Enzootic Pneumonia of Pigs: Identification of a Causative Mycoplasma in Infected Pigs and in Cultures by Immunofluorescent Staining

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ABSTRACT

Immunofluorescent staining has been used to identify Mycoplasma hyopneumoniae in smears of broth cultures, in infected pig testicle cell cultures, and in frozen cut sections of pneumonic lungs from field and experimentally produced cases of enzootic pneumonia. In the pneumonic pig lung, fluorescent staining was limited to the surface of the bronchial and bronchiolar epithelium and to the contained exudate. In a series of trials using experimentally infected pigs fluorescence was not detected until 25 days post-infection and was regularly seen in pigs killed thereafter. Porcine immune globulin precipitated from the serum of experimentally infected pigs and conjugated with fluorescein isothiocyanate was reactive and specific for the detection of M. hyopneumoniae. Immune globulin conjugates prepared from the serum of hyperimmunized rabbits were reactive but in some cases produced a faint non-specific staining of frozen tissue sections. No such non-specific reactions were noted on stained culture smears or cell cultures.

Fluorescence was not seen in known positive preparations stained with non-immune pig globulin conjugates or in preparations from uninoculated cell cultures or pigs, stained with non-immune or immune globulin conjugates.

Mycoplasma hyorhinis was detected by immunofluorescent staining with homologous conjugates, in smears of broth cultures and in tissue sections from pigs with polyserositis.

Immunofluorescent staining was found to be species specific and useful for the early species identification of mycoplasma isolated from pigs.

RÉSUMÉ

L'épreuve de l'immunofluorescence est utilisée pour l'identification de Mycoplasma hyopneumoniae un agent étiologique de la pneumonie enzootique du porc (pneumonie à virus). L'immunofluorescence permet de démontrer cette bactérie aussi bien sur les frottis de cultures que dans les cultures cellulaires et les poumons infectés. Dans le cas des porcs infectés on examine des coupes histologiques de lésions d'hépatisation pulmonaire provenant de cas cliniques et de cas produits par infection expérimentale. Quelle que soit l'origine des poumons affectés l'antigène immunofluorescent apparait sous forme d'une lisière étroite en marge de la muqueuse des bronches et des bronchioles et de granules dans l'exsudat qu'elles contiennent. Cet antigène sur la muqueuse est granuleux et d'un jaune-verdâtre intense tandis que la fluorescence dans l'exsudat se présente sous forme de granules individuels ou en amas associés aux débris cellulaires.

Lors d'une série de trois expériences sur des porcs infectés avec des cultures de M. hyopneumoniae le plus tôt que nous avons pu déceler de la fluorescence dans les poumons fut 25 jours après l'infection. Toutefois les porcs examinés après ce délai ont donné des réactions positives dans huit cas sur dix.

Les immunoglobulines, de porcs atteints de pneumonie enzootique à la suite d'une inoculation expérimentale, conjuguées avec l'isothiocyanate de fluorescéine se sont avérés efficaces et spécifiques pour déceler M. hyopneumoniae.

Des conjugués préparés avec du sérum de lapins hyperimmunisés sont efficaces mais ont tendance à réagir de façon non-spécifique lorsqu'utilisés pour colorer des sections de tissus porcins. Ces conjugués ne sont pas non-spécifiques lorsqu'utilisés sur des frottis de cultures ou sur des cultures cellulaires.

Aucune fluorescence non-spécifique n'est décelée lorsque des préparations positives sont teintes avec des conjugués préparés avec le sérum de porcs normaux. Il en est ainsi des cultures cellulaires non-inoculées et des tissus de porcs normaux teints avec des conjugués de sérum de porcs infectés ou normaux.

Nous avons réussi par l'immunofluorescence à déceler Mycoplasma hyorhinis dans des frottis de cultures en bouillon et dans des coupes de tissus de porcs atteints de polysérosite. Aucune réaction croisée n'est décelée entre les deux

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espèces de mycoplasma (M. hyopneumoniae et M. hyorhinis) lorsque l'une ou l'autre est présente soit sur un frottis ou une coupe de tissu. Cette immunofluorescence spécifique à l'espèce s'est avérée utile pour l'identification rapide et spécifique des mycoplasmas isolés de cas cliniques de pneumonie enzootique.

