CHEMOPROPHYLAXIS, CHEMOTHERAPY, AND ECONOMIC EFFECT OF SWINE ENZOOTIC PNEUMONIA

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INTRODUCTION

Confinement systems of swine management in which large numbers of pigs are in close contact may create an environment favorable for the spread of respiratory diseases. The most widespread of these diseases is variously termed "Virus Pneumonia of Pigs", "Infectious Pneumonia", "Enzootic Virus Pneumonia", "Mycoplasmal Pneumonia of Swine", and "Swine Enzootic Pneumonia". The etiologic agent is now known to be Mycoplasma hyopneumoniae; this suggests that descriptions containing the word "virus" are incorrect and should not be used. Several reports in the literature suggest that Mycoplasma hyorhinis plays an etiologically important role in chronic swine pneumonia; other more properly performed work suggests that this is not the case at all. The term "Mycoplasmal Pneumonia of Swine", while etiologically suggestive, does not differentiate between the primary or secondary nature of the incriminated agents. An etiologically neutral term gaining widespread use is "Swine Enzootic Pneumonia". This is the term that will be used to describe the chronic respiratory disease of swine induced by Mycoplasma hyopneumoniae, the organism used in these studies.

Swine enzootic pneumonia is a chronic pneumonia characterized by a persistent nonproductive cough, loss of condition, growth retardation, high morbidity and low mortality. Clinical signs are usually first manifested two to three weeks after exposure. Gross pneumonic lesions occur predominantly in the anterior lobes; microscopic examination reveals pronounced peribronchiolar and perivascular lymphoid hyperplasia, thickening of the alveolar walls, septal cell proliferation, and a moderate neutrophil infiltration.

The incidence of swine enzootic pneumonia has been reported often, but only a few studies have been made to determine the effect of the disease on performance. Within these studies, there is considerable variation in economic significance attributed to swine enzootic pneumonia.

Several antibiotics have been tested for their effect on the agent causing swine enzootic pneumonia, but no effective chemoprophylactic or chemotherapeutic regimen has been defined.

This study was undertaken in an attempt to determine the utility of chemoprophylactic and chemotherapeutic regimens in controlling or eradicating swine enzootic pneumonia; a determination of the economic significance of the disease under controlled conditions was also attempted.

REVIEW OF THE LITERATURE

This literature review covers five areas of the disease condition known as swine enzootic pneumonia. These are 1) a brief history of attempts to isolate and transmit the causative agent, 2) lesions observed in the disease and diagnosis of the condition, 3) incidence, economic effects, and ecological factors, 4) control and eradication procedures other than chemotherapy, and 5) chemotherapy.

History

The literature concerning swine enzootic pneumonia (SEP) has increased in volume in recent years as interest in the condition has increased. Much of the early literature has been reviewed by Betts (1953), L'Ecuyer (1962), Mare' (1965), and Mare' and Switzer (1966c).

Gulrajani and Beveridge (1951) recognized a chronic pneumonia of swine in Britain which differed from swine influenza. They could not isolate swine influenza virus from these cases even though they could reproduce the disease with bacteria-free filtrates. Macroscopic lesions of pneumonia were observed on the twelfth day after inoculation. It was believed that this pneumonia was probably identical to that described by Pullar (1948, 1949a, 1949b) in Australia. Pullar (1949b) was able to experimentally transmit the disease to pigs by exposing them to an aerosol of a suspension of pneumonic lung. He could not transmit the disease with material taken from the nares, tonsils, or bronchioles of an infected pig.

L'Ecuyer (1962), L'Ecuyer and Switzer (1963), and Betts and Whittlestone (1963) reported limited success in propagating the causative

agent in cell cultures. They were not able to reproduce the disease with materials transferred for more than a few passages in cell cultures. At this time the causative agent of swine enzootic pneumonia was still believed to be a virus, although Goodwin and Whittlestone (1963) suggested the possibility that the agent was a mycoplasma. Subsequently, Goodwin and Whittlestone (1964) produced enzootic pneumonia in pigs with an organism grown in a medium free from living cells.

In the United States, workers were also progressing toward definition of the causal agent. Small coccobacillary organisms were isolated from a pneumonic lung and grown in a cell-free medium. Reintroduction of these organisms into the nares of susceptible pigs resulted in the production of lesions typical of enzootic pneumonia. From these lesions organisms similar to those inoculated were isolated and characterized as mycoplasma. This work was summarized and published by Mare' and Switzer (1965), constituting the first published declaration of enzootic pneumonia as a disease proven to be caused by a mycoplasma. The name Mycoplasma hyopneumoniae was proposed for this agent. Several months later, research personnel in Britain also reported the isolation and characterization of a mycoplasma which was capable of inducing lesions of enzootic pneumonia (Goodwin et al., 1965). This work was essentially similar to that performed in the United States; the authors wished, however, to declare the name "Mycoplasma suipneumoniae" for the agent that they isolated.

The causative agent of swine enzootic pneumonia has been further characterized by Mare' and Switzer (1966a, 1966b, and 1966c). The organism was found to have a minimal reproductive unit size of 110-225 millimicrons and was coccobacillary in shape with a great tendency toward pleomorphism.

It was ether-sensitive *in vitro*, tetracycline-sensitive *in vivo*, and penicillin-resistant *in vitro*. Colonies typical of mycoplasma were grown on a solid medium; growth in cell-free media was substantiated. Goodwin and Whittlestone (1966) reported similar findings for the agent they had isolated. Later, Goodwin *et al.*, (1967) reported the results of a comparative study of several mycoplasma species and strains. They found that *Mycoplasma hyopneumoniae* and "*Mycoplasma suipneumoniae*" were indistinguishable in the metabolic inhibition test and in the growth inhibition test. These two isolates were shown to be distinct from other known swine mycoplasma. Goodwin *et al.* (1968) found that they could produce lesions of swine enzootic pneumonia with embryonated egg cultures of "*Mycoplasma suipneumoniae*". The experimental animals used were surgically-produced and colostrum-deprived while those used by L'Ecuyer and Switzer (1963) and Mare and Switzer (1966c) were naturally born and had received colostrum; the results are not completely comparable.

Goodwin *et al.* (1968) was able to recover "*Mycoplasma suipneumoniae*" from ninety-one percent of experimentally infected pigs. In examining twelve field outbreaks, however, this organism was only isolated on two occasions. *Mycoplasma hyorhinis* was commonly found in lung lesions of enzootic pneumonia; these authors believed that this secondary invader often prevented them from isolating the primary mycoplasma.

Betts and Whittlestone (1963), using the Cambridge "J" strain of the swine enzootic pneumonia agent, detected a cytopathic effect in plasma clot cultures prepared from the lungs and from the nasal mucosa of an infected pig. Goodwin and Whittlestone (1963) used similar plasma clot isolates in

pig lung monolayer cell cultures and produced a cytopathic effect; they could detect pleomorphic organisms in stained preparations of these cell cultures. Seventh passage cell culture fluids, representing a 10^{16} dilution of the original material were used to produce pneumonia in four pigs. L'Ecuyer and Switzer (1963) used seven-day passage intervals in cell cultures and obtained multiplication of an infectious agent but could not detect any effect on the cells. L'Ecuyer (1968), using fourteen-day passage intervals until an effect was observed, was successful in detecting a cytopathic effect upon tissue culture cells. It appeared that the prolonged incubation period either allowed the mycoplasma to adapt to growth in an artificial environment or simply allowed sufficient numbers to accumulate and deplete cell substrates. Considerable variation was observed in the ability of strains to adapt to cell cultures.

Refinements in procedures have not changed the basic finding that enzootic pneumonia of swine is caused by a mycoplasma distinguishable from all other swine mycoplasma known today. This advance is significant.

