Microorganisms Associated with Pneumonia in Slaughter Weight Swine

R.B. Morrison, C. Pijoan, H.D. Hilley and V. Rapp*

ABSTRACT

The lungs of 334 pigs were obtained from two slaughter plants in Minnesota and examined in detail. Macroscopic and microscopic evaluation, direct fluorescence for *Mycoplasma hyopneumoniae* and bacterial culture were done on all of them and a subsample of 50 were selected for virus culture.

***Mycoplasma hyopneumoniae, Pasteurella multocida* and *Haemophilus* spp. were detected in 24.0%, 34.1% and 27.0% of the lungs, commonly in conjunction with each other. One isolate of *Haemophilus pleuropneumoniae* serotype 2 was detected and this represents the first report of its presence in the United States. No virus was detected in any of the lungs.**

Lungs with both *M. hyopneumoniae* and *Pasteurella multocida* had the greatest amount of macroscopic pneumonia (9.8% of the lung). Lungs with *M. hyopneumoniae* or *P. multocida* alone had 4.9% and 5.2% of the lung involved with pneumonia respectively. Lungs with *Haemophilus* sp. Taxon "minor group" had 3.8% of the lung involved which was not significantly different from lungs with none of these organisms being detected (1.6%). There was a positive correlation between the extent of *M. hyopneumoniae* infection, as scored by FAT and the amount of macroscopic pneumonia present ($r = 0.46$; $P < 0.001$). Likewise, there was a positive correlation between the estimated concentration of *P. multocida* present, as scored by the relative number of colonies on blood agar and the amount of macroscopic pneumonia present ($r = 0.60$; $P < 0.001$).

Microscopically, the amount of lymphoreticular proliferation, polymorphonuclear cells and alveolar macrophages were evaluated. Lungs with no isolations had the lowest scores of all three components and lungs with *M. hyopneumoniae* combined with *P. multocida* had the highest. *Haemophilus* sp. Taxon "minor group" was associated with significantly more lymphoreticular proliferation and alveolar macrophages than sections with no isolations.

Key words: *Haemophilus, Mycoplasma hyopneumoniae, Pasteurella multocida*, pneumonia, swine.

RÉSUMÉ

Cette expérience consistait à effectuer une étude détaillée des poumons de 334 porcs envoyés à deux abattoirs du Minnesota. On utilisa à cette fin l'examen macroscopique, l'histopathologie, la recherche de *Mycoplasma hyopneumoniae*, par l'immunofluorescence directe et la culture, ainsi que l'examen virologique de 50 de ces organes.

L'examen bactériologique se solda par l'isolement de *M. hyopneumoniae, Pasteurella multocida* et *Haemophilus* spp., dans respectivement 24%, 34,1% et 27% des poumons; il révéla aussi que plus d'un de ces microorganismes se retrouvaient ensemble dans un bon nombre d'échantillons. L'isolement d'une souche du sérotype #2 d'*Haemophilus pleuropneumoniae* constitue le premier rapport de sa présence aux États-Unis. La virologie s'avéra constamment négative.

Les poumons desquels on isola *M. hyopneumoniae* et *P. multocida*, représentaient 9,8% des échantillons et affichaient le plus de lésions macroscopiques. Ceux desquels on isola seulement *M. hyopneumoniae* ou *P. multocida* arboraient des lésions de pneumonie dans respectivement 4,9% et 5,2% de leur parenchyme. Ceux desquels on isola *Haemophilus* sp. Taxon du groupe mineur présentaient des lésions de pneumonie dans 3,8% de leur parenchyme, lequel ne différait pas sensiblement de celui du 1,6% des poumons dont la bactériologie se révéla négative. On enregistra une corrélation positive entre la gravité de l'infection par *M. hyopneumoniae,* telle que la démontra l'immunofluorescence, et l'ampleur des lésions macroscopiques ($r = 0,46$; $P < 0,001$). On constata aussi une corrélation positive entre la concentration approximative de *P. multocida*, telle que la révéla le nombre relatif de colonies sur la gélose au sang, et l'ampleur des lésions macroscopiques ($r = 0,6$; $P < 0,001$).

L'histopathologie servit à évaluer l'intensité de la prolifération des cellules réticulo-endothéliales, des neutrophiles et des macrophages alvéolaires. Les poumons dont l'examen bactériologique donna des résultats négatifs affichèrent les plus faibles valeurs relatives à ces trois facteurs, contrairement à ceux desquels on isola simultanément *M. hyopneumoniae* et *P. multocida*. Les coupes histologiques des poumons qui recelaient *Haemophilus* sp. Taxon du groupe mineur révélèrent une plus grande prolifération de cellules réticulo-endothéliales et de macro-

*Department of Large Animal Clinical Studies, University of Minnesota, St. Paul, Minnesota 55108 (Morrison and Pijoan), Department of Food Animal and Equine Medicine, North Carolina State University, Raleigh, North Carolina 27606 (Hilley) and Veterinary Medical Research Institute, Iowa State University, Ames, Iowa 50011 (Rapp).

Reprint requests to C. Pijoan.

Part of a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D degree in Large Animal Clinical Sciences, University of Minnesota.

Submitted January 24, 1984.

phages alvéolaires que celles des poumons qui n'hébergaient pas de microorganismes.

Mots clés: *Haemophilus* spp., *Mycoplasma hyopneumoniae, Pasteurella multocida*, pneumonie, porcs.

INTRODUCTION

Etiological investigations have been previously conducted on swine lungs at slaughter (1-10). Osborne *et al* (4) recently reported the detailed microbiological findings from 347 pneumonic lungs and this work exemplified the myriad of organisms that can be detected in respiratory disease of swine as found at slaughter. Smith (11) analyzed comprehensive pathological and microbiological records collected from 109 pigs at slaughter and concluded that of the organisms detected, *Mycoplasma hyopneumoniae* and *Pasteurella multocida* were the most highly associated organisms with the development of lung lesions. Gois *et al* (2) also concluded that *P. multocida* was positively associated with pneumonic lesions but found no such association with *M. hyopneumoniae*. The role of *Haemophilus pleuropneumoniae* in respiratory disease has been demonstrated (1,7,9,12,13) but that of *Haemophilus* sp. Taxon "minor group" is unreported, despite the fact that it may be very common (14).