INTRODUCTION

A specific etiological diagnosis of enzootic pneumonia (EP) (virus pneumonia of pigs) was not possible until recently because of our inability to isolate the primary etiological agent in culture. The results of recent work (5, 8, 11, 18) make it clear that a new bacterial species, *Mycoplasma hyopneumoniae,* is a primary etiological agent of a condition previously diagnosed as EP on the basis of clinical observations with typical gross and histopathological lesions. The identification and propagation of *M. hyopneumoniae* in pure culture has made possible the development of serological tests for EP such as complement-fixation (2, 14, 16) and growth or metabolic inhibition (6).

The primary isolation of *M. hyopneumoniae* from pneumonic lungs, proven infectious by pig inoculation, is successful only in a small percentage of cases (7, 9). Several reasons for this difficulty are the extreme lability of *M. hyopneumoniae,* its very fastidious growth requirements and the presence in a majority of infected lungs of *Mycoplasma hyorhinis,* a secondary contaminant, which, because of its more rapid adaptation to artificial medium, will generally overgrow *M. hyopneumoniae.*

Because of the problems of identifying *M. hyopneumoniae* by bacteriological culture an alternate method of specifically recognizing it in pneumonic lungs was sought. It was felt that immunofluorescent staining might offer a rapid and relatively simple technique for the detection of the organism in tissues.

The objectives of this investigation were to determine the usefulness, the sensitivity, and the specificity of direct immunofluorescent staining for the detection of *M. hyopneumoniae* in the lungs of pigs experimentally infected with EP.

Whereas immunofluorescent staining has been used fairly extensively for the detection of viral antigens in tissues and for the identification of different mycoplasma from man and animals, to our knowledge there are no reports in the literature on the use of this technique for the detection of mycoplasma in porcine tissues. Goodwin *et al* (6) used labelled rabbit and pig antiserum to stain their EP isolate, *Mycoplasma suipneumoniae,* either in smears of concentrated antigens or in infected cell cultures. The technique was considered sensitive because conjugated pig and rabbit sera were active at fairly high dilutions and no cross reactivity occurred between different species of mycoplasma.

MATERIALS AND METHODS

Mycoplasma Strains

The origin of *M. hyopneumoniae* strains 28, 29 and 33, and of *M. hyorhinis* strain 31 have been described (8).

The prototype *M. hyopneumoniae* strain 11 (11) as well as *M. hyorhinis* strain 7 and *Mycoplasma granularum* strain 39 were obtained through the courtesy of Dr. W. P. Switzer[1]. *M. hyorhinis* strains E4737 and F3023 were recovered from field cases of acute polyserositis in young pigs.

Bacteriological Methods

The broth media used for the propagation, the routine of cultivation and the methods of storage of the mycoplasma have been previously described (8). Primary pig testicle cell cultures were prepared and cultivated as described (8). Cell cultures grown on coverslips were inoculated with 0.1 - 0.2 ml of an active broth culture of mycoplasma.

Smears for immunofluorescent staining were prepared by centrifuging 3 ml of broth culture in an angle head centrifuge at 6,800 x G for 15 minutes. The supernatant was discarded and the sediment was resuspended in a few drops of medium and smeared on a microscope slide. Smears were air dried and stored at 4°C until stained with or without prior acetone fixation. Mycoplasma antigens were prepared and stored as described previously (2, 8).

Animal Experimentation

All pigs used in experimental studies were from a Caesarean-derived, barrier-sustained herd. The pigs were naturally

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farrowed and reared and were from four to seven weeks old when used. The pigs in this herd have been shown by repeated pathological and serological examinations to be free of EP. However, some pigs from this herd have developed mild, focal, linear areas of consolidation in the dorsal portion of the lungs. These lesions are characterized histologically by the formation of granulomas containing giant cells as well as what appears to be plant material. These pneumonias could not be transmitted to other pigs and have been found negative for bacteria during routine examinations. This pneumonia is thought to be due to the inhalation of minute particles of plant material from the wood shavings used as bedding or from the feed.

The methods of inoculation, blood sampling, euthanasia and necropsy have been described (2, 8).

Five field cases of EP were included in the examinations made. Three were acute pneumonias in weanling pigs and the other two were collected at random from pigs at slaughter.