A clarification in scientific work is often striking because of the cloudiness of other work. Much of the work on swine enzootic pneumonia was and continues to be difficult to evaluate because of uncontrolled and poorly controlled variables. L'Ecuyer et al. (1961) found that the most common secondary invader in swine enzootic pneumonia lesions was Mycoplasma hyorhinis, a mycoplasma that requires less complex media for growth than does Mycoplasma hyopneumoniae. Mycoplasma hyorhinis also grows more rapidly and more abundantly than does Mycoplasma hyopneumoniae. Switzer (1967a) discussed this complication of the swine enzootic pneumonia problem. Many reports in the literature do not determine the species of mycoplasma re-

covered from the animals examined.

Hartwich and Niggeschulze (1966) found mycoplasma in seventy-five percent of healthy swine lungs examined; the species was not established. McKay et al. (1966) reported the production of enzootic pneumonia in swine with "protoplasts" of Hemophilus parainfluenzae. The origin of their experimental animals remains in question. They did not culture the examined tissues for mycoplasma. Lannek and Wesslén (1957) may have grown the causative agent in suspended cell cultures, as they reported producing gross lesions resembling enzootic pneumonia with such mycoplasma infected cultures. They failed to distinguish the agent from other mycoplasma. Kley and Mayr (1965) reported the isolation of mycoplasma from herds of swine that had enzootic pneumonia, but they failed to characterize the organisms sufficiently to rule out the occurrence of secondary swine mycoplasma or poultry mycoplasma in the embryonated eggs they used. Dinter et al. (1965) suggested that the swine enzootic pneumonia mycoplasma was closely related antigenically to Mycoplasma hyorhinis but was distinguishable from Mycoplasma granularum. The mycoplasma that they worked with did not induce typical lesions of swine enzootic pneumonia in experimental pigs, strongly suggesting that these workers may have been working with a strain of Mycoplasma hyorhinis. Another report by Bakos et al. (1962) is subject to similar criticism.

Takatori et al. (1964) reported on an agent isolated from a case of enzootic pneumonia which produced floating crystals soluble in organic solvents. The organism was not differentiated from Mycoplasma hyorhinis.

Scheer et al. (1967) reported on a Swedish "SEP" agent which produced only minimal histological changes in experimentally infected swine. Like-

wise, Hartwich et al. (1967) reported isolating mycoplasma from pigs with enzootic pneumonia. The isolate was not capable of reproducing the disease, suggesting that it was a secondary invader or a contaminant. Nardelli et al. (1966) also found mycoplasma in pneumonic lungs, but did not classify the isolate properly.

Estola and Schulman (1966a and 1966b) and Schulman and Estola (1966) reported on their efforts to recover causative agents. A twenty percent suspension of pneumonic lung inoculated into susceptible pigs was found to induce the formation of gross and microscopic lesions typical of enzootic pneumonia. A sterile lung suspension elicited no reaction when inoculated into pigs. Allantoic fluid containing the "SEP" mycoplasma produced microscopic lesions when inoculated into pigs; however, an inoculum of allantoic fluid alone produced microscopic and small macroscopic lesions reminiscent of those encountered in enzootic pneumonia. The production of polyserositis in pigs inoculated intraperitoneally with their "SEP" agent suggests that these people were working with Mycoplasma hyorhinis.

Monreal (1966) felt that the embryonated egg test was satisfactory in demonstrating the causal agent of swine enzootic pneumonia. He concluded that the agent was a mycoplasma but did not define the species. Kley (1966) reported on his findings concerning the causal agent of swine enzootic pneumonia in the nasal cavities of infected swine; the identity of this agent is not certain. In trials utilizing animals of questionable health status, Gois et al. (1968) believed that they had proven Mycoplasma hyorhinis to be a causative agent of enzootic pneumonia. No uninoculated control animals from the "clinically negative" source herd were incorporated into the experiment; it is difficult to interpret their results. Pillai

et al. (1967) reported the production of an interstitial pneumonitis, pleural thickening, and early granuloma formation within the lung parenchyma in colostrum-deprived pigs using a mycoplasma isolate. The mycoplasma species was not identified; the number of pigs used was very limited.

Lesions Observed in the Disease and Diagnosis of the Condition

Omar (1964) pointed out that the responses which the septal cells and the alveolar lining cells of the lung may show under various intensities of injury may be similar for various injurious substances, especially in acute conditions. In these cases too, the changes may be complicated by hematogenous infiltration of inflammatory cells. Jericho (1967) felt that the intrapulmonary lymphoid tissue met all the cellular and fibrillar requirements for performing functions usually attributed to normal lymphoid tissue in other parts of the body. Agents capable of eliciting hyperplasia of intrapulmonary lymphoid tissue may belong to a broad spectrum of biological and chemical entities. One must, in assessing the pathogenicity of specific invaders, use animals with lung tissue of known microbiological flora; also, the environmental conditions in which the animals are produced and maintained must be known and constant. Jericho (1968) stated that such factors as age and type of pig used, route and method of inoculation, volume of inoculum, frequency of inoculation, nature of the inoculation vehicle, and age to which a lesion may be allowed to progress, must be evaluated before accepting the pathogenicity of some reported inocula. Few, if any, reports in the literature meet all of these requirements.

Reports concerning the lesions of swine enzootic pneumonia have a common theme. The gross lesions were characterized by Betts (1952) as well-

outlined, plum-colored or greyish pneumonic areas in the apical and cardiac lobes. These areas resemble an atelectic lung; other lobes may be affected but are usually less frequently involved.

The microscopic lesions were characterized by Pattison (1956) as involving a gradation of changes beginning as an increase in cellularity of the interalveolar tissue with slight edema and a few large mononuclear cells free in the alveolar spaces. Later, an increase in cellular exudate, more obvious edema and frank lymphoid hyperplasia often related to the bronchi and bronchioles was observed. This culminated in obliteration of considerable areas of alveolar tissue by dense accumulations of lymphocytes related closely to bronchi and bronchioles. Neutrophils were scarce. It was evident that lymphoid hyperplasia could occur in the absence of alveolar reaction. Collapse of lobular tissue without an associated inflammatory reaction was also noticed--often being adjacent to an emphysematous area. No consistent abnormality of the bronchial wall was noticed. This general gross and microscopic picture has been consistently associated with swine enzootic pneumonia by subsequent workers.

Urman et al. (1958) reported on the microscopic differentiation of swine influenza from swine enzootic pneumonia. Their evaluation of the histopathology of swine enzootic pneumonia agreed with the findings of Pattison (1956). They reported that lesions did not consistently appear until thirteen days postinoculation. Tissue responses to the introduction of swine influenza virus were much more rapid. Within twenty-four hours after inoculation, scattered areas of edema and hyperemia were observed throughout the lung. By the third day, the alveoli and bronchi contained many neutrophils; many areas were filled with cellular debris. Degenera-

tive and regenerative changes were apparent by the fourth and fifth days. Up to the sixth day postinoculation, the mononuclear perivascular reaction was more pronounced than was the peribronchiolar reaction. Later, however, peribronchiolar cuffing became the more prominent lesion. Consolidated areas exhibited atelectasis. In these areas, some bronchi were found to contain cellular debris; the bronchial epithelial cells had degenerated or were necrotic. Swine lungs infected with swine influenza virus thus showed significant differences at the microscopic level when compared with swine enzootic pneumonia lesions.

Duncan (1965) and Duncan et al. (1966) described the pathology of experimentally-induced Bordetella bronchiseptica pneumonia in swine. The most striking lung lesions observed in swine inoculated with pure cultures of Bordetella bronchiseptica were vascular alterations and fibrosis; alveolar edema and hemorrhage were marked. Early vasculitis was followed by endothelial hypertrophy and hyperplasia and medial and adventitial hyperplasia. Fibrosis initiated in the perivascular, peribronchiolar, subpleural, and septal regions was found to follow the vascular changes. Sheets of fibrous tissue replaced portions of the lung parenchyma. Epithelialization of the alveoli occurred in later stages. Thus, the tissue changes associated with Bordetella bronchiseptica pneumonia have been reported to be considerably different from those associated with swine enzootic pneumonia.