It is difficult to compare the reported prevalence or extent of pneumonia between studies since techniques for scoring pneumonia have varied greatly. Aalund *et al* (15) and Willeberg *et al* (7) have reported the prevalence of pneumonia as a percentage of pigs affected. Backstrom *et al* (16) and Flesja *et al* (17) reported the prevalence by grading lungs as being mild, moderate or severe. This system is similar to that employed by Gois *et al* (2) and Osborne *et al* (4) who used a four point scale for severity. Goodwin (18) used a system whereby if all the anterior lobes, the accessory lobe and the anterior edge of the diaphragmatic lobes were pneumonic, a maximum score of 55 was allocated. Jericho *et al* (19) categorized lungs as: no externally visible consolidation, 0-5%, 5-10% and greater than 10% consolidation. Straw *et al* (20) estimated the percentage of each lobe involved but did not define how the total percent of lung involvement was determined.

Histology has been used to evaluate the cell mediated immune response in the lung and the predominant cells involved are macrophages, lymphocytes and polymorphonuclear cells (21-25). Roberts *et al* (6) described the histopathology associated with *P. multocida* and *Mycoplasma hyorhinis* in field cases of pneumonic swine lungs collected at slaughter. Jericho (26) summarized the potential problems in extrapolating histopathological experimental observations to field studies.

This investigation concentrated on the macroscopic and microscopic lesions associated with *M. hyopneumoniae, P. multocida* and *Haemophilus* species. An objective, detailed, macroscopic scoring technique was devised that allowed comparisons within this study's findings and between other future investigations that may employ the same technique. Histopathological evaluation was done to investigate whether microscopic evaluation of lungs collected at slaughter is an informative procedure.

MATERIALS AND METHODS

Examinations were conducted on 334 porcine lungs obtained at two local slaughter plants. The lungs were collected at the slaughter line without regard for the presence or absence of lesions. Following macroscopic evaluation, sections adjacent to each other were taken for virology, bacteriology, histology and fluorescent antibody testing (FAT). These sections were taken at the edge of a gross lesion or from the center of the right middle lobe if no lesions were visible.

MACROSCOPIC EVALUATION

In order to accurately assess the percent of lung involved with pneumonia, 11 lungs without any gross lesions were bluntly dissected along interlobar fissures. All lobes were individually weighed and mean values determined for the relative amount of lung that each lobe contributed.

All 334 lungs were scored by the same individual based on a subjective evaluation of the percent of the surface area of each lobe involved in the pneumonic process. These percentages were then multiplied by each lobe's relative weight in the normal lung and summed to equal the total percent of the surface area of each lung affected by macroscopic pneumonia. No record of color, consistency, or depth of the lesion was made. Lungs were also scored for the presence or absence of pleural adhesions.

MICROSCOPIC EVALUATION

Tissue samples were fixed in phosphate buffered 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin by routine procedures. One evaluator subjectively scored all specimens using modified previously discussed methods (6,21,23,25-28).

Lymphoreticular proliferation, polymorphonuclear cells, and alveolar macrophages were scored 0, 1, 2, or 3, depending on their relative abundance in each section. The lymphoid response was graded as follows:

0 — No lymphoreticular nodules in the submucosa of bronchi, bronchioles, or vessels.

1 — One or more lymphoreticular nodules in the submucosa of bronchi, bronchioles, or vessels, but not appearing to penetrate the muscularis mucosa.

2 — One or more lymphoreticular nodules extending through the muscularis mucosa and/or partially compressing the bronchus or bronchiole.

3 — Extensive perivascular and peribronchiolar lymphoreticular proliferation, which may completely occlude airways.

Polymorphonuclear cells and alveolar macrophages were graded 0, 1, 2, or 3 which corresponded respectively to none visible, few, moderate and very plentiful.

VIROLOGICAL EXAMINATION

Standard virological techniques were conducted on 50 randomly selected lungs from the sample of 334. Samples were ground up, diluted to 5-10X in PBS, sonicated and centrifuged. The supernatant was inoculated into primary porcine kidney (PPK) cell monolayers and incubated at 37°C. The culture was observed daily for gross cytopathic effects (CPE) for seven days. Cell culture with no CPE was then frozen and thawed three

times and passaged once to PPK cell monolayer and observed for another seven days.

BACTERIOLOGICAL EXAMINATION

After scorching the lung surface with a hot spatula, adjacent samples of approximately 1 cm^3 were collected from the edge of a macroscopic pneumonic lesion. A dilution technique with antibacterials added to the media, as described by Pijoan *et al* (14), was used to enhance the isolation of *Haemophilus* sp. Isolation of other bacteria was achieved by smearing the cut surface of lung directly onto 5% sheep blood agar and streaking for isolation. All broths and plates were incubated aerobically at 37° C overnight.

Colonies suspected to be *Pasteurella* species were visually identified according to de Jong *et al* (29) and their relative abundance was scored 1, 2, or 3 by one evaluator. Individual colonies were selected, confirmed as being *P. multocida* and capsular and somatic serotypes were identified, following previously described methods (30). Special effort was made to differentiate the primary somatic antigen, characterized by the heaviest precipitation line, from the secondary antigens (31).

For *Haemophilus* isolation, following overnight incubation, dilution broths were plated onto sheep blood agar and a nurse colony of *Staphylococcus epidermidis* was streaked at a right angle and the plate was incubated under the same conditions. Colonies showing satellitism were recorded and a sample of 45 strains were characterized further (14).

Selected urease positive strains were serotyped by rapid slide agglutination (RSA) (32) and indirect fluorescent antibody tests (33). For the agglutination test, hyperimmune antisera to the reference strains of *H. pleuropneumoniae* serotypes 1-5 was produced in rabbits. Two subcutaneous inoculations were given 14 days apart followed in 14 days by a series of three intravenous injections at two day intervals. The dose for each inoculation was the equivalent of 4 x 10^9 cfu of a five to seven hour culture grown on PPLO agar (34) and inactivated with 0.3% formaldehyde solution. The RSA test was conducted by mixing equal volumes of culture and antisera and agglutination was scored within five minutes. For the indirect fluorescent antibody (IFA) test, formalin fixed culture smears were reacted with specific antiserum, followed by fluorescein-isothiocyanate conjugated goat antirabbit IgG (Miles Laboratory, Elkhart, Indiana). Slides were counterstained with chelated Eriochrome black T dye for 30 seconds and examined at 1000 x magnification using an AO fluorescence microscope. Hyperimmune rabbit antisera to *H. pleuropneumoniae* serotypes 1-5 and *Haemophilus* sp. Taxon "minor group" used for IFA was supplied by Soren Rosendal, Ontario Veterinary College, Guelph, Ontario.