Ten experimentally infected pigs were examined during the first stages of this work. Two pigs had been inoculated with a lung suspension from a field case of EP whereas the other eight pigs received broth cultures of *M. hyopneumoniae* as follows: strain 28, two pigs; strain 29, one pig; strain 33, one pig; and strain 11, four pigs.

Conjugates were prepared from the serum of two pigs (2508 and 2510) experimentally infected with *M. hyopneumoniae* strain 29 and from the serum of one rabbit (297) hyperimmunized with this strain and one (294) with strain 28. Conjugates for *M. hyorhinis* were prepared from the serum of a pig (2505) experimentally infected with strain 31 and from one hyperimmunized rabbit (699).

Three trials were carried out to determine the sequence of appearance of fluorescence following exposure. Twenty-one pigs were used in these trials, five in the first, nine in the second and seven in the third trial. In each trial the pigs were inoculated intratracheally with 5 ml of a 48 hour broth culture of *M. hyopneumoniae* strain 29 and were housed together after inoculation. Individual pigs were then randomly selected for examination at predetermined intervals as indicated in Table II.

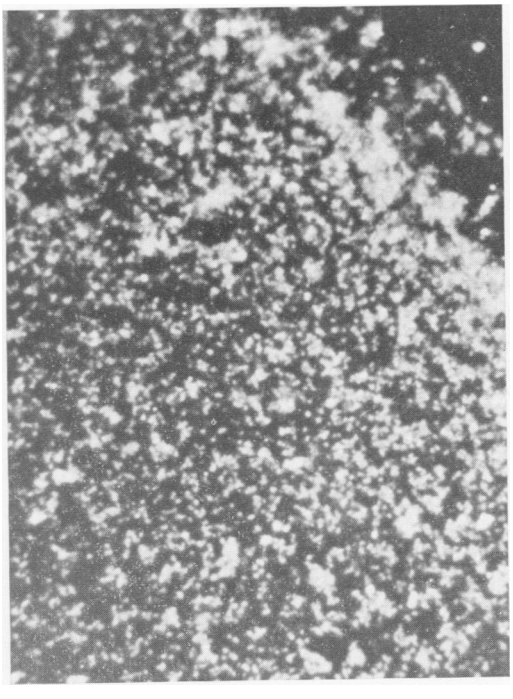

Fig. 1. Photomicrograph of a smear prepared from a broth culture of M. hyopneumoniae. Stained with a homologous immune conjugate. x640.

Fig. 2. Primary pig testicle cell culture infected with M. hyopneumoniae. Stained with a homologous immune conjugate. x640.

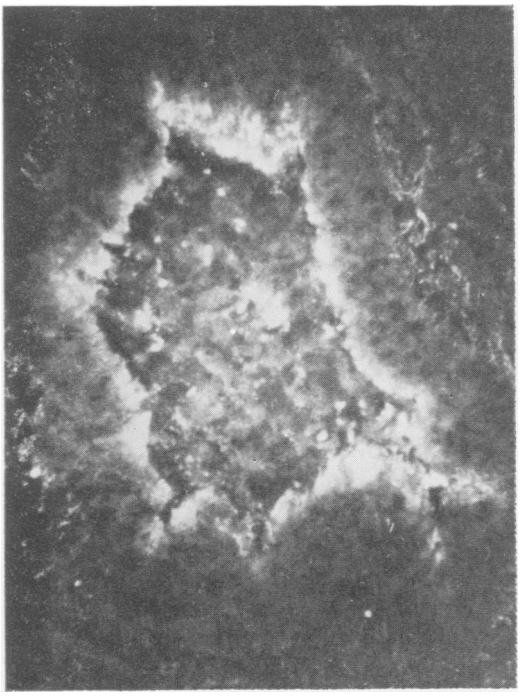

Fig. 3. Frozen section from a pneumonic pig lung illustrating the typical localization of immunofluorescent antigen on the surface of the epithelium of a small bronchiole and in the contained exudate. x640.

