer and Farrington, 1977). In pigs which have been infected by contact, positive titers to the test are detected at 4 to 12 weeks postcontact.

Indirect (Passive) Hemagglutination (IHA)

With this test, in experimentally infected swine, high titers of serum antibodies have been detected 5 to 7 weeks postinoculation and through a period corresponding to the healing of the macroscopic lung lesions (about 85 days postinoculation) (Kobisch, Tillon, and Vannier, 1978).

Fluorescent Staining Antibody

This technique has been reported as a rapid and reliable method, which has been applied to smears of broth culture, to infected pig testicle cell culture, and to frozen sections of consolidated swine lungs. In the latter case, the pig lungs tested were from animals that died from experimentally produced mycoplasmal pneumonia or were field cases of the disease. Fluorescence has also been demonstrated in mycoplasmas of porcine origin, grown in colony form on a filter membrane. The fluorescent antibody has proven to be species specific and has been suggested as an alternative to the bacteriologic culture for isolation and identification of porcine *Mycoplasma* sp., a difficult and time-consuming process (Armstrong, 1980; Kobisch, Tillon, and Vannier, 1978; L'Ecuyer and Boulanger, 1970; Meyling, 1971; Stone, 1976).

Direct FA. The direct fluorescent staining antibody method was used for identification of *M. hyopneumoniae* colonies, grown in lifeless media as well as sections of consolidated pig lungs, with results reported as satisfactory (L'Ecuyer and Boulanger, 1970; Meyling, 1971; Kobisch, Tillon, and Vannier, 1978). Porcine and/or rabbit globulins were used to perform the direct FA, and porcine globulins appeared more satisfactory to some workers (L'Ecuyer and Boulanger, 1970; Dobisch, Tillon, and

Vannier, 1978), while rabbit globulins appeared satisfactory to others (Meyling, 1970).

Indirect FA. *Mycoplasma* sp. of porcine origin grown in colony form on filter membrane has been identified by indirect fluorescent staining antibody method. This method has also been used to identify the mycoplasmal organisms in pig lung showing macroscopic and/or microscopic lesions. Rabbit globulins and goat-antirabbit globulins have been used and satisfactory results reported, which permits shortening the period of time for identification of porcine *Mycoplasma* sp. (Armstrong, 1980).

FA relationship with other conditions. In early stages of mycoplasmal pneumonia in swine, the results of the direct FA test have been negative. Convincing positive results were seen at the 25th day postinoculation and thereafter (L'Ecuyer and Boulanger, 1970). Reasons for this outcome could be due:

1) to the difficulty in obtaining representative sections of lung, when the areas of pulmonary consolidation are very limited,
2) to an insufficient amount of antigen being present to allow visualization, and
3) to washing out of the antigen from the epithelium of the air passages during the staining procedure.

(L'Ecuyer and Boulanger, 1970)

Other workers have reported positive results with the direct FA as early as 14 days postinoculation of experimentally infected pigs with mycoplasmal pneumonia (Livingston, Stair, Underdahl, and Mebus, 1972) and have added that *Mycoplasma* sp. organisms can be identified in thin sections of frozen lungs in both experimental and field cases which showed no clinical signs or macroscopic lung lesions (Kobisch, Tillon, and Vannier, 1978).

In pneumonic pig lungs, *Mycoplasma hyorrhinis* has been isolated while *M. hyopneumoniae* has been direct FA positive and culture negative. Such results have been interpreted as "the *Mycoplasma hyorrhinis* blocking and out-growing *Mycoplasma hyopneumoniae*" (Meyling, 1971).

In chronic cases of mycoplasmal pneumonia in swine, weak or negative direct FA results have been observed, and they have been attributed to the action of local antibody (Meyling, 1971; Kobisch, Tillon, and Vannier, 1978). As for the indirect FA, it correlates well with the findings of macroscopic and/or microscopic lung lesions (Armstrong, 1980).

In pneumonic swine lung, *M. hyopneumoniae* positive to FA staining, fluorescent organisms are clearly visible over the surface of the airways when observed with the fluorescent microscope.

Diagnosis

Nonspecific Diagnosis

Clinical signs and macroscopic lesions. More than one disease can produce similar clinical signs and macroscopic pathologic changes in pig lungs (Pattison, 1956; Whittlestone, 1957, 1973; Switzer and Ross, 1975). Similar macroscopic lung lesions produced by different diseases exhibit histopathologic differences (Pattison, 1956; Whittlestone, 1957, 1973; Goodwin and Whittlestone, 1960; Switzer and Ross, 1975; Jericho, 1968).

