Evaluation of Criteria for the Postmortem Diagnosis of Mycoplasmal Pneumonia of Swine

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ABSTRACT

Ten swine from each of five herds believed to be affected with mycoplasmal pneumonia of swine and ten swine from each of five herds believed to be mycoplasmal pneumonia-free were selected for postmortem study. Lungs from the 100 swine were examined; grossly and microscopically for lesions typical of mycoplasmal pneumonia of swine and culturally and by an indirect immunofluorescent procedure for the presence of *Mycoplasma hyopneumoniae*.

Nineteen of the lungs had both gross and microscopic lesions typical of mycoplasmal pneumonia of swine and 13 (68%) of these were infected, i.e. were culturally and/or indirect immunofluorescent positive. Absence of gross lesions did not prove freedom from mycoplasmal pneumonia, 14 of 73 (19%) grossly normal lungs were found to be infected with *M. hyopneumoniae*.

Comparison of the indirect immunofluorescent and cultural examination, as methods of diagnosing mycoplasma pneumonia, revealed that neither procedure alone was reliable in the case of negative results. Ten lungs were indirect immunofluorescent negative and culturally positive and seven were culturally negative and indirect immunofluorescent positive (11 lungs were positive by both procedures).

It was concluded that a definitive diagnosis of mycoplasmal pneumonia of swine requires that *M. hyopneumoniae* be visualized in indirect immunofluorescent stained lung sections or that it be recovered culturally.

Key words: *Mycoplasma hyopneumoniae, Mycoplasma flocculare*, mycoplasmal pneumonia of swine, enzootic pig pneumonia, specific pathogen free swine.

RÉSUMÉ

Cette expérience portait sur 50 porcs choisis dans cinq troupeaux apparemment aux prises avec la pneumonie à mycoplasme et sur 50 autres qui provenaient de cinq troupeaux exempts de cette maladie. Elle consistait à rechercher dans les poumons de tous ces porcs des lésions macroscopiques ou histologiques typiques de la pneumonie à mycoplasme et à les soumettre à la culture, ainsi qu'à un examen par l'immunofluorescence indirecte, dans le but de vérifier s'ils recelaient *Mycoplasma hyopneumoniae*.

Les poumons de 19 porcs présentaient des lésions macroscopiques et histologiques typiques de la pneumonie à mycoplasme, mais ceux de seulement 13, i.e. 68% de ces porcs, se révélèrent infectés, parce qu'ils donnèrent des résultats positifs à la culture et/ou à l'examen par l'immunofluorescence indirecte. L'absence de lésions macroscopiques ne correspondait toutefois pas à l'absence de la maladie, puisque les poumons apparemment normaux de 14 porcs, i.e. 19% d'un total de 73, se révélèrent infectés par *M. hyopneumoniae*.

Une comparaison entre la culture et l'immunofluorescence indirecte, comme méthodes de diagnostic de la pneumonie à mycoplasme, révéla que, prises individuellement, ni l'une ni l'autre ne saurait être fiable, lorsqu'elles donnent des résultats négatifs. En effet, les poumons de dix porcs s'avérèrent négatifs par l'immunofluorescence indirecte et positifs à la culture, tandis que ceux de sept autres donnèrent des résultats contraires et que ceux de 11 autres porcs s'avérèrent positifs par les deux méthodes précitées.

Les auteurs conclurent par conséquent qu'un diagnostic définitif de pneumonie porcine à mycoplasme repose sur la visualisation de *M. hyopneumoniae* dans les coupes de tissu pulmonaire, examinées par l'immunofluorescence indirecte, ou sur l'isolement du mycoplasme, en culture.

Mots clés: *Mycoplasma hyopneumoniae, Mycoplasma flocculare*, pneumonie porcine à mycoplasme, pneumonie enzootique porcine, porcs exempts d'agents pathogènes spécifiques.