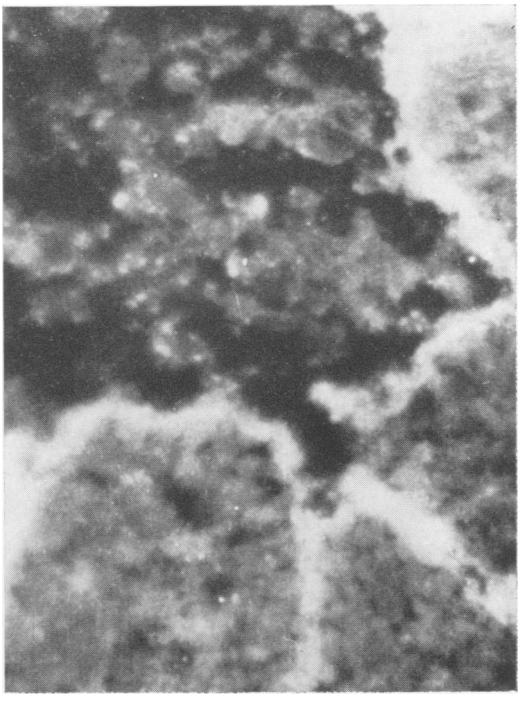

Fig. 4. Immunofluorescent stained section of a pneumonic pig lung illustrating, in one area of a bronchus, the concentration of fluorescent antigen on the surface and along the folds of the mucosa. x640.

Immunofluorescent Staining Technique

The methods described by Boulanger *et al* (1) were closely adhered to as regards: (1) the preparation of fluorescein isothiocyanate-labelled globulins from hyperimmunized rabbits or infected pigs; (2) the verification of these conjugates for specificity and staining activity; (3) the fixation and staining of smears of broth cultures, of cell culture coverslips, or of pig tissues; and (4) the equipment used for evaluating stained preparations.

Conjugates were also prepared from the serum of uninoculated rabbits and pigs and were used at the same dilution as the immune sera.

Tissues regularly collected for study were representative sections of lung, bronchial lymph node, spleen, and tonsil.

RESULTS

Reactivity of Conjugates

Immune globulin conjugates prepared from the serum of hyperimmunized rabbits were active at dilutions of 1:40 to 1:80 and were routinely used for staining mycoplasma preparations at dilutions between 1:5 and 1:10. These rabbit antisera had titers of 1:640 to 1:1280 (2) in the direct complement-fixation test with homologous antigens. Conjugates prepared from the serum of infected pigs were active at a dilution of 1:20 and were employed for staining at a dilution of 1:5. The modified direct complement-fixation titers of these pig sera varied from 1:160 to 1:320 (2).

Smears of Broth Culture

Smears of mycoplasma culture sediments stained with homologous, immune rabbit or pig conjugates appeared as intensely fluorescent, greenish-yellow bodies or clumps of bodies evenly distributed over the glass surface, Fig. 1. The intensity of the fluorescence was good whether the smears were prepared from washed antigens or from unwashed broth culture sediments. Comparative studies made between acetone fixed and unfixed smears indicated no advantage was gained by fixation, consequently subsequent work was done with unfixed smears.

Conjugates prepared against one strain of *M. hyopneumoniae* were equally sensitive in staining the other strains studied. Stained broth culture smears were usually included as positive controls when infected cultures or tissues were examined. No fluorescence was detected in any of the smears stained with non-immune rabbit or pig conjugates.

Mycoplasma-infected Cell Cultures

Pig testicle cell cultures infected with *M. hyopneumoniae* and stained with a homologous rabbit or pig conjugate contained large numbers of clear-cut, intensely fluorescent bodies. Fig. 2. The bodies appeared as individuals or as small clumps and seemed to be on the surface of the cells. Infected cell cultures treated with non-immune rabbit or pig conjugates and uninoculated cultures stained with both normal and immune conjugates did not contain any of these fluorescent bodies.

Appearance and Localization of Antigen in Infected Pig Tissues

The pattern of fluorescence in infected lungs was the same whether the lesions were from field cases or were the result of experimental inoculations with pneumonic lung suspensions or broth cultures.