DIRECT IMMUNOFLUORESCENCE

A section of lung approximately 1 cm^3, including a bronchus was taken and direct immunofluorescence for *Mycoplasma hyopneumoniae* was performed as described by Amanfu *et al* (35).

All slides were read by one individual and the intensity and location of fluorescence was scored as proposed by Piffer (27):

0 No significant fluorescence.

1^+ Scattered fluorescent particles lining bronchi but no fluorescence in bronchioli.

2^+ Thin coat of fluorescence lining bronchi but no fluorescence in bronchioli.

3^+ Thin coat of fluorescence lining bronchi and scattered fluorescent granules lining bronchioli.

4^+ Continuous thin coat of fluorescence lining bronchi and bronchioli.

Fluorescein-conjugated porcine IgG antibody against *M. hyopneumoniae*, negative and 4^+ control slides were kindly provided by R.F. Ross, V.M.R.I., Iowa State University, Ames, Iowa.

STATISTICAL ANALYSES

Standard microcomputer software (Microstat by Microsoft Inc. Indianapolis, Indiana) was used to tabulate data and perform descriptive statistics, frequency distributions and regression and correlation analyses.

RESULTS

MACROSCOPIC FINDINGS

The percentages of total lung (and standard errors of means) contributed by the right apical, right middle, right caudal, accessory, left apical, left middle and left caudal lobes were 11.9 (0.5), 7.5 (0.2), 30.0 (0.7), 4.6 (0.2), 7.1 (0.3), 6.9 (0.4) and 31.6% (0.6) respectively. These values were used in calculating the percent of pneumonia scores for each lung.

Two main types of pneumonic lesions were noted. The most common was anteroventral in its distribution and purple to grey in color. Affected areas felt firm and appeared atelectic. These lesions have been described as being typical of enzootic pneumonia in swine and have been usually associated with *M. hyopneumoniae* and/or *P. multocida* (36).

The other characteristic lesion observed in 1.8% of the lungs, consisted of firm, well circumscribed, reddish-black foci ranging in size from 1 cm^3 to 5 cm^3. This type of lesion has been described as being typical of *H. pleuropneumoniae* infection (13). Four lungs had lesions in the right caudal lobe only, one had lesions in the right caudal and left caudal lobes and one lung had lesions in the right caudal, right middle, left caudal and left middle lobes.

The percent of lung macroscopically involved in the pneumonic process ranged from 0 to 34.7% (Fig. 1) and the mean pneumonia score was 3.9% (standard deviation 5.7%). The right middle lobe was the most frequently affected lobe and also had the most involvement. The mean scores from all lobes were positively correlated ($p < 0.001$) with the total score (Table I).

MICROBIOLOGICAL FINDINGS

Mycoplasma hyopneumoniae was detected by FAT in 24.0% of the lungs and it was commonly found in conjunction with *P. multocida* infection. *Pasteurella multocida* was the only significant isolate in 15.9% of the lungs and was found in a total of 34.1% of lungs. Two hundred and twenty-two isolates of *P. multocida* were serotyped, of which 97.1% were capsular type A and 2.9% were capsular type D. Fourteen different somatic serotypes were detected with A:3,5 being the most common (Table II).

Ninety-one *Haemophilus* sp. were isolated with one lung yielding two

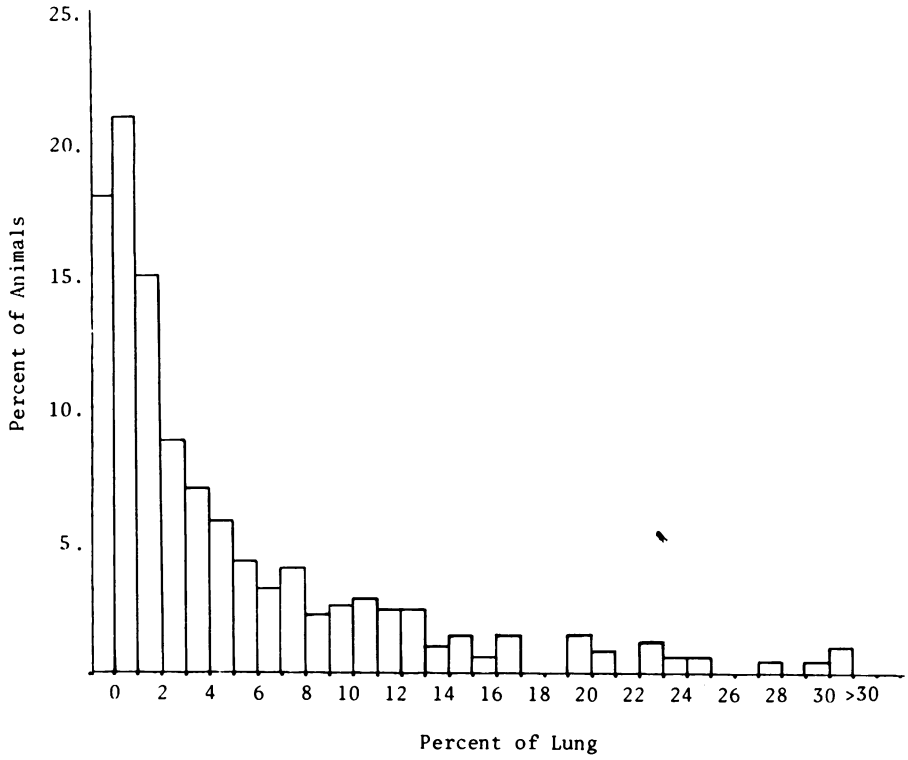

Fig. 1. Frequency distribution of pneumonia scores.