In field conditions a variety of bacteria such as *Pasteurella multocida, P. haemolytica, Bordetella bronchiseptica, Klebsiella pneumoniae,* and *Corynebacterium pyogenes* may be associated with mycoplasmal pneumonia in swine, contributing to the severity of the disease and type of gross pathology. Lungworms, swine influenza virus, and pseudorabies

can also complicate the disease picture, masking the primary changes (Whittlestone, 1957, 1973; Jericho, 1977; Switzer and Ross, 1975).

Specific Diagnosis

Respiratory disease in swine, suggestive of mycoplasmal pneumonia on histologic examination and consideration of clinicopathologic and epidemiologic data, could be confirmed to be mycoplasmal pneumonia only by recovery of the specific organism from the lung. Isolation and identification of the pathogenic agent of mycoplasmal pneumonia in swine is a time-consuming process, not suited for purposes of routine diagnosis.

Serologic procedures. The complement fixation test could be helpful if used as a predictor of macroscopic lung lesions, substituting or supplementing the slaughter inspection for a herd diagnosis (Switzer and Ross, 1975; Switzer and Farrington, 1977; McKean, Andrews, and Farrington, 1979). With regard to the latex agglutination tube test (LAT), with LAT serum antibody titers persisting even after the macroscopic lung lesions and CF serum antibodies disappearing, this serologic procedure could be used as a predictor of microscopic lesions. In such a way, it would be useful in determining or detecting chronic infected herds (Switzer and Farrington, 1977; Slavik and Switzer, 1979).

Fluorescent antibody staining. Identification of *M. hyopneumoniae* isolates in filter membrane (Armstrong, 1980) or in frozen sections of consolidated lungs, smears of broth culture and bronchial exudate (Armstrong, 1980; L'Ecuyer and Boulanger, 1970; Meyling, 1971) by visualization of the organism in the lung airways makes the FA a suitable supportive diagnostic procedure for diagnosis of mycoplasmal pneumonia in swine.

Differential Diagnosis

Swine influenza. For this pathologic condition there is sudden onset, severe clinical signs, pyrexia, anorexia, painful coughing and sneezing, and rapid loss of weight. Pathologic changes observed are pulmonary edema, diffuse acute inflammation, consolidated cranial lung lobes, and resolution of lesions in 2 to 3 weeks in uncomplicated cases. Microscopically, bronchial exudate, focal necrosis and degeneration of bronchial epithelium, peribronchial and perivascular inflammatory mononuclear cell infiltration, polymorphonuclear leukocytes, and focal coagulative necrosis are observed (Shope, 1931; Whittlestone, 1957). Isolation of swine influenza virus or demonstration of specific serum antibodies are sine qua non for diagnosis of swine influenza. *Mycoplasma hyopneumoniae* isolation by culture or visualization by FA staining in frozen sections of consolidated lung tissue will help in a differentiation of the disease.

Lungworms--pneumonia. Clinical signs described for this pathologic condition usually are: anorexia, constipation, diarrhea, emaciation, and coughing. On postmortem examination, areas of emphysema are observed in the diaphragmatic lung lobes, at the tips or free borders of these lung lobes; parasites can be found in the bronchiolar lumens. Microscopically, the lung lesions show hypertrophy of smooth muscle, lymphoid hyperplasia, giant cells and eosinophils, as well as lungworm eggs in the alveoli.

Pasteurellosis. In severe cases of this disease, there is fever, mucopurulent nasal discharge, anorexia, and depression. Exudative bronchopneumonia of lobular distribution is observed, as well as grayish lung consolidation and edema. Pleura and peritoneum occasionally show

serofibrinous inflammation. Microscopically, lymphocytes, macrophages, and neutrophils can be seen around the peribronchial and perivascular spaces. Neutrophils accumulate also in the alveolar spaces. *Pasteurella multocida* is almost always isolated. In complicated forms of the disease, there is a mucopurulent exudate which contains large numbers of neutrophils. Focal necrosis and abscessation are frequently observed. In septicemic pasteurellosis, the serous and mucous membranes show petechial and ecchymotic hemorrhages, and the kidney, liver, spleen, and intestine are congested. *Pasteurella* can coexist with mycoplasmal pneumonia in swine as well as with other pathogenic bacteria in the respiratory tract, such as *C. pyogenes, Haemophilus, Streptococcus* sp., and *M. hyorrhinis* (L'Ecuyer, Switzer, and Roberts, 1961).