Fluorescence was limited to the surface of the epithelium of the bronchi and of the bronchioles and to the exudate which filled the smaller passages. The antigen appeared as a granular, intensely fluorescent, yellow-green layer on the surface of the bronchial and the bronchiolar epithelium and it closely followed the epithelial folds which occur in the larger air passages, Fig. 3 and 4. The fluorescence seen in the bronchiolar exudate occurred as individual brightly fluorescent spots or clumps and seemed to be mainly concentrated on or around the cells and cellular debris. In some cases it was felt that the fluorescent granules represented individual mycoplasma cells but mostly it appeared to represent clumps of the organisms in the surface exudate. The intensity of the fluorescence varied considerably from pig to pig and from one area to another within the same lung. In general, the fluorescence was most marked in the lungs of field cases of pneumonia which appeared active macroscopically (extensive, moist lesions). It was less intense in chronic field cases collected at slaughter-weight and in experimentally infected pigs in which the disease is generally milder and the lesions less extensive. In no case was fluorescence seen in the lungs of normal control pigs stained either with immune or normal conjugates. No fluorescence was seen when sections of pneumonic lungs from *M. hyopneumoniae* infected pigs were treated with conjugates prepared with immune globulins against *M. hyorhinis* strain 31 or with globulins from control pigs.

Tissues from 14 pigs with typical gross lesions of EP were examined by the direct immunofluorescence technique. Fluorescence was seen in three of the four field cases. The fourth pig had extensive lesions but the lungs were very edematous. Fluorescence was detected in all of the ten remaining pigs, seven of which were examined and found to have histological lesions characteristic of EP, whereas three also had foreign body granulomas. Although five different strains of *M. hyopneumoniae* were used to produce pneumonia in these pigs, fluorescence was seen with the pig serum conjugate prepared against one of them (strain 29). No fluorescence was seen in the tissues of seven uninoculated, enzootic pneumonia-free, control pigs.

Representative lung sections, sections of tonsil, bronchial lymph node and spleen were examined from all experimentally infected pigs. In the early stages of the study, using rabbit conjugates, some apparently specific fluorescence was seen on the surface and in the crypts of the tonsil and in the germinal centers of the bronchial lymph nodes and of the spleen. However, when tissues from pigs experimentally infected with the same strain of *M. hyopneumoniae* were stained with porcine conjugates no fluorescence could be detected in organs other than the lung.

Specificity of the Immunofluorescent Staining Technique

When unknown tissues were examined the specificity of the rabbit and pig immune conjugates was regularly verified by including uninoculated cell cultures or tissues from uninoculated pigs among the stained preparations. It was found that conjugates prepared from normal or hyperimmune rabbit serum tended to give a faint yellow-white coloration to the tissue sections. This non-specific staining was

randomly located in the tissues, it was of low intensity, and did not cause confusion with the intense, granular, specifically located yellow green fluorescence in the bronchiolar epithelium.

The non-specific staining properties could be removed by repeated absorption with pig liver powder and its disappearance was accompanied by a decrease of intensity of the specific staining properties of the conjugate. Because of this difficulty rabbit conjugates were used only during the early stages of this study. Non-specific staining of frozen tissue sections was not seen in preparations stained with the various porcine globulin conjugates.

The specificity of the immunofluorescent staining was further verified by the routine use of conjugates prepared from normal pig serum on duplicate smears, cell cultures or tissue sections. These normal conjugates did not at any time, stain the various preparations examined.

As can be seen from Table I, the immunofluorescent staining technique was species specific in that immune conjugates for *M. hyopneumoniae* and *M. hyorhinis* reacted only with strains of the homologous mycoplasma species. The staining of smears of broth cultures of isolates from infected pigs was found to be a rapid and reliable method for the species identification of mycoplasma. The technique was also found to be species specific when duplicate cell cultures or pig tissue sections infected with *M. hyopneumoniae* or *M. hyorhinis* were stained with homologous or heterologous immune globulin conjugates.

Sequential Appearance of *M. hyopneumoniae* in the Lungs of Experimentally Infected Pigs

The presence of gross lesions and *M. hyopneumoniae* fluorescent antigen in the lung of 21 pigs experimentally infected in three trials are summarized in Table II. Gross lesions were observed in two (12 and 20 days PI) of ten pigs examined within 21 days after infection. Immunofluorescent antigen was absent from the lungs of all ten pigs. However, ten of the 11 pigs which were examined from the 25th to the 49th day after infection had slight to extensive gross lesions and mycoplasma antigen was demonstrated by immunofluorescence in eight of these lungs.