TABLE I. Distribution Frequency and Extent of Pneumonic Lesions in 334 Swine Lungs Collected at Slaughter

Lobe	% Frequency of Involvement	% Mean Score (Standard Error)	Correlation With Total % Score ($p < 0.001$)
Right cranial	44.3	8.2 (0.8)	0.912
Right middle	67.7	14.7 (0.8)	0.773
Accessory	38.3	8.6 (0.5)	0.804
Right caudal	26.0	1.0 (0.1)	0.836
Left cranial	27.2	3.0 (0.2)	0.811
Left middle	62.0	10.4 (0.6)	0.842
Left caudal	23.7	0.9 (0.1)	0.810

TABLE II. Amount of Pneumonia Associated with Serotypes of *P. multocida* in Slaughter Weight Swine

Serotype	Number of Isolates	Pneumonia (Mean % of Lung)	(Standard Error)
A:3,5	15	4.7	1.5
A:3,4,5,12	10	5.5	1.9
A:3,4,5	6	5.0	3.0
A:3,5,12	9	5.8	2.3
A:3	2	4.4	3.1
A:5	4	3.9	2.4
A:3,4,5,7,12	3	2.8	2.3
A:3,4	1	3.7	—
A:4,5,12	1	0.7	—
A:4,5	1	0.0	—
D:3,4,5,7,12	1	1.5	—

strains. Forty-five isolates were further characterized biochemically. Thirty-nine were identified as *Haemophilus* sp. Taxon "minor group" as they were nonhemolytic on blood agar, negative (or weakly positive) CAMP, urease and ALA test positive, fermented dextrose, lactose and sucrose with the production of acid, and were negative for mannitol and xylose. One isolate was hemolytic, CAMP, urease and ALA positive, lactose negative and fermented dextrose, sucrose, mannitol and xylose with the production of acid and was thus identified as *H. pleuropneumoniae*. Serotyping by RSA and IFA indicated the isolate to be serotype 2. Five isolates were identified as *H. parasuis* on the basis of being nonhemolytic, CAMP negative, ALA positive, urease, lactose, mannitol and xylose negative and producing acid from dextrose and sucrose.

Twenty-nine of the *Haemophilus* sp. Taxon "minor group" isolates were serotyped by RSA using antisera to *H. pleuropneumoniae* serotypes 1-5. Twenty-five isolates showed no reaction, three isolates reacted with serotype 3 antisera and one isolate cross reacted with serotype 3 and 5 antisera. Sixteen of these 29 isolates were serotyped by IFA using antisera to serotypes 1-5 and to strain 202, the reference strain for the Taxon "minor group". Eight isolates reacted with strain 202 antisera, while two isolates reacted with serotype 2 antisera, one isolate reacted with both strain 202 and serotype 2 antisera and five isolates showed no reaction.

No virus was detected in any of the 50 lung samples that were checked.

PATHOLOGICAL FINDINGS

The amounts of pneumonia associated with *M. hyopneumoniae* and *P. multocida* were significantly more than lungs with no isolations but were not significantly different from each other (Table III). There was a significant positive correlation between the *M. hyopneumoniae* score ($r = 0.46$; $p \leqslant 0.001$) and also the *P. multocida* score ($r = 0.60$; $p \leqslant 0.001$) and their respective percentages of pneumonia. The combination of *M hyopneumoniae* and *P. multocida* was associated with approximately twice as much pneumonia as with either of these organisms alone. The presence of these two organisms accounted for 41% of the variation observed in the amount of pneumonia in these slaughter weight pigs ($r = 0.64$; $p \leqslant 0.001$).

TABLE III. Macroscopic and Microscopic Observations in Swine Lungs Collected at Slaughter

		no isolation	*Mycoplasma hyopneumoniae*	*Pasteurella multocida*	*M. hyopneumoniae* + *P. multocida*	*Haemophilus* sp. Taxon "minor group"
Macroscopic findings						
Number of lungs		124	22	44	39	15
Mean percent pneumonia (± s.e.)		1.6(0.2)	4.9(1.1)[a]	5.2(0.9)[a]	9.8(1.5)[b]	3.8(1.4)
Percent pleuritic lungs		22.0	36.3[a]	31.8[a]	33.3[a]	47.0[a]
Microscopic findings						
Number of lungs		124	22	42	38	15
Lymphoreticular	0-1	55	10	10	5	1
	2-3	69	12	32 [a]	33 [a]	14 [a]
Polymorphonuclear	0-1	114	19	29	22	14
	2-3	10	3	13 [a]	16 [ac]	1
Alveolar macrophages	1-0	94	8	17	13	4
	2-3	30	14 [a]	25 [a]	25 [a]	11 [a]

[a] Indicates significant difference from lungs with no isolation ($p \leq 0.05$)
[b] Indicates significant difference from lungs with *M. hyopneumoniae* or *P. multocida* ($p \leq 0.05$)
[c] Indicates significant difference from lungs with *M. hyopneumoniae* ($p \leq 0.05$)

The amount of pneumonia associated with *Haemophilus* sp. Taxon "minor group" (3.8 ± 1.4%) was not significantly more than lungs with no isolations (1.6 ± 0.2%). The lung from which *H. pleuropneumoniae* serotype 2 was isolated had 0.2% of the lung involved with pneumonia but the lesions were more typical of enzootic pneumonia rather than those usually associated with *H. pleuropneumoniae*. *Haemophilus parasuis* was found in combination with *M. hyopneumoniae* and/or *P. multocida* but insufficient numbers were confirmed to present those data.

The mean amounts of pneumonic tissue in lungs where *P. multocida* but not *M. hyopneumoniae* was detected and the corresponding *P. multocida* capsular and somatic serotypes were compared (Table II). There were no significant differences in the amount of pneumonic lung associated with each of the serotypes listed.

MICROSCOPIC FINDINGS

Histological sections of lung were scored on a four point scale for the relative presence of lymphoreticular and polymorphonuclear cells and alveolar macrophages. Significant relationships for each organism were determined and then for clarity, the results were collapsed into two categories for each cell type (Table III). *Haemophilus* sp. Taxon "minor group", *P. multocida*, *M. hyopneumoniae* and *M. hyopneumoniae* and *P. multocida* combined were compared. All other organisms were not included in the data due to insufficient numbers.

Lungs with *M. hyopneumoniae* in combination with *P. multocida* and those with *Haemophilus* sp. Taxon "minor group" were associated with the greatest extent of lymphoreticular proliferation. Sections with *P. multocida* by itself were intermediate, whereas those with *M. hyopneumoniae* and those with no isolations had the least. For the polymorphonuclear score, lungs with *P. multocida* by itself and especially in combination with *M. hyopneumoniae* were associated with the most response. *Haemophilus* sp. Taxon "minor group" and lungs with no isolations had the least and *M. hyopneumoniae* lungs were intermediate. Lungs with no isolations had the least associated alveolar macrophage response and all those lungs with isolates had significantly elevated macrophage scores but these were not significantly different from each other.

DISCUSSION

The method of macroscopically scoring lungs in this study was considerably more accurate than previously reported grading techniques (2,4, 18,19). It allows for estimations to be made on the percent of lung involved with pneumonia and the association with various organisms.