Roberts et al. (1962) found in a survey of swine herds with pneumonia that Pasteurella multocida isolations from lungs were associated with a marked neutrophilic reaction throughout the lung and with septal cell proliferation. They found no definite histological changes associated with

the isolation of Mycoplasma hyorhinis from swine lungs. In eighty-nine percent of the pneumonic lungs examined microscopically, a lymphoid reaction similar to that observed in swine enzootic pneumonia was present. This suggested to these workers that in these field cases, swine enzootic pneumonia was the primary condition with other organisms being superimposed upon it as secondary invaders.

Grace et al. (1963) felt that clinical history, gross lesions, and microscopic lesions should all be considered in a diagnosis of swine enzootic pneumonia. This technique limited practical diagnosis to a herd basis.

Koper (1964) explored the use of the radiograph in diagnosing swine enzootic pneumonia. The mechanics of this technique would pose many problems if widespread application were to be attempted.

Roberts (1968) reported using a complement-fixation test to detect antibodies against Mycoplasma hyopneumoniae. These antibodies were produced in pigs infected with a broth culture of Mycoplasma hyopneumoniae. Cross reactions occurred with the mycoplasma reported by Goodwin, et al. (1965) which they named "Mycoplasma suipneumoniae", confirming the similarity of the two isolates.

Boulanger and L'Ecuyer (1968) have reported using the modified direct and indirect complement-fixation tests to detect antibodies for the enzootic pneumonia mycoplasma and for Mycoplasma hyorhinis in the serum of infected pigs and immunized rabbits. These workers found that only the modified direct complement-fixation test in which guinea pig complement was supplemented with fresh, normal, unheated calf serum was suitable for the detection of mycoplasma antibodies in the sera of infected swine. The

close correlation between the production of typical lung lesions in experimentally infected pigs and the appearance of significant serum antibody titers suggests that a sensitive, specific *in vitro* method for the detection of enzootic pneumonia in the live pig is at hand. The test also permitted the *in vitro* differentiation of the mycoplasma causing enzootic pneumonia from *Mycoplasma hyorhinis* as no cross reactions were found to occur in swine serum. Antibodies in swine serum were demonstrable only with the homologous antigen.

Complement-fixation antibody against *Mycoplasma hyopneumoniae* was also demonstrated in the sera of pigs experimentally or naturally infected with the agent of swine enzootic pneumonia by Takatori *et al.* (1968). These workers found that antibody first appeared two to three weeks after inoculation and tended to increase for at least nine weeks. Swine sera containing complement-fixation antibody against *Mycoplasma hyopneumoniae* did not react with *Mycoplasma hyorhinis* or with *Mycoplasma granularum* antigen. Likewise, swine sera containing antibody against *Mycoplasma hyorhinis* or *Mycoplasma granularum* did not react with *Mycoplasma hyopneumoniae* antigen.

The recent successes in detection of swine enzootic pneumonia through serological tests are not without blemishes. The tests are difficult to perform and are complex. The procomplementary effects of swine serum must be accounted for as must be the anticomplementary effects of some antigen preparations. With refinements these tests should aid considerably in combatting swine enzootic pneumonia at the pig level instead of at the herd level; epidemiological investigations should also be enhanced.

Incidence, Economic Effect, and Ecological Factors

Incidence

Switzer (1967a) suggested that, based on the clinical course, gross lesions, and microscopic lesions, swine enzootic pneumonia was present throughout the world. He found the incidence in Iowa to be between thirty-five and sixty percent. Betts (1952) believed that swine enzootic pneumonia was the most common pathological condition in the pig in England. In two separate groups of one thousand pigs each, enzootic pneumonia was observed in forty-two and sixty-one percent of the animals. Whittlestone (1967) reported a similar morbidity in England. Carter and Schroder (1956) reported the occurrence of swine enzootic pneumonia in Canada. Cilli and Scatozza (1962) confirmed the presence of the disease in Italy. Audi et al. (1962) felt that the morbidity of swine enzootic pneumonia in Yugoslavia approached one hundred percent. Coman et al. (1966) of Roumania believed that enzootic pneumonia was the most common pneumotropic disease of that country. Kono et al. (1964) reported the occurrence of swine enzootic pneumonia in Japan, but gave no figures on morbidity; later Kono et al. (1967) stated that the incidence was in the range recognized by observers of other countries. Rees (1964) of New Zealand, examined three hundred twenty-six pigs at slaughter weight. He observed pneumonia in eighty-five percent of these animals; over eighty percent of these pneumonic lungs were diagnosed as typical cases of swine enzootic pneumonia. Mugera (1967) reported that fifty-six percent of the pneumonia cases observed in Kenya resembled swine enzootic pneumonia.

An additional aspect of incidence has also appeared in the literature.

Pullar (1948) recognized a declining incidence of infectious pneumonia in pigs when older groups were sampled; whereas up to sixty-eight percent of market-weight pigs were affected with the infectious pneumonia, only thirty-two percent of older adult animals exhibited similar lesions. Macpherson and Shanks (1955) reported a similar finding in which the differences were even more striking. Of six hundred seventy-five sows examined, only five and eight-tenths percent possessed lesions of swine enzootic pneumonia--most lesions being diminutive in nature; however, fifty-five and four-tenths percent of one thousand market weight pigs examined showed lesions typical of swine enzootic pneumonia--some four hundred of these being quite extensive in nature.

Economic effect

Young et al. (1959) found that pigs with both atrophic rhinitis and enzootic pneumonia took about one month longer to reach market weight than did animals free of these maladies. They reported, however, that the incidence and severity of atrophic rhinitis seemed to have little relationship to the growth rate of the afflicted swine, while the rate of gain was closely correlated with the incidence and extent of swine enzootic pneumonia lesions. This report and that of Pearce and Roe (1967) suggest strongly that of these two disease entities, atrophic rhinitis and enzootic pneumonia, only one (enzootic pneumonia) is correlated with adverse economic effects.

Betts (1952) estimated that enzootic pneumonia caused a five percent reduction in rate of weight gain. With an estimated fifty percent of the swine population involved, he attributed a two and one-half percent overall decline in average daily gain to enzootic pneumonia. Betts and Beveridge

(1953) conducted experimental inoculations to determine the effect of enzootic pneumonia on rate of weight gain during both summer and winter months. They concluded from the results of these trials that the reduction in rate of weight gain and feed efficiency for pigs with enzootic pneumonia was approximately twenty-five percent or, again, twelve and one-half percent for each pig produced in the United Kingdom. Other experiments by Betts et al. (1955) resulted in similar conclusions; these experiments suggested that infected pigs have a sixteen percent reduction in rate of weight gain and a twenty-two percent reduction in feed efficiency.

Goodwin (1963) estimated the cost of enzootic pneumonia per affected pig in England at about five dollars. Goodwin (1966) reported that eradication of enzootic pneumonia should result in a twenty percent increase in feed efficiency. Hradil (1966) also felt that enzootic pneumonia was one of the main factors keeping swine herds from maximizing their genetic potential.

The results of other studies do not concur with those of the English workers just cited. Eikmeier and Mayer (1965) analyzed three hundred four swine with a forty percent incidence of enzootic pneumonia and found no economic effect of the disease either on rate of gain or on feed conversion. They felt that the lack of effect may have been due to excellent management procedures. Englert and Eisenack (1964) reported that there was no statistical significance in the variation in weight gain between pneumonic and pneumonia-free pigs utilized in their enzootic pneumonia research. However, weight increases were reduced in the animals affected with enzootic pneumonia and the variation in weight gain among animals with enzootic pneumonia was statistically significant, suggesting that the degree of

pneumonia might affect the rate of weight gain. Björkland and Henricson (1965) found no difference in daily gain for diseased versus nondiseased animals in trials related to enzootic pneumonia. They attributed this to the high quality animals used and to good management.