INTRODUCTION

An accurate method of diagnosing mycoplasmal pneumonia of swine (MPS) is essential to the success of a disease control scheme such as the specific pathogen free (SPF) swine program. While several serological tests could theoretically satisfy this need, none in the present state of development is entirely reliable due to lack of sensitivity and/or specificity. Consequently, MPS is diagnosed primarily on postmortem evidence of infection, i.e. on the presence of typical gross and microscopic lesions, on the visualization of *Mycoplasma hyopneumoniae* in immunofluorescent stained lung sections and/or on cultural recovery of *M. hyopneumoniae* from lung tissue. The reliability of these methods of diagnosing MPS was investigated. The results are reported and their

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practical application in monitoring SPF swine herds for freedom from MPS is discussed.

MATERIALS AND METHODS

The study was initiated by identifying five herds (designated A, B, C, D and E) believed to be affected with MPS (referred to hereafter as MPS-positive herds) and five herds (designated V, W, X, Y and Z) believed to be MPS-free (referred to hereafter as MPS-negative herds). Owner participation was obtained with the understanding that all data acquired would be recorded in a coded form and that results of the study would not affect the herd's SPF accreditation status.

Herds B and C were SPF accredited at the onset of the study. Herd E was a former SPF herd that had lost accreditation due to atrophic rhinitis (AR). These three herds were placed in the MPS-positive category because previous indirect immunofluorescent (IIF) and/or cultural studies had proven that they were infected with *M. hyopneumoniae*. The remaining two herds, A and D, were former SPF herds that had lost accreditation due to MPS.

Three of the five MPS-negative herds (V, Y and Z) were SPF accredited. Herd W had lost accreditation due to AR. These four herds were placed in the negative category because there was no previous IIF and/or cultural evidence of *M. hyopneumoniae* infection. The fifth herd (X) was not associated with the SPF organization. It recently had been established with swine believed to be MPS-free. It was included in the study because a fifth SPF herd of suitable size whose owner was willing to participate in the study could not be located.

Ten swine from each herd (five swine three and a half to five months of age and five swine six to seven months of age) were selected for postmortem studies, i.e. for pathological, cultural and immunofluorescent examination.

POSTMORTEM STUDIES

Gross Examination — Lungs were examined grossly for lesions typical of MPS (1). At the completion of the gross examination specimens were collected sequentially for cultural, IIF and histological studies.

Cultural Examination — Areas to be sampled were seared lightly with a propane torch to destroy surface contaminants. A block of tissue, approximately 1 cm^3, was collected from the advancing edge of the lesion in each of the four anterior lobes of the lung. In the absence of lesions, tissue was collected from the ventral margin of the lobe. The tissues from the four lobes were combined, macerated and cultured for mycoplasmas as described by Armstrong and Friis (2) using Friis' broth supplemented with 0.5 mg/mL of cycloserine and 5% anti-*Mycoplasma hyorhinis* serum to suppress the growth of *M. hyorhinis* (3).

Indirect Immunofluorescent Examination — A block of tissue approximately 1.5 cm^3 was collected from each of the four anterior lobes adjacent to the specimens harvested for cultural studies. Each block of tissue was sectioned, stained and examined as described by Armstrong *et al* (4).

Histopathological Examination — A block of tissue about 1 cm^3 was collected from each of the four anterior lobes adjacent to the specimen collected for immunofluorescent studies. Hematoxylin and eosin stained sections were prepared and examined microscopically for lesions typical of MPS (1).

RESULTS

GROSS AND MICROSCOPIC LESIONS

Results of examining the lungs for gross and microscopic lesions are summarized in Table I. Thirty-two percent of the MPS-positive swine had both gross and microscopic lesions and 48% had neither (overall agreement of 80%). In the disagreement category, 10% of the lungs were grossly positive and microscopically negative and 10% were microscopically positive and grossly negative.

Fewer lesions were observed in the lungs from the MPS-negative swine. Six percent of these swine had both gross and microscopic lesions and 72% had neither (overall agreement of 78%). In the disagreement category, 6% of the lungs were grossly positive and microscopically negative and 16% were microscopically positive and grossly negative.

There was no significant difference in lesion status of the three and a half to five month and the six to seven month old swine in either the MPS-positive or the MPS-negative group.

CULTURAL AND IMMUNOFLUORESCENT STUDIES

Results of cultural examinations are summarized in Table II. All of the MPS-positive herds and one herd in the MPS-negative category were infected with *M. hyopneumoniae*. There was no significant difference in the rate of infection between the three and a half to five month and the six to seven month age group.