Summary

Specifically, mycoplasmal pneumonia in swine and the different names given to the disease through the years have been considered. Steps which led to the isolation and identification of *Mycoplasma hyopneumoniae* as the specific etiologic agent of this disease have been described, as well as the organism's requirements for growth and its distinctive morphologic features. Epidemiologic behavior of the disease, the clinical signs and macroscopic and histologic lesions, serologic tests such as CFT, indirect (passive) hemagglutinating angibody (IHA), latex agglutination tube test, and immunofluorescent antibody staining tests have been discussed. A review of the literature has defined the value of the pathologic changes observed in the lungs of animals which died of the disease, as well as the value of each serologic test individually as it is used to establish the diagnosis of mycoplasmal pneumonia in swine. Pathogenic agents other than *Mycoplasma* sp. were considered for differential diagnosis.

MATERIALS AND METHODS

Lung Specimens

Twenty-two pig lungs were collected at a local abattoir,[a] of which 19 had macroscopic lesions. Eight additional pig lungs were collected at the Animal Health Diagnostic Laboratory (AHDL), College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, from pigs received for diagnostic purposes. Of these pigs, only 1 had macroscopic lesions. The 30 pig lungs collected were those available when the search for specimens was made, and no particular purpose existed regarding the age of the pigs. In Table 1 are presented the pig lungs collected, distributed by presence or absence of macroscopic lesions and the pigs' age in weeks.

Antiserum-Conjugate-Controls

Antisera against *M. hyopneumoniae* produced in rabbits and thin lung sections of experimentally infected pigs to be used as controls were produced by the Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, using as antigen *M. suipneumoniae* "J" strain ATCC No. 25934 (Armstrong, 1980). These materials were provided through the courtesy of the above-mentioned Dr. Armstrong, as well as fluorescein isothiocyanate (FITC) conjugate goat antirabbit globulin, which is commercially available.[b]

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[a]Milligan Packing Plant.

[b]Antibodies, Inc., Davis, CA.

Table 1. Number of pig lungs collected and lesions

Age of Pigs (weeks)	With Macroscopic Lesions	Without Macroscopic Lesions	Subtotals
3	-	3[a]	3
5	-	1[a]	1
6	-	1[a]	1
8	1[a]	1[a]	2
16	-	1[a]	1
20	16[b]	-	16
24	3[b]	3[b]	6
Totals	20	10	30

[a]Collected at the Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, MI.

[b]Collected at a local abattoir.

Normal rabbit serum was collected from rabbits at the Animal Health Diagnostic Laboratory (AHDL) and used for negative control. Phosphate buffered solution (PBS)[c] adjusted to pH 7.3, PBS-bovine serum albumin (PBS-BSA)[c] used for diluting sera and conjugate, as well as buffered glycerin,[c] pH 8.5, used for mounting slides, were all prepared at the AHDL.

Macroscopic Lung Evaluation

The lungs collected at the commercial abattoir, as well as those collected at the AHDL, were evaluated according to percentage of consolidation exhibited by each of the right and left anterior lung lobe fields,

[c]See Appendix, page 56.

and a grade was assigned according to a scheme for grading designed by the author and here presented (Table 2).

Table 2. Grading scheme of pig lung lesions according to percent average of consolidated right and left anteroventral lung lobe fields

Percent Average of Consolidated Anterior Lung Lobe Fields	Grade
1-5	1
6-15	2
16-30	3
31-50	4
>50	5

Histopathology

Sections of the right and left apical lung lobes as well as the right and left cardiac lobes were taken and fixed in buffered 10% formalin, trimmed, embedded in paraffin, and sections 6 µm thick made and stained with hematoxylin-eosin (H&E) for histopathologic examination.

Tissues for Immunofluorescence Staining

From those lungs with macroscopic lesions, sections of consolidated tissue were taken, and from lungs without macroscopic lesions, tissue sections were taken at the tip of the apical and/or cardiac lobes. The lung samples thus collected were placed in plastic bags and stored at -20 C for periods of 1 to 3 weeks, until examined.

Indirect Immunofluorescent Staining Antibody (IFA)

From each pig lung, portions 0.4 x 0.2 cm were frozen onto sectioning blocks and, with a cryostat-equipped microtome,[d] 2 sections 6 μm thick each, made and placed on 1 slide, dried for 8 to 10 minutes, fixed in acetone for 10 minutes, and air dried.

Antiserum and normal rabbit serum, respectively, were added to individual tissue sections to be tested, as well as to the tissue sections used as controls. They were then incubated in a moist chamber for 30 minutes at room temperature. The slides were washed with PBS for FA for 10 minutes, dried, and stained with fluorescein-isothiocyanate conjugated antirabbit globulin, incubated in a moist chamber for 30 minutes at room temperature, washed with PBS for FA for 10 minutes, and then mounting media added and coverslips applied. The slides were examined by fluorescent microscope.[e]

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[d] International Equipment Co., Needham Heights, MA (cat. no. 31875).