The determination of the economic effects of swine enzootic pneumonia is clouded somewhat by the different experimental conditions used by various investigators. There is a general consensus that management is important in stemming the detrimental effects of swine enzootic pneumonia.

Ecological factors

Nikolić et al. (1965) tested the effect of housing types upon the incidence of swine enzootic pneumonia. No significant effect of housing was uncovered. This work utilized quite small numbers of pigs which may have led to invalid conclusions; Eernstman (1963) found that high temperature and high humidity sharply reduced enzootic pneumonia. The results of his work also suggested that high humidity and low temperatures as well as low humidity regardless of temperature were detrimental to the health of the swine respiratory system.

Kosztolich (1966) found a clear connection between air space per pig and enzootic pneumonia. A reduction in air space per pig resulted in an increased incidence of enzootic pneumonia. This was believed to be because of an increased concentration of the etiologic agent in the air. These experiments did not result in the detection of any effect due to other factors such as ammonia or relative humidity on swine enzootic pneumonia, but work in this area was not complete. Gordon (1963a) found that the number of bacteria in the air was lowest in those pig houses which had the highest absolute humidity. The value of sedimentation in reducing the

bacterial complement of the air was shown in a striking fashion. Gordon (1963b) also demonstrated that the incidence and degree of enzootic pneumonia was lower in pigs kept in an environment of high temperature and humidity than in pigs kept in ambient conditions. Two thousand pigs were utilized in this investigation.

Jericho (1967) discussed several aspects of the role of environment on respiratory problems of young pigs. He found that the lowering of relative humidity to fifty percent was associated with coughing and respiratory distress due to a drying of the mucous blanket covering the tracheal and bronchial epithelium. The optimal relative humidity for young pigs was felt to be about eighty percent; optimal temperatures were from seventy-nine degrees Fahrenheit for piglets under eleven pounds to seventy degrees for older pigs up to sixty-five pounds. The prevailing temperature and humidity was thought to determine the penetration of pathogens by influencing the size of particles in the air. The degree of ventilation also affects the concentration of infectious agents in the air. Heat loss from young pigs was felt to be more critical than for older, larger pigs in reducing resistance to respiratory infections.

To the various interactions of the pig with his macroscopic environment must be added interaction with various agents within the respiratory system itself. Mackensie (1963) observed that the extensiveness of swine enzootic pneumonia lesions was greatly increased in the presence of swine lungworm infection.

Underdahl and Kelley (1957) obtained a tenfold increase in the size of lesions in pigs that were infected simultaneously with enzootic pneumonia and ascarid larvae over those pigs infected with enzootic pneumonia alone.

L'Ecuyer (1963) found that in the majority of cases swine lungs were bacteriologically sterile unless enzootic pneumonia was present. The primary infection appeared to create the proper conditions for other agents to become established.

Control and Eradication Procedures Other Than Chemotherapy

Eikmeier and Mayer (1967) believed it was possible to reduce the effects of swine enzootic pneumonia through improved hygiene and management. They did not believe eradication was necessary.

Goret et al. (1963) considered ultraviolet rays efficient in preventing transmission in a contaminated piggery.

Young and Caldwell (1958) suggested that swine farms with enzootic pneumonia be depopulated, disinfected, and repopulated with surgically-derived piglets. They felt that this procedure would definitely break the disease cycle.

Pond et al. (1967) reported that swine herds could be freed of enzootic pneumonia through isolating the young piglets immediately after birth, a procedure which bypasses the hysterectomy or caesarean operations but which retains the rearing problems encountered with colostrum-deprived, motherless pigs.

Goodwin (1965) strongly opposed hysterectomy or caesarean-based repopulation schemes as a procedure for general application. He felt that the costs involved in the lowered production due to hand-raising the first generation, in waiting for the second generation to be farrowed and reared naturally, and in disease outbreaks--which occurred too frequently despite the original intent--were too high. Also, he suggested that pigs derived

by these artificial means may be very susceptible when reintroduced to normal swine microorganisms. In addition, he had misgivings about the proposed disease-free state of pigs so gained.

Lannek and Börnfors (1957) detected an apparent immunity in some of the pigs with which they experimented. These workers infected twenty-nine pigs with an inoculum felt to contain the etiologic agent of swine enzootic pneumonia. An x-ray diagnosis led them to believe that twenty-eight animals developed lung lesions; of these, fourteen were reported as recovering and being immune to further challenge with the enzootic pneumonia agent. Koper (1964) suggested that while x-ray diagnosis may be useful, its specificity was limited.

Betts (1952) attempted to induce immunity to enzootic pneumonia by utilizing a vaccine containing the infectious agent in a lung suspension. The subcutaneous route of immunization was ineffective. A similar finding was reported by Pullar (1949b) and by Börnfors and Lannek (1958).

Pullar (1948) observed that the incidence of enzootic pneumonia was much less in sows than in young pigs. Macpherson and Shanks (1955) reiterated this finding, reporting a tenfold reduction in the incidence of enzootic pneumonia in sows as compared to market-weight pigs. How much of this reduction is due to selection of healthy breeding stock by the pig producer and what can be attributed to acquired immunity has not been determined. Goodwin (1965 and 1966) and Beveridge (1962) both commented on the possible use of this reduced incidence with age in relation to control and eradication programs.

Betts *et al.* (1955) discussed several principles for eradication of enzootic pneumonia from swine herds. Whatever the scheme, these authors

felt that the principle factor for success was the unflagging enthusiasm of the owner and an intelligent appreciation of the principles involved in the control plan. Another fundamental requirement was the provision of accommodations for isolating pigs in such a way as to prevent transfer of airborne infection. This method of eradication of enzootic pneumonia consisted of the following stages: 1) farrowing sows in isolation to insure that any infection of the litter came only from the dam; 2) determination of whether the litter was infected or not by clinical examination, supplemented by post-mortem examination of one or more weaned pigs per litter if necessary and possible--in this way carrier sows are detected; 3) grouping of litters judged to be free from the disease but retaining the group in isolation; 4) examination at slaughter of lungs from a considerable proportion of the group as a further check when they reach market weight; and 5) replacement of the original breeding stock with healthy progeny as soon as possible. This procedure is tedious but is considered more economical than the previously cited depopulation-repopulation schemes. The possible addition of serological tests as reported by Roberts (1968), Boulanger and L'Ecuyer (1968), and Takatori et al. (1968) would increase the efficiency of this program even more.

Barber et al. (1955), Whittlestone and Betts (1955) and Dannenberg (1965) all confirmed the possibility of success through such an eradication program.

Chemotherapy

Penny (1954) and Pugh (1956) attempted treatment of swine enzootic pneumonia with chloramphenicol. Both concluded that the drug had value

only in controlling secondary bacterial invaders.

Betts and Beveridge (1952) recognized that the infectious agent of swine enzootic pneumonia was susceptible to chlortetracycline but not to penicillin or to sulfa compounds. Wesslén and Lannek (1954) reported an in vitro susceptibility of the swine enzootic pneumonia agent to chlortetracycline and to oxytetracycline.

Börnfors and Lannek (1955) considered sulfamethazine, penicillin, oxytetracycline, and chlortetracycline to be of equal value in combatting enzootic pneumonia. With each drug they noted a marked increase in the growth rate but did not note any significant resolution of lesions that could be attributed to the action of drugs.

Lannek and Börnfors (1956) felt that tetracycline in daily doses of twenty, fifteen, and ten milligrams per kilogram body weight had a marked prophylactic effect. Swine given tetracycline in these amounts did not develop lesions of enzootic pneumonia when placed in contact with known infected animals. Daily drug dosages of five milligrams and one milligram per kilogram body weight resulted in incomplete inhibition of lesion development. No tests were conducted to determine if the lesionless animals were infected.