Cultural studies also revealed that six herds were infected with *Mycoplasma flocculare* and four herds were infected with *M. hyorhinis*. The latter figure is regarded as being artificially

TABLE I. Results of Examining 100 Lungs for Gross and Microscopic Lesions Typical of Mycoplasmal Pneumonia of Swine (MPS)

	MPS Lesion Status			
Herd[a]	Grossly Pos Micro Pos	Grossly Pos Micro Neg	Grossly Neg Micro Pos	Grossly Neg Micro Neg
A	7	0	2	1
B	1	3	0	6
C	1	1	3	5
D	1	1	0	8
E	6	0	0	4
Total	16	5	5	24
V	0	0	2	8
W	3	1	6	0
X	0	2	0	8
Y	0	0	0	10
Z	0	0	0	10
Total	3	3	8	36

[a]Herds A through E = MPS-positive and herds V through Z = MPS-negative

TABLE II. Results of Examining 100 Lungs Culturally for *M. hyopneumoniae, M. flocculare* and *M. hyorhinis*

Herd[a]	Number of Lungs Yielding		
	M. hyopneumoniae	*M. flocculare*	*M. hyorhinis*
A	4	0	1
B	2	4	1
C	4	2	2
D	2	3	0
E	7	0	0
Total	19	9	4
V	0	1	0
W	2	1	0
X	0	0	0
Y	0	0	0
Z	0	2	1
Total	2	4	1

[a]Herds A through E = MPS-positive and herds V through Z = MPS-negative

low inasmuch as a culture medium inhibitory to *M. hyorhinis* was used in the isolation studies.

The reliability of diagnosing MPS by culture versus IIF was compared (Table III). Twenty-eight of the 100 lungs were culturally and/or IIF positive. Eleven were positive by both procedures, ten were culturally positive and IIF negative and seven were IIF positive and culturally negative. Eight of the ten culturally positive-IIF negative lungs were devoid of gross lesions.

CORRELATION OF LESIONS WITH *M. HYOPNEUMONIAE* INFECTION

The relationship between lung lesions and *M. hyopneumoniae* infection is summarized in Table IV. Nineteen of the 100 lungs examined had both gross and microscopic lesions. Thirteen (68%) of these were infected, i.e. were IIF and/or culturally positive for *M. hyopneumoniae*. A lower percentage of lungs was proven to be infected when gross or microscopic lesions alone were present. There was no significant difference in the infection rate for the two age groups.

DISCUSSION

The presence of typical gross and microscopic lesions was a good but not an absolute indicator of *M. hyopneumoniae* infection; 68% of such lungs were IIF and/or culturally positive. About one-third of the lungs with typical gross and microscopic lesions were IIF and culturally negative. This finding indicates that the gross and microscopic lesions associated with MPS are not entirely specific; an observation reported by others (1,5).

TABLE III. Results of Examining 100 Lungs for *M. hyopneumoniae* by Culture and by Indirect Immunofluorescence (IIF)

Herd[a]	*M. hyopneumoniae* Status			
	Cult Pos IIF Pos	Cult Pos IIF Neg	Cult Neg IIF Pos	Cult Neg IIF Neg
A	3	1	4	2
B	0	2	2	6
C	2	2	0	6
D	0	2	0	8
E	4	3	1	2
Total	9	10	7	24
V	0	0	0	10
W	2	0	0	8
X	0	0	0	10
Y	0	0	0	10
Z	0	0	0	10
Total	2	0	0	48

[a]Herds A through E = MPS-positive and herds V through Z = MPS-negative

However, failure to confirm infection in lungs with typical gross and microscopic lesions may have been due, in some cases, to lack of sensitivity of the IIF and cultural procedures.

Comparison of the efficacy of the IIF and the cultural examination as methods of diagnosing MPS revealed that neither test alone was reliable in the case of negative results, i.e. some IIF negative lungs were culturally positive and some culturally negative lungs were IIF positive. Gois *et al* (6) and Friis and Meyling (7) have reported similar observations. Our data also indicated that the cultural examination may be more sensitive than IIF in the absence of gross lesions; eight of ten IIF negative and culturally positive lungs were devoid of gross lesions. This apparent difference in sensitivity probably is due to sampling error, i.e. to the relatively small area of the lung examined by the IIF procedure as opposed to a much larger portion examined culturally.