[e] Zeiss standard microscope, 100 W halogen lamp transmitted light, infrared wavelength, heat absorption filter, exciter filter, barrier filter for FITC.

RESULTS

Macroscopic Lesions

The lesions described below were common to all 20 pig lungs with variation more in degree of severity than in type of lesion. The anteroventral parts of the apical and cardiac lobes showed macroscopic lesions with a symmetrical tendency in distribution. Areas of consolidation ranged from 5 to 60% of the lung lobe fields and appeared as plum-colored discolored areas with a fairly firm feeling, which were clearly demarcated from other areas of grossly normal appearing, healthy looking lung. Consolidation was observed extending from the tips of the affected lobes towards the hilus. When graded for lesions, the 20 pig lungs appeared distributed as reported in Table 3. The types of macroscopic lesions can be observed in Figures 1 through 3.

Histopathology

Pig Lungs with Macroscopic and Microscopic Lesions

The pig lungs which had macroscopic lesions showed a variety of microscopic lesions when histologic examination was done. A summary of morphological diagnosis is presented for each pig lung examined (Table 4).

1) Lung sections were examined from 1 pig 8 weeks old and 5 pigs 20 weeks old each. The identification numbers of these pigs in Table 4 are 1 through 6, respectively. Marked peribronchial and peribronchiolar

Table 3. Grading scheme of pig lung lesions according to percent average of consolidated right and left anteroventral lung lobe fields (number of pig lungs by grade)

Percent Average of Consolidated Anterior Lung Lobe Fields	Grade	No. of Pig Lungs
1-5	1	2
6-15	2	3
16-30	3	9
31-50	4	4
>50	5	2

Figure 1. Pneumonic pig lung. Bilateral and symmetrical consolidation of the anteroventral parts of the apical and cardiac lobes.

Figure 2. Pneumonic pig lung. Consolidated right apical and cardiac lobes.

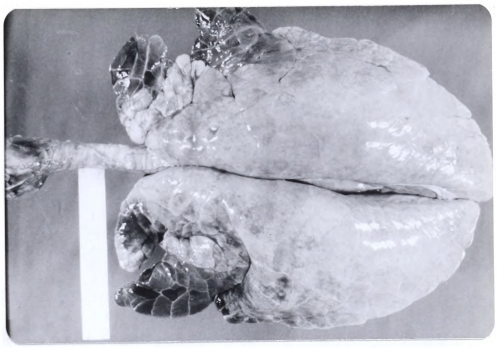

Figure 1

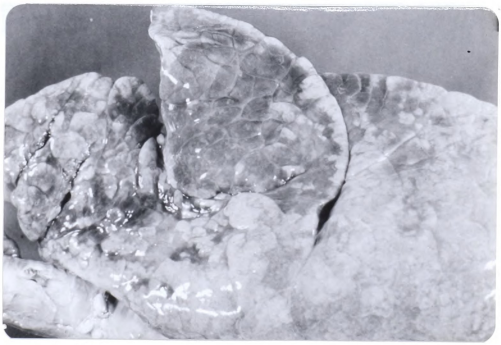

Figure 2

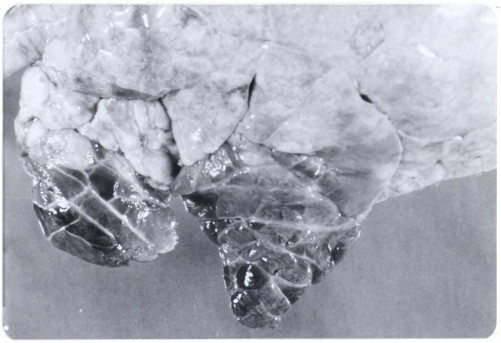

Figure 3. Pneumonic pig lung. Consolidated left apical and cardiac lobes. Lobular distribution of the lesions. Clear-cut separation between affected lung and normal appearing healthy lung.

Table 4. Summary of morphologic diagnosis and age of each study pig; lungs with macroscopic and microscopic lesions

Pig Lung No.	Age (weeks)	Subacute Interstitial Pneumonia	Subacute to Chronic Interstitial Pneumonia	Subacute Suppurative Pneumonia
1	8	X		X
2	20	X		X
3	20	X		X
4	20	X		X
5	20	X		X
6	20	X		X
7	20		X	
8	20		X	
9	20		X	
10	20		X	
11	20		X	
12	20		X	
13	20		X	
14	20		X	
15	20		X	
16	20		X	
17	20		X	
18	24		X	
19	24		X	
20	24		X	

lymphoid hyperplasia was seen in some areas, and in other areas a fairly marked thickening of the alveolar walls with proliferation and infiltration of lymphocytes, with scarce or no indication of neutrophil infiltration was observed. Lymphocytes were also observed in the peribronchial and peribronchiolar spaces. Some areas of the bronchial and/or bronchiolar epithelium had loss of cilia and desquamated epithelium, while other areas had a hyperplastic epithelium. In other sections of lung, large numbers of neutrophils were observed in the alveolar spaces accompanied by some edema. For these 6 pig lungs the microscopic characteristics were those of a subacute interstitial pneumonia and subacute suppurative pneumonia.