Betts and Campbell (1956) reported that drugs in the tetracycline group had a marked prophylactic effect with regard to swine enzootic pneumonia. Sulfamethazine, pencillin, streptomycin, and chloramphenicol had no prophylactic effect. None of the drugs tested had any apparent curative action. The period of treatment was four to seven days in each case. The authors felt that technical problems may have influenced results. Goret et al. (1960) and Goodwin and Whittlestone (1960) also felt that the

tetracycline group of antibiotics was the only means of preventing the development of swine enzootic pneumonia in susceptible animals. This antibiotic susceptibility suggested strongly to Goodwin and Whittlestone (1960) that the proposed virus etiology of enzootic pneumonia (Betts, 1953) might, indeed, be in error.

Takatori et al. (1967) believed that they had revealed a sensitivity of the swine enzootic pneumonia agent to oxytetracycline and to dihydrostreptomycin and a resistance to penicillin and sulfamonomethoxine. This work was performed with a very limited number of animals.

Mare' (1965) concluded from several trials that Tylosin tartrate had no prophylactic or therapeutic action on swine enzootic pneumonia. Likewise, furaltadone was ineffective against the disease agent. Mare' and Switzer (1966a) did show that chlortetracycline was effective in preventing the formation of the lesions of enzootic pneumonia when given at four hundred grams per ton in the feed.

Both Betts and Campbell (1956) and Mare' and Switzer (1966a) reported an inability to demonstrate the agent inducing enzootic pneumonia in the respiratory tissues of those animals treated prophylactically with the tetracycline group of drugs, but neither gave a detailed description of these recovery attempts.

The possibility that the agent inducing swine enzootic pneumonia may not be killed by drug treatments must be entertained. Slotkin et al. (1967) reported that chlortetracycline delayed colonization of Mycoplasma pneumoniae in Syrian hamsters but did not completely kill all organisms. Larin et al. (1967) confirmed this finding using an in vitro system. These results using a mycoplasma causing a disease in hampsters and man similar

to that caused by Mycoplasma hyopneumoniae in swine indicate the need for caution in evaluation of the prophylactic or curative effect of drugs.

Messersmith et al. (1967) reported that tissue residues in swine fed chlortetracycline at rates up to five hundred grams per ton for fourteen weeks were less than one part per million at all times, suggesting that the drug is safe to use up to marketing time. No adverse effects were observed with any treatment.

Dearborn et al. (1957) concluded that calcium ions interfere with the absorption of tetracycline drugs. MacDonald et al. (1964) concurred with this finding and suggested that food and milk be withheld for at least one-half hour after administration of the drug to permit maximal absorption.

Owen (1965) reported that placental transfer of the tetracycline drugs is efficient; blood levels of newborn pigs (six hours) were equal to those of the dam. The concentration of tetracyclines in the amniotic fluid was found to be twenty-five times the blood level.

Maddock et al. (1953) and Glawisching (1963) both reported that tetracycline compounds given to sows orally or intramuscularly were found in the milk of the sows and in the serum of pigs that suckled the sows. Little correlation was found between blood levels in sows and levels of drug excreted in the milk.

Schipper et al. (1956) found that they could treat enteritis in young pigs effectively by treating the sows with one to two grams of chlortetracycline. The young piglets presumably gained the drug through the sow's milk.

Mare' and Switzer (1966a) reported the causal agent of swine enzootic pneumonia, Mycoplasma hyopneumoniae, to be ether sensitive as determined by

in vitro tests. Another infectious agent known to be ether sensitive is the canine distemper virus. Donovan (1967) found that diethyl ether appeared to be an effective antiviral agent when added to the therapy for canine distemper. Ether inhalation therapy was used on fourteen dogs with classical signs of canine distemper; twelve made a rapid, complete, and uneventful recovery. Donovan (1968) reaffirmed this finding in experiments involving known canine distemper virus inoculum and susceptible dogs. All inoculated untreated control animals developed signs of canine distemper while none of the inoculated animals treated with ether inhalation became ill. The author believed that ether destroyed the lipid containing membrane of the virus *in vivo*; a chemotherapeutic approach to canine distemper treatment using diethyl ether was advocated.

MATERIALS AND METHODS

Swine Enzootic Pneumonia Agent (Mycoplasma hyopneumoniae)

L'Ecuyer (1962) transfered an agent to experimental pigs from a pig which originated in a large herd with chronic pneumonia. This material originated from the eleventh herd that he studied and was identified as number eleven. The apical and cardiac lobes of the lungs from which the inoculum was obtained were consolidated. Intranasal inoculation of respiratory disease-free pigs with this lung suspension consistently resulted in well demarcated, reddish-grey pneumonic lesions, generally involving the apical, cardiac and intermediate lobes. The microscopic lesions characteristically elicited by this agent were peribronchiolar and perivascular lymphoid hyperplasia, extensive alveolar interstitial thickening, and alveolar exudation of septal cells, mononuclear leucocytes, and in early cases, neutrophils.

Mare' (1965) found that an increase in virulence occurred when the pneumonia agent was serially passaged in pigs. During early passages, lesions were elicited in thirty to sixty percent of inoculated pigs. After seven serial passages, lesions were observed in eighty to one hundred percent of susceptible, inoculated pigs. During the course of his experiments, Mare' (1965) serially passaged the agent from the third to the tenth passage level in pigs.

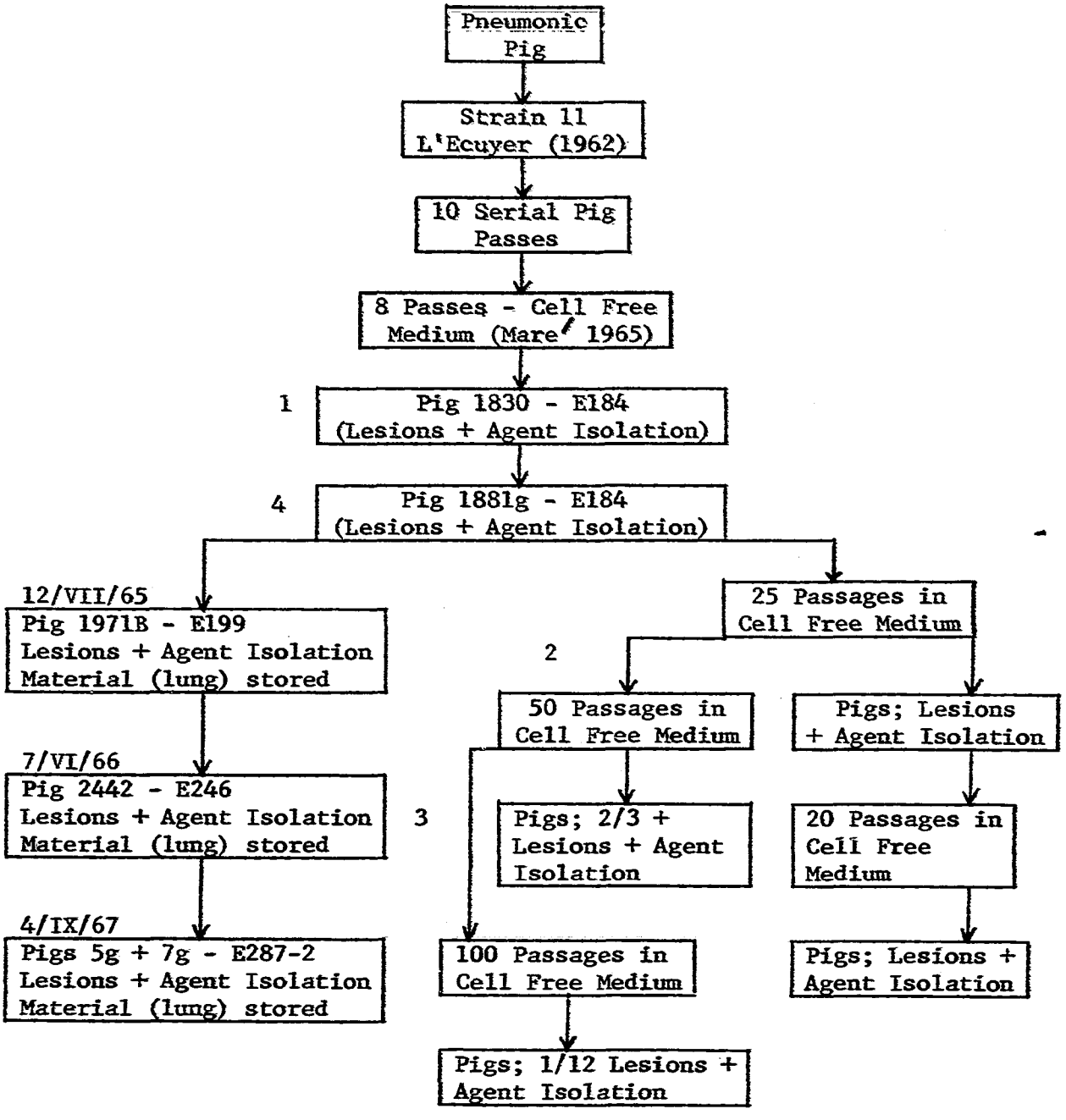
Both Mare' (1965) and L'Ecuyer (1962) serially transmitted this pneumonia utilizing the same general technique. Lesions from experimentally infected pigs were aseptically removed, cut into small pieces and stored at minus thirty degrees Centigrade. Routine bacteriological examination of