Previous investigations into pneumonia at slaughter have demonstrated that more organisms than just *M. hyopneumoniae* can be isolated from the lesions described as being enzootic pneumonia (36). This investigation concentrated on *M. hyopneumoniae*, *P. multocida* and *Haemophilus* species and found all three to be commonly found alone, or in various combinations. Failure to detect a particular organism did not prove its absence and more effective tests or more samples from the same lung may have increased the detection of the specified organisms.

The prevalence of pneumonia at slaughter reported in other countries has varied from 5.4% to 70% of the animals (17,37). Factors that may account for this great variation include different sampling methods, season of year when the investigation is conducted, environmental and management conditions, age at slaughter and different etiologies present. A major reason may be that the scoring system employed has varied dramatically between studies. The technique used in this investigation was designed to detect the slightest amount of macroscopic pneumonia and even if less than 1% of the lung was involved, it was counted as being pneumonic.

Similarly, a broad range exists in the literature on the prevalence of *M. hyopneumoniae* and *P. multocida* in swine lungs at slaughter. The factors that influence the prevalence of pneumonia, in addition to different microbiological techniques, will also influence the detection of microorganisms.

Since techniques and conditions may vary dramatically between studies, it seems potentially misleading to compare the prevalence of macroscopic lesions or microbiological findings between studies without accounting for differences in materials and methods.

No virus was detected in any of the lungs that were tested. Hog cholera virus, pseudorabies virus, adenovirus, swine influenza virus and possibly enterovirus may be involved in pneumonia in swine (11,38-42). However, whether any of these viruses were involved in the development of pneumonia in these pigs is unknown. Despite the fact that none was detected, one or more viruses may have been present at a younger age and since has been eliminated. Demonstrating infection by testing acute and convalescent sera and then attempting isolation at slaughter may be more informative.

Gram positive cocci were commonly detected in this investigation, but were not found to be associated with an increased amount of pneumonia. No attempt was made to identify these cocci and it is possible that one or more Gram positive bacterial species may have been associated with pneumonia and the effect may have been missed in the analyses.

Immunofluorescent staining for the identification of *Mycoplasma* spp. has been demonstrated to be rapid, reliable and species specific for *M. hyopneumoniae* and *M. hyorhinis* (43).

Detection of *M. hyopneumoniae* was positively associated with the severity of pneumonic lesions. That is, lungs from which it was detected had significantly more pneumonia than lungs with no isolations. This is consistent with experimental studies where *M. hyopneumoniae* was inoculated into pigs and resulted in pneumonic lesions (28,44,45). However, Gois *et al* (2) reported that the detection ratio of *M. hyopneumoniae* was greater in lungs with low lesion scores than high. They concluded that *M. hyopneumoniae* is not a species of marked pathogenic activity. The positive correlation ($r = 0.46$; $p < 0.001$) between the *M. hyopneumoniae* FAT score and the percent macroscopic pneumonia detected in this investigation, supports the experimental findings that *M. hyopneumoniae* alone is capable of causing lung damage. It should be noted that Gois *et al* employed isolation techniques to detect *M. hyopneumoniae* while FAT was used in this investigation. It seems possible that other organisms present in the lungs with high lesion scores, may have decreased Gois *et al*'s isolation of *M. hyopneumoniae* leading to the conclusion that the organism was absent.

When complicated by *P. multocida* infection, the consequent macroscopic pneumonia is significantly increased. This is consistent with experimental studies reported by Gois *et al* and Smith *et al* (2,46) and represents a confirmation of these findings at field level. Similar relationships have been shown to exist in other species with different organisms. Although a synergistic effect has been experimentally demonstrated to exist between *Mycoplasma bovis* and *Pasteurella haemolytica* and between bovine herpesvirus 1 and *P. haemolytica* in calves (47,48), this field study found that the effect between *M. hyopneumoniae* and P. multocida was only additive.

This investigation was purely observational and since pneumonia in swine is a dynamic process involving a complex etiology, some of the results obtained may not be consistent with experimental studies. For example, experimentally it has been proposed that the normal pig lung appears to be capable of efficiently coping with even a massive dose of *P. multocida* resulting in neither clinical signs nor macroscopic lesions (1,49). However, this study found 5.2% ($\pm$ 0.9) pneumonia associated with *P. multocida* alone. It seems possible that these lungs may have been exposed earlier to an immunosuppressive organism which may include *M. hyopneumoniae* and this event then predisposed them to *P. multocida* infection. Alternatively, a concurrent infection with *M. hyopneumoniae* may have been present but was not detected by the FAT.

The isolation of *Haemophilus* sp. Taxon "minor group" in North America was previously reported (14). Kilian *et al* proposed the formation of *Haemophilus* sp. Taxon "minor group" based on four strains that could not be characterized into existing species. This Taxon's distinguishing characteristics are its lack of hemolytic activity on plain blood agar or in the CAMP test (although some strains in this study were weakly CAMP positive), ability to ferment melibiose and soluble starch but not ribose, xylose and mannitol and a relatively low guanine plus cytosine content of DNA (50). At present the prevalence of this organism, and its exact role in the porcine respiratory disease complex are not known. It has been suggested that 17 isolates detected by Little in Great Britain from 120 grossly pneumonic lungs and named *Haemophilus parainfluenza* (12), may in fact be *Haemophilus* sp. Taxon "minor group" (13). Three of these 17 isolates (343,294,349) have been confirmed as "minor group" (50). These 17 isolates should not be confused with an organism isolated from a case of porcine pneumonia and initially called "haemophilus-like organism" by Pattison *et al* in 1957 (51). Unfortunately, this isolate was lost, but Matthews and Pattison isolated a similarly reacting one several years later (52). Based on cultural characteristics, serological reactions and pathogenicity, it was concluded to be the same organism as the "haemophilus-like organism" and was called *H. parainfluenzae*. However, Kilian (50) proposed that these two strains have cultural and biochemical characteristics similar enough to consider them belonging to the *Haemophilus pleuropneumoniae* species.

Biberstein *et al* (53) isolated one strain of *Haemophilus* sp. Taxon "minor group", which was denoted as being serotype 202 and it is this serotype that currently serves as the "minor group" type strain. The reactions observed by RSA of some of the Taxon "minor group" isolate to antisera of *H. pleuropneumoniae* reference serotypes could reflect reactions to common antigens since antisera prepared against inactivated whole cell cultures was used. Both *H. pleuropneumoniae* and Taxon "minor group" are urease positive and, unless complete biochemical characterization is performed, there is the possibility of misidentification.