2) At histologic examination of sections of lungs of 11 pigs, 20 weeks old each, identified in Table 4 with the numbers 7 through 17, there was an accumulation of lymphocytes, macrophages, and plasma cells within the alveolar spaces. There was infiltration of lymphocytes and macrophages in the alveolar walls. Loss of cilia and desquamation of bronchial and bronchiolar epithelium were evident, and mononuclear cells were accumulated in the peribronchial and peribronchiolar spaces. Lymphoid hyperplasia in the bronchial tree was marked. In some lung areas the bronchiolar epithelium was hyperplastic. Proliferating fibroblasts were seen in the alveolar walls. The histologic lesions described for these 11 pig lungs were considered as a morphological expression of a subacute to chronic interstitial pneumonia.

3) Three pigs with identification numbers 18, 19 and 20 in Table 4 had lymphocytes, macrophages and plasma cells in the alveolar lumens on histologic examination. There were accumulations of mononuclear cells in the alveolar walls as well as in the peribronchial and peribronchiolar spaces, with prominent lymphoid hyperplasia in the bronchial tree and

proliferating fibroblasts in the alveolar walls. The characteristics of the microscopic lesions observed and described for these 3 pig lungs were those of a subacute to chronic interstitial pneumonia.

Pig Lungs with Only Microscopic Lesions

Ten pig lungs had no macroscopic lesions but did have microscopic lesions at histologic examination. A summary of the morphologic diagnosis for these 10 pig lungs is presented in Table 5.

Table 5. Summary of morphologic diagnosis and age of each study pig; pig lungs showing only microscopic lesions

Pig Lung No.	Age (weeks)	Subacute Interstitial Pneumonia	Subacute Nonsuppurative Pneumonia	Subacute Suppurative Pneumonia
1	3	X		
2	3	X		
3	3	X		
4	5	X		
5	6	X		X
6	8	X		
7	16	X		X
8	24	X	X	
9	24	X	X	
10	24	X	X	

1) Lung sections were examined from three 3-week-old pigs, one 5-week-old pig, and one 8-week-old pig, identified in Table 5 with the numbers 1, 2, 3, 4 and 6, respectively. There were accumulations of mononuclear cells in the peribronchiolar and perivascular spaces. The alveolar walls appeared thickened and infiltrated by proliferations of lymphocytes. The bronchiolar epithelium showed a moderate loss of cilia. Collapsed lung tissue was observed in some areas of the lung sections, and a few mononuclear cells were observed within the alveolar spaces. The microscopic characteristics for the above pig lung sections examined were those of a subacute interstitial pneumonia and bronchiolitis.

2) Lung sections of 2 pigs, 6 and 16 weeks old, identified by the numbers 5 and 7, respectively, in Table 5, were examined. There was infiltration of mononuclear cells in the alveolar spaces with marked thickening of the alveolar walls and infiltration by mononuclear cells with little or no indication of neutrophil infiltration. There were fairly marked areas of peribronchiolar and perivascular accumulations of mononuclear cells. Loss of cilia of bronchial and bronchiolar epithelium was observed. In other sections of the lung in both pigs, there were accumulations of neutrophils and lymphocytes in the alveolar spaces with some edema. In addition, there were areas showing moderate peribronchiolar lymphoid hyperplasia. The lung sections of the 2 pigs showed microscopic characteristics of a subacute interstitial pneumonia, subacute suppurative pneumonia, and bronchiolitis.

3) In lung sections of three 6-month-old pigs examined histologically, there were accumulations of lymphocytes within the alveolar spaces as well as a few macrophages. These pigs are identified in Table 5 by numbers 8 through 10. In the peribronchiolar spaces there was

accumulation of mononuclear cells (lymphocytes), as well as peribronchiolar lymphoid hyperplasia. Within the alveolar walls there was infiltration of lymphocytes and a few macrophages. The bronchial and bronchiolar epithelium showed loss of cilia. Proliferating fibroblasts were observed in the alveolar walls. The microscopic characteristics in the lung sections of the 3 pigs were those of a subacute to chronic interstitital pneumonia and subacute nonsuppurative pneumonia.