the infective lung material was performed by inoculation of the following media: 1) horse blood agar plates streaked with a micrococcal nurse colony, 2) modified MacConkey's agar (Ross, 1965), and 3) beef heart infusion broth with hemoglobin, swine gastric mucin, and turkey serum (Ross and Switzer, 1963). This strain of the swine enzootic pneumonia agent was consistently shown to be free of Mycoplasma hyorhinis, Mycoplasma granularum, Hemophilus spp., Pasteurella multocida, Bordetella bronchiseptica, and Streptococcus spp. It was shown to be free of swine influenza virus by inoculation of embryonated hen's eggs and intranasal inoculation of mice. No cytopathogenic viruses were isolated in primary swine kidney cell culture inoculated with the agent.

This strain of the swine enzootic pneumonia agent was identified as a mycoplasma and was characterized by Mare' (1965) and Mare' and Switzer (1965, 1966a, 1966b, 1966c). This is the type species and is referred to as Mycoplasma hyopneumoniae. Serological evidence accumulated by Roberts (1968) and Takatori et al. (1968) has confirmed the incrimination of this mycoplasma as the causative agent of swine enzootic pneumonia.

The majority of experimental inoculations made in the course of the present work were suspensions of pneumonic lung containing Mycoplasma hyopneumoniae; the agent underwent five passages in pigs since Mare' (1965) grew it in a cell-free medium. A detailed history of the swine enzootic pneumonia agent used is presented in Diagram 1.

Mycoplasma hyopneumoniae was observed to be more infectious for experimental pigs if stored in pneumonic lung tissue at minus thirty degrees Centigrade as compared to storage in an artificial medium. It was also advantageous to use lung lesion material as inoculum in order to change the



This strain has been used by

[1] Mare' and Switzer (1966a)

[2] Roberts (1968)

[3] Takatori et al. (1968)

and [4] Huhn (author).

Diagram 1. Passage history of M. hyopneumoniae (strain 11) used in the present study

infectivity and antigenicity of the organism as little as possible during the course of experimentation; this procedure kept the organism in its most natural medium, the pig, and minimized contact with artificial conditions.

Suspensions of infective lung used for inoculation of pigs and artificial media were prepared by grinding the tissue in a glass tissue grinder with Dulbecco's phosphate buffer (Dulbecco and Vogt, 1954) plus twenty percent swine serum, five-tenths percent lactalbumin hydrolysate (enzymatic), one percent yeast extract (Mare', 1965), and one-fourth percent dextrose. A ten percent suspension prepared in this way was centrifuged at two thousand G for ten minutes to sediment cellular debris. The supernatant fluid was either used directly or was filtered through cellulose membranes[1] of eight hundred millimicrons average pore diameter.

In order to determine the infective titer of the suspension of pneumonic lung used, one of the major collections used as inoculum in this work was titered in experimental pigs. Four pigs per dilution were inoculated intranasally with two milliliters of tenfold dilutions of a pneumonic lung suspension. It was found that one milliliter of the unfiltered lung suspension contained approximately five million lesion producing doses$_{50}$ (LPD$_{50}$). The method of Reed and Muench (1938) was used in deriving this figure.

Artificial Media

Swine mycoplasma media

Attempts were made to propagate Mycoplasma hyopneumoniae from lung

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[1]Millipore Filter Corporation. Bedford, Massachusetts.

lesions in many of the experiments. Growth in fluid medium was evaluated by examination for turbidity, sediment, pH change, and by microscopic examination of Giemsa-stained sediment for evidence of mycoplasma. Growth on solid media was determined by microscopic examination using a binocular dissecting microscope and oblique lighting. The inoculum was usually a ten percent suspension of pneumonic lung clarified by centrifugation and sometimes by filtration through membranes of eight hundred millimicrons average pore diameter. Up to ten blind passes were made in liquid media at two to five day intervals before a negative finding was declared.

Several types of fluid media were employed in this study. A brief description of them follows.

Beef heart infusion medium The beef heart infusion medium (BHI) described by Ross and Switzer (1963) for the growth of Mycoplasma granularum and Mycoplasma hyorhinis was utilized; this medium is satisfactory for detection of these two common swine mycoplasma. This medium contained two-tenths percent hemoglobin, five-tenths percent swine gastric mucin, twenty percent turkey serum, penicillin, and thallium acetate prepared as described by Ross and Switzer (1963).

Dulbecco phosphate buffer medium The Dulbecco phosphate buffered medium (DPB) used for the isolation of Mycoplasma hyopneumoniae was prepared as follows:

1. Dulbecco's phosphate buffer[1]	79 ml.
2. swine serum[2]	20 ml.

[1] Prepared as described by Dulbecco and Vogt (1954).

[2] Swine serum was obtained from respiratory-disease free pigs and was unheated.

3.	yeast extract[1]	1 ml.
4.	lactalbumin hydrolysate (enzymatic)[2]	.5 gm. / 100 ml.

The ingredients were mixed together and stirred until the lactalbumin hydrolysate had dissolved. After the pH was adjusted to 7.2-7.4, the medium was sterilized by passage through a Selas number 02 filter and dispensed into tubes or flasks. This medium was incubated twenty to forty-eight hours to check for contaminants.

DPB - BHI medium This medium consisted of equal volumes of BHI liquid medium and DPB liquid medium. The mixture was adjusted to a pH of 7.2-7.4, sterilized by passage through a Selas number 02 filter, and dispensed into tubes or flasks.

DPB - dextrose medium The basic DPB liquid medium was enriched with twenty-five hundredths percent dextrose[3], by weight.

DPB - dextrose, acid-adjusted swine serum medium This medium had the DPB - dextrose liquid medium as its base. The unheated swine serum from the respiratory-disease free pigs was replaced by swine serum which had gone through an acid adjustment procedure as follows:

1. Thaw frozen swine serum (fresh serum can be used).
2. Using 1 N hydrochloric acid, bring pH of serum to 4.2-4.6 (do not go below 4.2).
3. Refrigerate serum (4°C.) for one to eighteen hours.

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[1] Prepared as described by Mare' (1965).

[2] Nutritional Biochemicals Corporation. Cleveland, Ohio.

[3] Dextrose (Bacto-Dextrose) Difco Laboratories. Detroit, Michigan.

4. Centrifuge at 2,000 RPM for fifteen minutes.
5. Filter through Whatman glass fiber paper (G/A).
6. Clarify further by passing serum through Selas filters--numbers 10, 01, and 02.
7. Bring pH to 7.0, using 1 N NaOH.
8. Freeze for further use.