Five of the nine pigs which had no gross lung lesions at necropsy were examined histologically. Two of these had histological lesions characteristic of EP and one of them also had some foreign-body granulomas. None of these lungs contained fluorescent antigen. Eight of the 12 pigs with gross lung lesions were examined histologically. Seven of these lungs, four of which contained fluorescent antigen, had histological lesions characteristic of EP whereas the eighth also had some foreign-body granulomas and contained fluorescent antigen.

Immunofluorescence in Tissues of Pigs Infected with *M. hyorhinis*

Tissue sections from the three pigs, respectively infected with *M. hyorhinis*

TABLE I. Results of Immunofluorescent Staining of Smears from Broth Cultures of Mycoplasma

Conjugate			Culture Smears						
Species	Strain	Source[a]	M. hyopneumoniae				M. hyorhinis		M. granularum
			11[b]	28	29	33	7	31	39
M. hyopneumoniae	29	R297	...[c]	...	+	...	−	−	−
M. hyopneumoniae	29	P2508	...	...	+	+	−	−	−
M. hyopneumoniae	29	P2150	+	+	+	+	...	−	...
M. hyopneumoniae	28	R294	...	+	+	...	...	...	...
M. hyorhinis	31	R699	...	...	−	...	+	+	−
M. hyorhinis	31	P2505	−	...	−	...	+	+	−
NA[d]	NA	NR	...	−	−	...	−	−	−
NA	NA	NP	−	...	−	−	−	−	−

[a] R = hyperimmunized rabbit; P = infected pig; NR = normal rabbit; NP = normal pig.
[b] Refers to strain 11, strain 28, etc.
[c] ... = not done; + = fluorescence detected; − = no fluorescence.
[d] NA = not applicable.

TABLE II. Results of Trials on the Time of Appearance of Immunofluorescence in the Lungs of Experimentally Infected Pigs[a]

Days PI	Pig #	Trial #	Gross Lesions[b]	Fluorescence detected[c]
7	5909	3	−	−
8	4907	2	−	−
	2408	1	−	−
12	2404	1	+	−
14	4910	2	−	−
	5902	3	−	−
16	2407	1	−	−
20	2402	1	−	−
	4904	2	+	−
21	6001	3	−	−
25	4901	2	+	++++
26	2503	1	+	++++
28	4905	2	+	++
	5905	3	+	−
	6103	3	+	+
35	4911	2	+	++
42	4903	2	+	+
	4908	2	+	−
	5906	3	−	−
	6002	3	+	+++
49	4909	2	+	++

[a] **M. hyopneumoniae** strain 29 grown in cell-free broth medium was used to infect pigs in these tr als.
[b] − = no lesions; + = gross consolidation.
[c] − = no fluorescence; + to ++++ refers to the amount of fluorescent material in bronchioles as well as the number and proportion of affected bronchioles.

strains 31, F3023, and E4737, were stained with an immune porcine globulin conjugate prepared from a pig infected with *M. hyorhinis* strain 31. Pig 3601 infected with *M. hyorhinis* strain 31 had a serofibrinous pleuritis and peritonitis at day seven PI. A layer of bright yellow-green, granular fluorescence was seen in and on the cells of the pleura. This granular fluorescence extended into the lung parenchyma for a short distance. There was no fluorescence in the depth of the lung tissue nor was any seen in or on the epithelium of the bronchioles. A few cells in the bronchial lymph node contained fluorescent granules and a similar though less intense fluorescence was seen on the surface of the spleen, the only abdominal organ examined. Pig 3304 inoculated with *M. hyorhinis* strain F3023, was killed 23 days PI and had early adhesive pericarditis, pleuritis and peritonitis as well as a serous arthritis with synovial proliferation. A small amount of granular fluorescence was seen on the surface of the pleura and in some of the pleural cells and in cells in the bronchial lymph node. There were some fluorescing cells in sections of the synovial membrane from the arthritic hock joint. In the case of pig 3301 inoculated 23 days before with *M. hyorhinis* strain E4737, the lesions of polyserositis were extensive but were mostly adhesive in nature. In this pig fluorescence was seen only on the surface of the synovial membrane from one of the arthritic joints. No fluorescence was seen in tissue sections from these pigs when stained with normal conjugates or with a conjugate prepared against *M. hyopneumoniae*.