Summary of Histopathology

In pig lungs with or without macroscopic lesions, the histologic lesions observed were: accumulations of lymphocytes in peribronchial-peribronchiolar perivascular spaces and alveolar lumens (Figures 4 through 9). Peribronchiolar lymphoid hyperplasia and discrete fibroblast proliferation (Figure 6), neutrophils and lymphocytes in bronchiolar lumens (Figure 7), thickened alveoli with infiltration of lymphocytes (Figure 8), lymphoid hyperplasia and highly dense accumulations of lymphocytes in peribronchial-peribronchiolar and perivascular spaces (Figure 10) and loss of cilia of bronchial epithelium were seen.

Indirect Immunofluorescent (IFA) Staining Results

Discrete fluorescent particles aggregating in masses or in clusters over the surface of the bronchial and bronchiolar epithelium were interpreted as *M. hyopneumoniae* organisms. A summary of IFA results for pig lungs which had macroscopic and microscopic lesions is presented in Table 6. In this category, 1 pig 8 weeks of age showed marked fluorescence over the bronchial and bronchiolar epithelial surface. Of sixteen 20-week-old pigs, fluorescence was observed over the surface of the lung airways in 14, and 2 failed to show any fluorescence. Three 24-week-old pigs had fluorescence over the surface of the lung airways.

Figure 4. Photomicrograph of pig lung. Notice accumulations of lymphocytes in peribronchiolar and perivascular spaces. H&E stain, 64X.

Figure 5. Photomicrograph of pig lung. Notice accumulations of lymphocytes in peribronchiolar spaces and alveolar lumens. H&E stain, 160X.

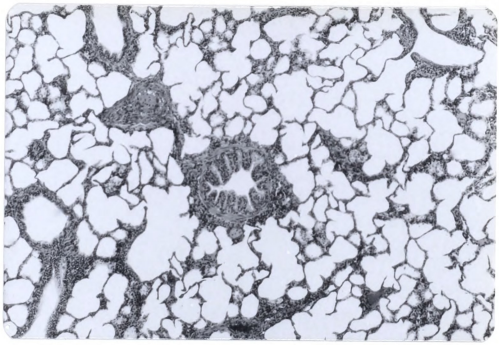

Figure 4

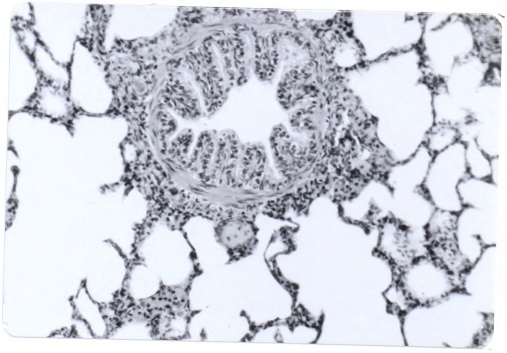

Figure 5

Figure 6. Photomicrograph of pig lung. Peribronchiolar cuffing with lymphocytes. Peribronchiolar lymphoid hyperplasia. Fibroblasts. H&E stain, 64X.

Figure 7. Photomicrograph of pig lung. Debris, lymphocytes, and neutrophils in bronchiolar lumen. H&E stain, 160X.

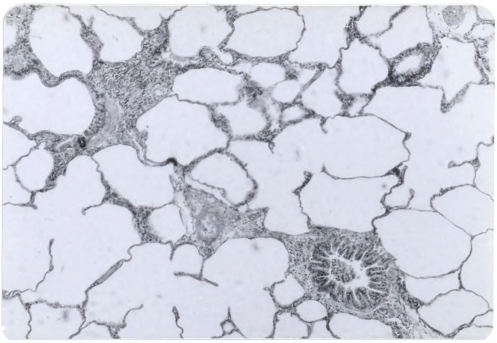

Figure 6

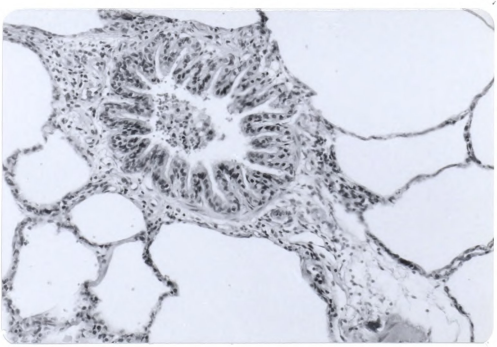

Figure 7

Figure 8. Photomicrograph of pig lung. Notice infiltration of lymphocytes in alveolar walls and bronchiolar spaces. H&E stain, 64X.

Figure 9. Photomicrograph of pig lung. Peribronchiolar accumulations of lymphocytes. H&E stain, 160X.