The medium was otherwise prepared in the same manner as the DPB - dextrose liquid medium.

DPB - agar medium *Mycoplasma hyopneumoniae* was cultured on a solid medium several times during the experiments. This medium was prepared as follows:

Solution A.

1. Noble agar[1]	1 gm.
2. Dulbecco's phosphate buffer solution	50 ml.

Solution B.

1. Dulbecco's phosphate buffer solution	29 ml.
2. Swine serum (unheated)	20 ml.
3. Yeast extract	1 ml.
4. Lactalbumin hydrolysate	0.5 gm.

Solution A was autoclaved (fifteen pounds of pressure for fifteen minutes) and then cooled to 45°C. Solution B was mixed, adjusted to pH 7.2, filter sterilized and warmed to 45°C. Solutions A and B were then mixed aseptically and plates of agar were poured and incubated for a sterility check before using.

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[1]Difco Laboratories. Detroit, Michigan.

Inoculations onto solid medium were made with a ten percent suspension of pneumonic lung or with a broth culture of Mycoplasma hyopneumoniae. The inoculum was allowed to dry onto the agar surface; a streak inoculum of a micrococcus was then made across the plate.

All inoculated media were incubated at thirty-seven degrees Centigrade.

Histological Procedures

Tissue sections

Tissues were collected and immersed in fixative within ten minutes after death in all experiments except the trial involving animals from commercial swine herds. In the latter case, the tissues were fixed within one hour after collection from the packing plant. Ten percent buffered formalin was used as the fixative. In all cases a minimal fixation time of forty-eight hours was allowed. Tissues were then removed, trimmed and processed immediately, or were stored in seventy percent ethyl alcohol. Tissues were embedded in Paraplast[1] tissue embedding medium, sectioned at six microns and mounted on glass slides with an albumin fixative. Sections were stained with Giemsa or with Harris' hematoxylin and counterstained with eosin Y as described in the U. S. Armed Forces Institute of Pathology (1960) "Manual of Histologic and Special Staining Technics".

Touch preparations

Touch preparations were prepared by gently pressing clean microscope slides against freshly cut surfaces of swine enzootic pneumonia lung lesions. The impressions were then dried using very mild heat. After

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[1] ALOE Scientific. St. Louis, Missouri.

fixing in methyl alcohol for three minutes, the slides were stained for ninety to one hundred twenty minutes in two percent Giemsa solution in distilled water. The slides were then washed in tap water and air-dried prior to microscopic examination.

Liquid culture stains

Culture media suspected of containing organisms were centrifuged at four thousand G for fifteen to twenty minutes; the supernatant fluid was removed, and smears were made from the button in the centrifuge tube. The smears were dried using mild heat, fixed in methyl alcohol for three minutes and stained with Giemsa solution as described for touch preparations.

Experimental Pigs

The pigs used in experiments involving artificial exposure to Mycoplasma hyopneumoniae were procured from three sources. A few were obtained from the Department of Veterinary Microbiology and Preventive Medicine, Iowa State University; these were surgically-derived, colostrum-deprived pigs reared in isolation.

The majority of the animals used for experiments were procured from the respiratory disease-free herd maintained at the Veterinary Medical Research Institute. This herd of Hampshire and Yorkshire breeds was established in 1951 with surgically derived breeding stock; all subsequent introductions into the herd have been surgically derived. The experimental pigs from this source were naturally farrowed and were raised with the sows until weaning. Clinical, pathological, and microbiological examinations performed annually on several hundred pigs from this herd have con-

firmed it to be free of Mycoplasma granularum, Mycoplasma hyorhinis, Mycoplasma hyopneumoniae, Pasteurella spp., and Bordetella bronchiseptica. Occasionally, a Hemophilus spp. was isolated from the nares of animals raised in this herd but such isolations were never made from lung material in the course of experimentation.

For one of the experiments, groups of eleven to twenty-six pigs were purchased from several purebred Yorkshire herds in central Iowa, northern Iowa, and southern Minnesota. These herds were selected on the basis of owner cooperation and because of attempts of the owners to maintain good health standards in their herds. Two of the herds had originated from surgically derived stock in a depopulation-repopulation scheme. The selection of these herds was also based on the possibility that they would have some animals with swine enzootic pneumonia.

In all possible cases, pigs were selected and placed in their respective experimental group through utilization of an approved randomization method.

The intranasal route of inoculation was used to infect the pigs unless otherwise stated. The pigs were held in a vertical position with the snout pointing upward. The inoculum was then administered through the use of a syringe with an attached urethral catheter which was placed in the nare of the pig. Small amounts of inoculum were administered with each respiratory effort of the pig. Anesthesia was never used in the inoculating procedure.

Pigs were necropsied after observation periods ranging from three days to sixteen or more weeks. The usual procedure was to electrocute and then exsanguinate the pigs, the exceptions being those animals that died before termination of an experiment. All animals were examined for gross lung

lesions; specimens were collected for microscopic examination. Where possible, specimens were also collected aseptically for microbiological examination. If the microbiological examination was not carried out immediately, the tissues were stored at minus thirty degrees Centigrade.

Drug Sensitivity

Ether

An experiment was performed to determine the in vivo sensitivity of Mycoplasma hyopneumoniae to diethyl ether[1]. The experiment was designed to determine the effect of diethyl ether administered by inhalation on the development of swine enzootic pneumonia lesions.

A total of twenty-two pigs approximately four to six weeks of age were used. These were divided into four groups of four pigs each and one group of six pigs; each group was housed in strict isolation. Three groups were challenged intranasally with two milliters of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae; one group of pigs served as uninoculated control animals, while the last group was challenged with a lung suspension gained from one of the artificially inoculated, etherized group of pigs.

One of the artificially challenged groups of pigs served as inoculated untreated controls. Another group was subjected to ether inhalation on the fourth and sixth days postinoculation. The third group of artificially inoculated pigs was subjected to ether inhalation on the sixth and eighth

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[1]Ether, anhydrous. J. T. Baker Chemical Company. Phillipsburg, New Jersey.

days postinoculation.

Ether-induced anesthesia was obtained by using a nose cone partially filled with gauze. Liquid ether was poured over the gauze while the cone was held over the pig's nose. Light to medium depth surgical anesthesia was maintained for fifteen minutes each time that the pigs were anesthetized. Three weeks postinoculation, pigs from four groups were killed and examined for gross lesions of swine enzootic pneumonia. The location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

A lung suspension was prepared from material collected at necropsy from the pigs that were anesthetized on the sixth and eighth days after challenge. This suspension was given intranasally (two milliliters each) to the fifth group of four pigs. These pigs were killed and examined for gross lesions of swine enzootic pneumonia three weeks after challenge. The location and extent of lesions was recorded. Specimens for histological and microbiological examination were collected.

Tylosin

An experiment was performed to determine the in vivo sensitivity of Mycoplasma hyopneumoniae to Tylosin tartrate[1] (water-soluble). This experiment was designed to determine the effect of Tylosin tartrate administered in the drinking water on the development of lesions of swine enzootic pneumonia.

Seven pigs approximately eight to ten weeks old were divided into two groups, each housed in separate isolation units. One group was put on drug

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[1]Tylosin Tartrate. Elanco Products Company. Indianapolis, Indiana.

(2 gm./gallon) for the duration of the experiment; this same group was challenged with a total of three milliliters of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae. The intranasal route of inoculation was used. The other inoculated group of pigs received untreated water.

Twenty-four days after inoculation all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Erythromycin

An experiment was performed to determine the in vivo sensitivity of Mycoplasma hyopneumoniae to erythromycin[1]. The experiment was designed to demonstrate the effect of erythromycin given in the drinking water on the development of lesions of swine enzootic pneumonia.