When serotyping of the Taxon "minor group" strains by IFA was done, antisera to the reference strain 202 was available. Eight of 16 isolates reacted with strain 202 antisera, while

two isolates reacted with *H. pleuropneumoniae* serotype 2 antisera, one with both strain 202 and serotype 2 and 5 isolates were nonreactive. The positive IFA reactions to serotype 2 antisera were unexpected. It is perhaps notable that the single *H. pleuropneumoniae* isolation was a serotype 2 culture. The fact that five of the isolates failed to react with strain 202 antisera indicate that antisera to this strain may not have complete reactivity for all field isolates.

The gross and histopathological findings associated with *Haemophilus* sp. Taxon "minor group" have not been described previously. In this investigation its presence was associated with significant histological changes and a nonsignificant increase in macroscopic pneumonia compared to lungs with no isolations. It appears that *Haemophilus* sp. Taxon "minor group" is capable of infecting the lower respiratory tract of pigs which results in a lymphoid response. Given a sufficient dose it may be capable of causing macroscopic lesions but this warrants further experimental studies to confirm.

This report on the isolation of *H. pleuropneumoniae* serotype 2 is the first in the United States. Elsewhere in the world, diagnostic laboratories have isolated it commonly from acute cases; 73%, 94% and 2% were reported from Sweden, Switzerland and Ontario, respectively (33,34,54).

No cultures of serotype 2 were identified in two studies where a total of nearly 200 field isolates of *H. pleuropneumoniae* from swine from the midwest United States were serotyped (32,55). Its failure to have been detected at diagnostic laboratories may reflect recent introduction into the U.S. Alternatively, it may not be causing significant mortality and may not have been detected despite its presence. The significance of the three isolates of *Haemophilus* sp. Taxon "minor group" isolated in this study that cross reacted with *H. pleuropneumoniae* serotype 2 antisera on IFA is unknown. Since serological surveys incorporating random sampling have not been conducted for haemophilus serotypes in the United States, it is impossible to speculate on its true prevalence.

This particular isolate of *H. pleuropneumoniae*, serotype 2 was detected in a lung without typical lesions of haemophilus pneumonia. Little and Harding (1), using a similar dilution technique as was used here also detected *H. pleuropneumoniae* in lungs without typical lesions. It appears that *H. pleuropneumoniae* may be found in the lower respiratory tract but may not be detected unless techniques are used to suppress and dilute overgrowth by less fastidious organisms.

It has been suggested that *P. multocida* serotype differences may explain why some cases of pasteurellosis are more severe than others (56). This study found no significant differences between the seven most commonly isolated serotypes. However, the number of some of the isolates is quite low and significant differences may not have existed as a consequence. It has been suggested that mouse inoculation techniques may enhance the isolation of D strains from nasal turbinates of swine (29), but this has been demonstrated not to be the case in swine pnemonia (57).

The primary cell types involved in the immune response that can be histologically examined from lung sections are the alveolar macrophage, lymphocytes and polymorphonuclear cells of which the predominant type is the neutrophil. This investigation found a positive association between the isolation of *P. multocida* and the presence of polymorphonuclear cells. This is consistent with other studies which infer that these granulocytes play a major role in the clearance of Gram negative bacteria from the lung (6,21,58).

Alveolar macrophage scores were significantly elevated for *M. hyopneumoniae, P. multocida* and *Haemophilus* sp. Taxon "minor group". This suggests that these cells are recruited in an attempt to eliminate these organisms. Studies in laboratory animals have indicated that under certain circumstances, both granulocytes and macrophages are attracted, whereas in other circumstances, only one of these cells is recruited. The Gram type of the infecting organism has been shown to be a factor, but the inoculum size has also been demonstrated to be an important determinant (59,60). Using *Staphylococcus aureus*, Onofrio *et al* (60) suggested that low doses are rapidly cleared by alveolar macrophages but as the dose was increased, polymorphonuclear cells were recruited to assist in the elimination. It seems likely that these relationships occur in pigs also. This points towards a potential problem in conducting extensive histopathological studies with the objective of distinguishing organisms.

In this investigation, no significant increase in lymphoid proliferation was noted in *M. hyopneumoniae* sections compared to those with no isolations. Similar results were obtained by Roberts *et al* (6). This is in distinct contrast to experimental inoculation studies (44,45) and two explanations for this apparent discrepancy are possible. It may be that most or all of the lungs with no isolations that had lymphoid proliferation had been previously infected with *M. hyopneumoniae* but the infection had been cleared or not detected on FAT. It seems equally as likely that since other organisms and inhaled organic material can stimulate the lymphoreticular system (61) the effect that *M. hyopneumoniae* has would be missed in the analysis when examining conventionall raised pigs.

Jericho stated that the histopathological changes are important, although inconclusive guidelines and that misinterpretation of these changes may lead to undue simplification or complication in the study of porcine respiratory disease (26). In our experience, conducting extensive histopathological surveys of conventionally raised market hogs with no history of previous exposures does not appear to be overly rewarding.

The results of this study emphasize the complex nature of pneumonia in market weight swine. Microscopic examination indicates a much higher prevalence of inflammation but it may be only when the immune system is overwhelmed that macroscopic pneumonia results.

Many organisms can be detected, but without having any information on disease incidence rates, it is impossible to speculate on their relative importance. Pneumonia in market weight swine from conventional herds will inevitably involve *M. hyopneumoniae, P. multocida*, possibly *H.*

pleuropneumoniae if present in the herd and various other secondary invaders. Investigations of this type can generate a wealth of information on organisms involved in the respiratory disease complex, but research efforts towards clarifying when and why the pig becomes infected and how to reduce each organism's incidence rate may be more rewarding.

ACKNOWLEDGMENTS

We thank Dr. R.F. Ross of the Veterinary Medical Research Institute, Ames, Iowa for providing continual assistance. Without his cooperation this investigation could not have been conducted as it was. We also acknowledge the cooperation of Dr. H.S. Joo in performing the virological techniques and Dr. F. Martin for his advice on statistical analysis. Technical assistance provided by B. Beatty and G. Brelie was invaluable. Iowa Pork slaughter plant at South St. Paul and Hormel Co., Austin, Minnesota were kind enough to allow us access to the slaughter area and to remove viscera for further laboratory workup.