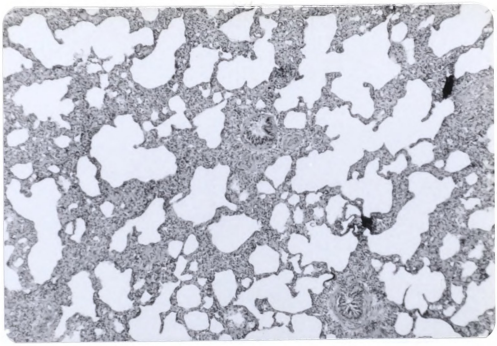

Figure 8

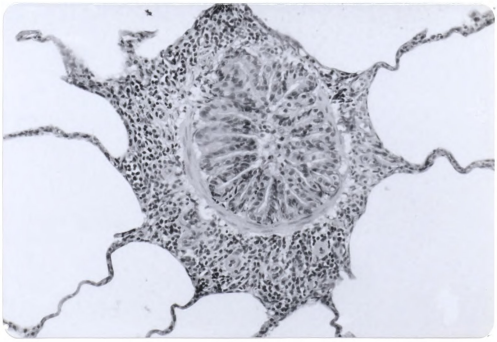

Figure 9

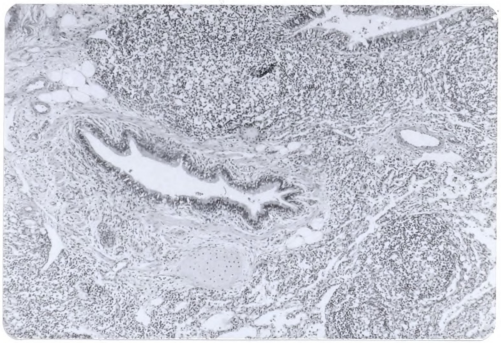

Figure 10. Photomicrograph of pig lung. Notice accumulations of lymphocytes in peribronchial-peribronchiolar-perivascular spaces. Peribronchial-peribronchiolar lymphoid hyperplasia. H&E stain, 64X.

Table 6. Summary of indirect FA test in pig lungs with macroscopic and microscopic lesions and age of each study pig

Pig Lung No.	Age (Weeks)	Indirect FA Test
1	8	Positive
2	20	Positive
3	20	Positive
4	20	Positive
5	20	Negative
6	20	Positive
7	20	Positive
8	20	Positive
9	20	Positive
10	20	Positive
11	20	Positive
12	20	Positive
13	20	Positive
14	20	Positive
15	20	Positive
16	20	Negative
17	20	Positive
18	24	Positive
19	24	Positive
20	24	Positive

In Table 7 is presented a summary of IFA test results for pig lungs which had no macroscopic lesions but did have microscopic lesions.

Table 7. Summary of indirect fluorescent antibody test (IFA) in pig lungs which had no macroscopic lesions but did have microscopic lesions, and age of each study pig

Pig Lung No.	Age (Weeks)	Indirect FA Test
1	3	Positive
2	3	Positive
3	3	Negative
4	5	Positive
5	6	Positive
6	8	Positive
7	16	Positive
8	24	Positive
9	24	Positive
10	24	Negative

In this category of pig lungs, in 3-week-old pigs fluorescence was seen over the surface of the lung airways in 2 and the third failed to show any fluorescence. Four pigs, 5, 6, 8 and 16 weeks of age, respectively, had fluorescence over the surface of the lung airways of each one. Of the three 24-week-old pigs, 2 had discrete fluorescence on the surface of the lung airways while the third one failed to show any fluorescence.

The immunofluorescent test was repeated a second time for all the pig lungs which showed only microscopic lesions. The results were as with the first test, with the exception of pig 7, which was negative on

the first test and positive on the second. Those pig lungs with macroscopic and microscopic lesions which were immunofluorescent negative were tested a second time with identical results in all cases.

DISCUSSION

Of 10 pig lungs which showed only microscopic lesions, three 3-week-old pigs showed lesions suggestive of an early *M. hyopneumoniae* infection based on histopathologic findings, but fluorescence on the surface of the lung airways was observed in only 2 of these 3 pigs, while the third failed to show any fluorescence.

Positive results by means of the direct fluorescent staining procedure have been reported in experimental cases of the disease as early as the 14th day postinoculation (Livingston et al., 1972) and by the 21st day postinoculation (L'Ecuyer and Boulanger, 1970). No data could be found in the literature about diagnosis of field cases of mycoplasmal pneumonia of swine by means of fluorescent antibody staining in very young piglets.