No fluorescence was seen in lung sections of four field cases of enzootic pneumonia treated with porcine immune globulin con-

jugates prepared from the serum of pigs experimentally infected with *M. hyorhinis* strain 31 even though this organism was later recovered from these tissues by broth culture. None of these pigs had lesions of polyserositis at necropsy.

DISCUSSION

Direct immunofluorescent staining would appear, on the basis of the studies reported herein, to provide a rapid, reasonably simple and specific method for the diagnosis of EP in tissue sections. By specifically allowing the visualization of the causative agent *M. hyopneumoniae,* immunofluorescent staining could confirm at necropsy the serological diagnosis established by the complement-fixation test (2, 14, 16) and possibly also by the growth or the metabolic inhibition test (6). Our experience (9) and that of Goodwin *et al* (7) has been that recovery of *M. hyopneumoniae* was difficult, unreliable and time consuming even when attempted from pigs experimentally infected with EP. In addition one is faced with the problem of identifying the species of the isolate upon recovery of the mycoplasma. Immunofluorescent staining appears to provide a simpler and more reliable technique than isolation for identifying *M. hyopneumoniae* in pneumonic pig lungs. It would appear that this method is sensitive for the detection of *M. hyopneumoniae* in established cases of EP but is not reliable during the early stages of the disease. The absence of fluorescence early in the disease may 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; or (3) to washing out of the antigen from the epithelium of the air passages during the staining procedure. This washing effect may be more pronounced at this stage due to the absence of the plug of bronchiolar exudate which often fills the air passages at later stages.

The species identification of mycoplasma isolates has been a problem because morphology and growth requirements and characteristics were the only criteria available. Immunofluorescent staining of smears from broth cultures has proven to be very specific in our hands and can be used early in the isolation procedure without it being necessary to thoroughly adapt the isolate to artificial medium. Although we have used the technique only on smears from broth culture it would probably work equally well on colonies from solid medium as in the case for other mycoplasma (3, 17).

The reliability of the staining reactions obtained with *M. hyopneumoniae* infected lungs would appear to be assured by: (1) the presence of intense apparently specifically located fluorescence in infected lungs stained with homologous, immune, porcine conjugates; (2) the species specificity of our various porcine and rabbit immune conjugates when applied to smears of broth cultures of *M. hyopneumoniae, M. hyorhinis* or *M. granularum;* (3) the absence of such fluorescence in sections of infected lungs stained with normal conjugates or with immune conjugates for *M. hyorhinis;* (4) the absence of fluorescence in sections from normal control lungs stained with porcine immune or normal globulin conjugates.

Rabbit globulin conjugates were less satisfactory when used to stain tissue sections since they tended to produce a faint, nonspecific fluorescence. In view of the potential development of antibodies in animals against medium components in mycoplasma antigens used in their immunization (17), we feel that it is preferable to use serum from experimentally infected pigs rather than the serum of hyperimmunized pigs or rabbits. The heterologous cross reactivity shown by rabbit antisera in the modified direct complement-fixation test (2) was not present when these sera were used in the immunofluorescence test.

The localization, as determined by immunofluorescent staining, of *M. hyopneumoniae* on the surface of the epithelium of the bronchi and bronchioles of infected pig lungs is similar to that reported (10) for the human pathogen *Mycoplasma pneumoniae* in the chick embryo and (4) the hampster and for the mouse pathogen (13) *Mycoplasma pulmonis* in gnotobiotic mice. This localization is also in agreement with the finding (15) that in thin sections of infected pig lungs, *M. hyopneumoniae* tends to occur as microcolonies on the epithelium of the small bronchioles.

For the detection of *M. hyorhinis* it would appear that the direct immunofluorescent technique is specific and sensitive but will be useful only during the acute and early convalescent stages of polyserositis. It may be that during resolution of the lesions the organism had been eliminated or was in too small numbers to be detected by immunofluorescent staining. *M. hyorhinis*

did seem to persist for longer periods in the arthritic joints but the amount of fluorescence in sections of the synovial membrane was limited. This technique may prove useful for the rapid and specific differential diagnosis of serositis caused by *M. hyorhinis* and by *Hemophilus suis* (Glasser's disease). Isolation of *M. hyorhinis* and *H. suis* in culture requires specialized techniques and media, which for the latter organism is unreliable when attempted from field specimens (12).

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