REFERENCES

1. **LITTLE, TWA, HARDING JDJ.** The interaction of *Haemophilus parahaemolyticus* and *Pasteurella multocida* in the respiratory tract of the pig. Br Vet J 1980; 136: 371-382.
2. **GOIS M, KUKSA F, SISAK F.** Microbiological findings in the lungs of slaughter pigs. Proc Cong Int Pig Vet Soc Copenhagen, Denmark, 1980; 6: 214.
3. **LITTLE TWA.** Respiratory disease in pigs: A study. Vet Rec 1975; 96: 540-544.
4. **OSBORNE AD, SAUNDERS JR, SEBUNYA TK.** An abattoir survey of the incidence of pneumonia in Saskatchewan swine and an investigation of the microbiology of affected lungs. Can Vet J 1981; 22: 82-85.
5. **GOIS M, SISAK F, SOVADINA M.** Incidence and evaluation of the microbial flora in the lungs of pigs with enzootic pneumonia. Zentralbl Veterinaermed 1975; 22: 205-219.
6. **ROBERTS ED, SWITZER WP, L'ECUYER C.** Influence of *Pasteurella multocida* and *Mycoplasma hyorhinis* (PPLO) on the histopathology of field cases of swine pneumonia. Cornell Vet 1961; 52: 306-327.
7. **WILLEBERG P, GERBOLA MA, KIRKEGAARD PETERSON B, ANDERSON JB.** The Danish pig health scheme: Computer-based abattoir surveillance and follow-up at the herd level. Prev Vet Med (in press).
8. **NOGUEIRA RHG, RFZENDE M, REIS R, NASCIMENTO EF, CHQUILOTF MAG.** Pulmonary lesions in swine. IV: Microbiologic and pathologic diagnosis in swine enzootic pneumonia (SEP) in the state of Minas Gerais, Brazil. Proc Cong Int Pig Vet Soc Mexico, 1982; 7: 87.
9. **SZAZADOS I, KADAS I.** Identification of *Haemophilus parahaemolyticus* pleuropneumonia among normal slaughter pigs. Acta Vet Acad Sci Hung 1981; 29: 301-316.
10. **BOLSKE G, MARTINSSON K, PERSON N.** The incidence of mycoplasma and bacteria from lungs of swine with enzootic pneumonia in Sweden. Proc Cong Int Pig Vet Soc Copenhagen, Denmark, 1980; 6: 213.
11. **SMITH JE.** Analysis of autopsy data on pig respiratory disease by multivariate methods. Br Vet J 1977; 133: 281-291.
12. **LITTLE TWA.** *Haemophilus* infection in pigs. Vet Rec 1970; 87: 399-402.
13. **NICOLET J, SCHOLL E.** *Haemophilus* infections. In: Leman AD, Glock RD, Mengeling WL, Penny RHC, Scholl E, Straw B, eds. Diseases of swine, 5th ed. Ames, Iowa: Iowa State University Press, 1981: 368-377.
14. **PIJOAN C, MORRISON RB, HILLEY HD.** Dilution technique for isolation of *Haemophilus* from swine lungs collected at slaughter. J Clin Microbiol 1983; 18: 143-145.
15. **AALUND O, WILLEBERG P, MANDRUP M, RIEMANN H.** Lung lesions at slaughter: Associations to factors in the pig herd. Nord Vet Med 1976; 28: 487-495.
16. **BACKSTROM L, BREMER H.** Disease registrations on pigs at slaughter as a method of preventive and therapeutic veterinary medicine in swine production. Svensk Vet 1976; 28: 312-336.
17. **FLESJA KI, ULVESAETER HO.** Pathological lesions in swine at slaughter. Acta Vet Scand 1979; 20: 498-514.
18. **GOODWIN, RFW.** Activity of tiamulin against *Mycoplasma suipneumoniae* and enzootic pneumonia of pigs. Vet Rec 1979; 104; 194-195.
19. **JERICHO KWF, DONE SH, SAUNDERS RW.** Pneumonia and efficiency of pig production. Can Vet J 1975; 16: 44-49.
20. **STRAW BE, BURGI EJ, HILLEY HD, LEMAN AD.** Pneumonia and atrophic rhinitis in pigs from a test station. J Am Vet Med Assoc 1983; 182: 607-611.
21. **PIERCE AK, REYNOLDS RC, HARRIS GD.** Leukocytic response to inhaled bacteria. Am Rev Respir Dis 1977; 116: 679-684.
22. **WILKIE BN.** Respiratory tract immune response to microbial pathogens. J Am Vet Med Assoc 1982; 181: 1074-1079.
23. **JERICHO KWF, DARCEL CleQ.** Response of the respiratory tract of calves kept at controlled conditions to bovine herpesvirus 1 in aerosol. Can J Comp Med 1978; 42: 156-167.
24. **DEGRÉ M, SOLBERG LA.** Synergistic effect in viral-bacterial infection. Acta Path Microbiol Scand 1971; 79: 129-136.
25. **JERICHO KWF, MAGWOOD SE.** Histological features of respiratory epithelium of calves held at differing temperature and humidity. Can J Comp Med 1977; 41: 369-379.
26. **JERICHO KWF.** Interpretation of the histopathological changes of porcine enzootic pneumonia. Vet Bull 1977; 47: 887-890.
27. **PIFFER IA.** Effect of age on the susceptibility of pigs to *Mycoplasma hyopneumoniae* pneumonia. MS Thesis. Iowa State University, Ames, Iowa. 1981.
28. **WHITTLESTONE P.** The role of mycoplasmas in the production of pneumonia in the pig. In: Pathogenic mycoplasmas. London: Associated Scientific Publishers, 1972: 263-283.
29. **deJONG MF, OEI HL, TETENBURG GJ.** Atrophic rhinitis pathogenicity tests of *Pasteurella multocida* isolates. Proc Cong Int Pig Vet Soc Copenhagen, Denmark, 1980; 6: 211.
30. **PIJOAN C, MORRISON RB, HILLEY HD.** Serotyping of *Pasteurella multocida* isolated from swine lungs collected at slaughter. J Clin Microbiol 1983; 17: 1074-1076.
31. **CARTER GR, CHENGAPPA MM.** Recommendations for a standard system of designating serotypes of *Pasteurella multocida*. Proc Am Assoc Vet Lab Diagnost 1981; 24: 37-42.
32. **RAPP VJ, ROSS RF, ZIMMERMANN B.** Serotyping of *Haemophilus pleuropneumoniae* by rapid slide agglutination and indirect fluorescent antibody tests in swine. Am J Vet Res 1985; 46: 185-192.
33. **ROSENDAL S, LOMBIN L, DeMOOR J.** Serotyping and detection of *Haemophilus pleuropneumoniae* by indirect fluorescent antibody technique. Can J Comp Med 1981; 45: 271-274.
34. **NICOLET J.** *Haemophilus* infection in pigs. III. Serologic studies on *Haemophilus parahaemolyticus*. Zentralbl Bakteriol I Abt orig 1971; 216: 487-495.
35. **AMANFU W, WENG CN, BARNES HF, ROSS RF.** Direct immunofluorescence technique for detection of *M. hyopneumoniae* in swine lungs. Proc Cong Int Pig Vet Soc Copenhagen, Denmark, 1980; 6: 16A.
36. **BOLSKE G, MARTINSSON K, PERSON N.** The incidence of mycoplasma and bacteria from lungs of swine with enzootic pneumonia in Sweden. Proc Cong Int Pig Vet Soc Copenhagen, Denmark, 1980; 6: 213.
37. **LAMONT HG.** The problems of the practitioner in connection with the differential diagnosis and treatment of diseases of young pigs. Vet Rec 1938; 50: 1377-1390.
38. **PIJOAN C, OCHOA G.** Interaction between a hog cholera vaccine strain and *Pasteurella multocida* in the production of porcine pneumonia. J Comp Pathol 1978; 88: 167-170.
39. **FUENTES M, PIJOAN C.** Studies on the interaction between vaccinial and pathogenic Aujeszki's virus and *Pasteurella multocida* in young pigs. Proc Cong Int Pig Vet Soc Ghent, Belgium 1984: 28.
40. **KASZA L, HODGES RT, BETTS AO, TREXLER PC.** Pneumonia in gnotobiotic pigs produced by simultaneous inoculation of a swine adenovirus and *Mycoplasma hyopneumoniae*. Vet Rec 1969; 84: 262-267.
41. **CHARLEY B.** Interaction of influenza virus with swine alveolar macrophages: Influence of anti-virus antibodies and cytochalasin B. Ann Virol 1983; 134: 51-59.