As for the negative fluorescent results in pig lungs showing microscopic lesions and given *M. hyopneumoniae* infection, reasons could be, according to L'Ecuyer and Boulanger (1970): 1) an insufficient amount of antigen being present to allow visualization or 2) washing out of the antigen from the epithelium of the lung airways during the staining procedure.

Pathogenic agents other than *M. hyopneumoniae* may be the cause of microscopic lung lesions suggestive of mycoplasmal pneumonia infection but which actually are not (Pattison, 1956; Switzer and Ross, 1975; Jericho, 1968, 1977).

In the lungs of 2 pigs, 5 and 8 weeks old, respectively, the microscopic lesions observed were suggestive of mycoplasmal pneumonia infection, and both pigs showed fluorescence in the lung airways.

In 3 pigs 24 weeks old, the microscopic lung lesions were suggestive of mycoplasmal pneumonia, but in addition there were microscopic changes related to bacterial pneumonia in a stage of resolution. Two of these pigs showed fluorescence in the lung airways, and 1 failed to show lung fluorescence.

Of 20 pig lungs which showed macroscopic and microscopic lesions, the lungs of 1 pig, age 8 weeks, and 5 pigs, age 20 weeks, showed a variety of lesions suggestive of mycoplasmal pneumonia and superimposed suppurative bacterial pneumonia. Fluorescence was observed in the lung airways of 5 of these pigs; 1 pig (age 20 weeks) failed to show any fluorescence.

In 11 pigs, age 20 weeks, and 3 pigs, age 24 weeks, the lesions observed suggested mycoplasmal pneumonia and mild to severe superimposed bacterial pneumonia. Of this group, 13 pigs showed fluorescence in the lung airways and 1 pig (age 20 weeks) was immunofluorescent negative.

It should be pointed out that cultural procedures for isolation and identification of pathogenic bacterial agents might have been helpful in interpretation of the pathologic picture in the lungs. On the basis of the studies here reported, it would appear that the indirect immunofluorescent staining tests provides a rapid, relatively simple method for the visualization of *M. hyopneumoniae* organisms in lung sections. At necropsy and/or histopathologic examination of either pig lungs which showed only microscopic lesions or pig lungs which showed macroscopic and microscopic lesions, a suggestive diagnosis of mycoplasmal pneumonia could be confirmed by means of indirect fluorescent antibody staining,

by specifically allowing the visualization of the causative agent.

For routine diagnostic purposes, isolation from tissues of *M. hyopneumoniae* has been reported as difficult (Armstrong, 1980; L'Ecuyer and Boulanger, 1970; McKean, Andrews, and Farrington, 1979) and, once isolated, the species identification is a time-consuming process (Armstrong, 1980; L'Ecuyer and Boulanger, 1970).

The reliability of the staining reactions obtained with pig lungs showing microscopic lesions only or macroscopic and microscopic lesions suggestive of *M. hyopneumoniae* infection would appear to be assured by: 1) the presence of fluorescence in the lung airways stained with homologous immune globulins produced in rabbit and fluorescein isothiocyanate conjugated (FITC) antirabbit globulins produced in goat, 2) the absence of such fluorescence in sections of lungs infected and with lesions of *M. hyopneumoniae* when normal rabbit globulins are used, and 3) the presence of apparently specific fluorescence in the bronchial and bronchiolar epithelial surface in pig lungs infected with *M. hyopneumoniae* and stained with homologous immune globulins and fluorescein isothiocyanate conjugated antirabbit globulins produced in goats.

The FA methods have proven to be species specific (Armstrong, 1980; Kobisch, Tillon, and Vannier, 1978; L'Ecuyer and Boulanger, 1970; Meyling, 1971).

For etiologic definitions of macroscopic and/or microscopic lesions as caused by *M. hyopneumoniae* in pig lungs by means of the indirect fluorescent antibody test (IFA), the author recommends that further studies should be done to ascertain the degree of sensitivity of the test.

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VITA

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The author will work in Central America.

APPENDIX

APPENDIX

Working PBS for FA

Na_2HPO_4	24.0 grams
$NaH_2PO_4 \cdot H_2O$	4.4 grams
NaCl	170.0 grams
Deionized water	2000.0 ml

Each ingredient dissolved separately

Add 4 grams of NaN_3

pH: 7.4

PBS-BSA (for diluting serums and conjugates)

1 gram of bovine serum albumin (BSA)[a]

200 ml of working PBS

Dissolve

Pass through a 0.20 µm filter

Store at 4 C

Mounting Fluid

9 parts glycerine[b] and 1 part PBS

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[a]Grand Island Biological Company, 3175 Staley Road, Grand Island, NY 14072.

[b]Fort Dodge Laboratories, 800 Fifth Street, NW, Fort Dodge, IA 50501.