It was interesting to note that *M. flocculare* was recovered from six of ten herds, i.e. from three MPS-positive and three MPS-negative herds. *Mycoplasma flocculare* is seemingly nonpathogenic (8). However, its presence in accredited SPF herds is disconcerting for two reasons: 1) the mechanism whereby *M. flocculare* gains access to a herd could also permit the entry of pathogenic mycoplasmas (including *M. hyopneumoniae*) and 2) *M. flocculare* induces antibodies that cross-react with *M. hyopneumoniae* antigen in serodiagnostic tests (9).

The National SPF Swine Accrediting Agency relies primarily on slaughter-inspection to detect MPS in member herds. The absence of gross lesions typical of MPS at slaughter is interpreted as indicating freedom from *M. hyopneumoniae* infection, i.e. lungs are not examined further and the herd maintains its SPF accreditation. Results of the present study indicated that this concept is invalid. Fourteen of 73 grossly normal lungs (19%) were found to be infected with *M. hyopneumoniae*. It is clear that false negative diagnoses can occur when gross lesions are used as the sole criterion of MPS. McKean *et al* recognized the fallacy of ruling-out MPS on the basis of grossly normal lungs (10). They

TABLE IV. Correlation of Lesions with *M. hyopneumoniae* Infection

Lesion Status		No. Infected[a]		No. Noninfected[b]	
Gross	Microscopic	Young	Old	Young	Old
MPS-Positive Herds					
Pos	Pos	7	6	0	3
Pos	Neg	1	1	1	2
Neg	Pos	2	2	1	0
Neg	Neg	4	4	9	7
MPS-Negative Herds					
Pos	Pos	0	0	0	3
Pos	Neg	0	0	0	3
Neg	Pos	2	0	4	2
Neg	Neg	0	0	19	17
Combined Date from MPS-Positive and Negative Herds					
		No. Infected		No. Noninfected	% Infected
Pos	Pos	13		6	68.42
Pos	Neg	2		6	25.00
Neg	Pos	6		7	46.15
Neg	Neg	8		52	13.34

[a]Infected = number of lungs IIF and/or culturally positive for *M. hyopneumoniae*
[b]Noninfected = lungs IIF and culturally negative for *M. hyopneumoniae*

indicated that lesion resolution and small sample size could result in false-negative diagnoses and suggested that a minimum sample for slaughter inspection be ten swine.

Guidelines of the SPF organization specify that herds that fail slaughter-inspection due to MPS type lesions be placed in an "On-Hold" or "Not Passed" category. These herds cannot sell stock as being SPF accredited while in this status. However, accreditation is reinstated if two subsequent slaughter inspections yield negative results, i.e. if all lungs inspected are grossly normal. Data presented herein indicate that this is an unsound practice; it provides the potential for a fully accredited herd to sell *M. hyopneumoniae* infected stock to other SPF herds. This could have happened with herds B and C. Both of these herds had previously failed slaughter inspections, had subsequently been reinstated in the program and were proven to be infected with *M. hyopneumoniae* during the course of the study.

It seems clear that the methods currently used by the SPF organization to detect MPS in members herds are inadequate. Date reported herein indicate that all lungs examined at slaughter inspection should be subjected to laboratory study, i.e. to IIF (or direct immunofluorescent) examination and in the event of negative immunofluorescent results, to cultural examination. The immunofluorescent examination is specific (11,12,13,14) so positive results are reliable. However, as reported in this paper and elsewhere (6,7) false-negative results sometimes occur. In such cases the cultural examination should be completed before a negative report is rendered.

This study emphasizes the need for a reliable, inexpensive serodiagnostic test that could be applied on a herd basis. The ELISA is extremely sensitive and therefore promising for this purpose (15,16). However, the ELISA in its present state of development is not entirely specific (9,17). Efforts to enhance the specificity of the assay are in progress in our laboratories and elsewhere.

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