42. **SANFORD SE, JOSEPHSON GKA, KEY DW.** An epizootic of swine influenza in Ontario. Can Vet J 1983; 24: 167-171.
43. **L'ECUYER C, BOULANGER P.** Enzootic pneumonia of pigs: Identification of a causative mycoplasma in infected pigs and in cultures by immunofluorescent staining. Can J Comp Med 1970; 34: 38-46.
44. **LIVINGSTON CW, STAIR EL, UNDERDAHL NR, MEBUS CA.** Pathogenesis of mycoplasmal pneumonia in swine. Am J Vet Res 1972; 33: 2249-2258.
45. **UNDERDAHL NR, KENNEDY GA, RAMOS AS Jr.** Duration of *Mycoplasma hyopneumoniae* infection in gnotobiotic pigs. Can Vet J 1980; 21: 258-261.
46. **SMITH IM, HODGES RT, BETTS AD, HAYWARD AHS.** Experimental infections of gnotobiotic piglets with *Pasteurella septica* (sero-group A) alone or with *Mycoplasma hyopneumoniae.* J Comp Pathol 1973; 83: 307-321.
47. **HOUGHTON SB, GOURLAY RN.** Synergism between *Mycoplasma bovis* and *Pasteurella haemolytica* in calf pneumonia. Vet Rec 1983; 113: 41-42.
48. **JERICHO KWF.** Histological changes in the respiratory tract of calves exposed to aerosols of bovine herpesvirus 1 and *Pasteurella haemolytica.* J Comp Pathol 1983; 93: 73-82.
49. **FARRINGTON DO.** Pasteurellosis. In: Leman AD, Glock RD, Mengeling WL, Penny RHC, Scholl E, Straw B, eds. Diseases of swine, 5th ed. Ames: Iowa State University Press, 1981: 378-385.
50. **KILIAN M, NICOLET J, BIBERSTEIN EL.** Biochemical and serological characterization of *Haemophilus pleuropneumoniae* (Matthews and Pattison 1961) Shope 1964 and proposal of a neonate strain. Int J Syst Bacteriol 1978; 28: 20-26.
51. **PATTISON IH, HOWELL DB, ELLIOT J.** A *Haemophilus*-like organism isolated from pig lung and the associated pneumonic lesions. J Comp Pathol 1957; 67: 320-333.
52. **MATTHEWS PRJ, PATTISON IH.** The identification of a *Haemophilus*-like organism associated with pneumonia and pleurisy in the pig. J Comp Pathol 1961; 71: 44-52.
53. **BIBERSTEIN EL, GUNNARSON A, HURVEL B.** Cultural and biochemical criteria for the identification of *Haemophilus* spp from swine. Am J Vet Res 1977; 38: 7-11.
54. **GUNNARSON A.** Evaluation of different antigens in the complement fixation test for diagnosis of *Haemophilus pleuropneumoniae* (*para-haemolyticus*) infections in swine. Am J Vet Res 1979; 40: 564-567.
55. **SCHULTZ RA, ROSS RF, GUNNARSSON A, NIELSEN R.** Serotyping of 50 different isolates of *Haemophilus pleuropneumoniae* from swine pneumonia in Iowa and surrounding states. Vet Med Small Anim Clin 1983; 78: 1451-1453.
56. **COLLINS FM.** Mechanisms of acquired resistance to *Pasteurella multocida* infection: A review. Cornell Vet 1977; 67: 103-138.
57. **PIJOAN C, LASTRA A, RAMIREZ C, LEMAN AD.** Isolation of toxigenic strains of *Pasteurella multocida* from lungs of pneumonic swine. J Am Vet Med Assoc 1984; 185: 522-523.
58. **SNELLA MC, RYLANDER R.** Lung cell reactions after inhalation of bacterial lipopolysaccharides. Eur J Respir Dis 1982; 63: 550-557.
59. **TOEWS GB, GROSS GN, PIERCE AK.** The relationship of inoculum size to lung bacterial clearance and phagocytic cell response in mice. Am Rev Respir Dis 1979; 120: 559-566.
60. **ONOFRIO JM, TOEWS GB, LIPSCOMB MF, PIERCE AK.** Granulocyte-alveolar macrophage interaction in the pulmonary clearance of *Staphylococcus aureus.* Am Rev Respir Dis 1983; 127: 335-341.
61. **CORNER AH, JERICHO KWF.** Pneumonia associated with inhaled plant material in swine. Vet Pathol 1972; 9: 384-391.