Twelve pigs approximately eight to ten weeks old were divided into three groups of four; each group was housed in a separate isolation unit.

Two of the three groups were inoculated intranasally with two milliliters of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae. One of the inoculated groups was placed on the drug erythromycin (460 mg./gallon) for three days prior to inoculation; this treatment continued until the animals were removed from the trial. The other inoculated group received untreated water.

Twenty-one days after inoculation all pigs were killed and examined

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[1]Erythromycin. Amdal Company, Division of Abbott Laboratories. North Chicago, Illinois.

for gross lesions of swine enzootic pneumonia. The location and extent of lesions were recorded; specimens were collected for histological and microbiological examination.

Chlortetracycline

An experiment was performed to determine if pigs receiving feed containing varied levels of chlortetracycline[1] at the time of inoculation with Mycoplasma hyopneumoniae would develop lesions of swine enzootic pneumonia. The drug treatments continued throughout the trial.

Sixteen pigs three to five weeks of age were divided into groups of four which were housed in separate isolation units. The treatments were as follows:

1. Basal ration including two hundred grams of chlortetracycline per ton.
2. Basal ration including one hundred grams of chlortetracycline per ton.
3. Basal ration including fifty grams of chlortetracycline per ton.
4. Basal ration alone, free of antibiotics.

The groups were placed on the treatments one week before being challenged with a suspension of lung tissue containing Mycoplasma hyopneumoniae. Each pig received six milliliters of this suspension, two milliliters being administered on three occasions.

Twenty-six to thirty-four days after challenge all pigs were killed

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[1] Aureomycin (chlortetracycline). American Cyanamid Company. Pearl River, New York.

and examined for gross lesions of swine enzootic pneumonia. The location and extent of lesions were recorded; specimens were collected for histological and microbiological examination.

Age Susceptibility

A belief common to many concerned with swine enzootic pneumonia is that young piglets are uniformly susceptible to the causative agent. Two experiments were performed to determine the validity of this assumption.

Procedures common to these two experiments as well as to several following experiments involving very young piglets were as follows: pregnant sows from the Veterinary Medical Research Institute herd were brought to isolation units to farrow. The newly born pigs were allowed to remain with the sow for ten to twelve days. Each piglet was given an intramuscular injection of iron[1] at three and at ten days of age. When removed from the sow, the piglets were transferred in sterilized containers. They were placed in wire-bottomed rearing boxes which were housed in isolation units. These boxes were supplied with heat lamps, self feeders and a continuous flow water system.

Initially the young pigs received a dry starter ration and water *ad libitum* plus a milk replacer[2] given daily at four spaced intervals. The number of milk replacer feedings was gradually reduced until the pigs were eating only the dry feed. The pigs remained in the rearing boxes until the

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[1] Iron, Armidexan. 100 mg/cc. Bradley Products Company. Bradley, Illinois.

[2] SPF-Lac. Borden Chemical Company. Norfolk, Virginia.

termination of the experiment.

Inoculation at three days

A sow was allowed to farrow in an isolation unit. When the piglets (7) were three days old, each was given three-quarters milliliter of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae; the route of inoculation was intranasal.

At ten days of age the infected piglets were removed from the sow and placed in rearing boxes. Twenty-one days postinoculation, all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Inoculation at sixteen hours

A sow was allowed to farrow in an isolation unit. When sixteen hours old the piglets (8) were each inoculated intranasally with three-quarters milliliter of a ten percent suspension of lung containing Mycoplasma hyopneumoniae.

When ten days of age the infected piglets were placed in rearing boxes for the remainder of the experiment. Twenty-one days after inoculation all pigs were killed and examined for gross lesions of swine enzootic pneumonia. The location and extent of lesions were recorded; specimens were collected for histological and microbiological examination.

Treatment Schemata

Several treatment schemes were investigated using chloretetracycline

or a derivative of it, demethylchlortetracycline[1].

Demethylchlortetracycline without milk

An experiment was performed to determine the in vivo sensitivity of Mycoplasma hyopneumoniae to demethylchlortetracycline. Six, ten days old piglets were placed in an isolation unit. All piglets were challenged with one milliliter of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae. At the time of inoculation four of the pigs were placed on demethylchlortetracycline (one hundred fifty milligrams given per os at two day intervals); two piglets were left untreated. The pigs were fed milk replacer four hours after administration of the antibiotic; milk replacer was never given with the antibiotic. The inoculated but untreated control pigs remained in contact with the treated animals.

Twenty-four days after inoculation with Mycoplasma hyopneumoniae, all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Chlortetracycline without milk

Experiment one An experiment was performed to determine the in vivo sensitivity of Mycoplasma hyopneumoniae to chlortetracycline and to determine the ability of this antibiotic to clear infected pigs of the organism.

Twenty-eight, ten day old piglets were placed in several isolation units. Five piglets housed separately served as uninoculated control

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[1]Aureomycin (chlortetracycline) and Declomycin (demethylchlortetracycline). American Cyanamid Company. Pearl River, New York.

animals; two of these animals received seventy-five milligrams chlortetracycline per os daily; three were untreated. The twenty-three remaining piglets were all inoculated intranasally with one milliliter of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae. Twelve pigs were placed on a treatment of seventy-five milligrams chlortetracycline per os daily. Eleven of the challenged animals were left untreated. Untreated and treated animals were in the same isolation unit but were in different rearing boxes.

At three, seven, ten, fourteen, seventeen, and twenty-one days post-inoculation, two pigs each were taken from the treated and untreated rearing boxes. These pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Fifty-three pigs were placed in isolation units; four to seven piglets occupied each unit. Each unit of pigs was inoculated intranasally with two milliliters of a ten percent suspension of lung material collected from each two piglets killed in the primary portion of this trial. These inoculated piglets were then kept in the isolation units for three weeks; no antibiotic treatment was administered during this time. After three weeks observation all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Experiment two An experiment was performed to determine the ability of chlortetracycline to not only prevent development of lesions but also to clear swine lungs of Mycoplasma hyopneumoniae. Eight, sixteen day old piglets were placed in an isolation unit. All piglets were inoculated

intranasally with two milliliters of a suspension of lung tissue containing Mycoplasma hyopneumoniae. Six of the eight piglets were given one hundred milligrams chlortetracycline per os daily from the time of inoculation. No milk replacer was given within four hours of treatment.

Three weeks after inoculation, two treated pigs and the two infected untreated control animals were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

The four remaining treated pigs were treated for two more weeks (five weeks total) and were maintained without treatment for three more weeks. They were then killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Chlortetracycline with milk

Experiment one An experiment was performed to determine the effectiveness of chlortetracycline given orally with milk in preventing the development of swine enzootic pneumonia lesions in pigs inoculated with Mycoplasma hyopneumoniae. Eight, twelve day old piglets were placed in an isolation unit. Each pig was inoculated intranasally with one milliliter of a suspension of lung tissue containing Mycoplasma hyopneumoniae. Four pigs were given a daily oral treatment of fifty milligrams chlortetracycline; immediately after treatment, these pigs were allowed to drink milk replacer ad libitum. The remaining four pigs were not given the antibiotic. Treated and untreated animals were kept in the same isolation unit but were placed in separate rearing boxes.

Twenty-eight days after initial exposure to Mycoplasma hyopneumoniae,

all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the extent and location of lesions were recorded. Specimens were collected for histological and microbiological examination.

Experiment two An experiment was performed to determine the effectiveness of chlortetracycline administered orally to suckling pigs in preventing the development of swine enzootic pneumonia lesions. Seven, four-hour old piglets were inoculated intranasally with one-half milliter of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae. Two days postinoculation, four piglets were marked and treated with fifty milligrams chlortetracycline per os daily until the termination of the experiment. The pigs were weaned and moved to an isolation unit at ten days of age where milk replacer was given with the drug.

Twenty-one days after exposure to Mycoplasma hyopneumoniae all pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Postinoculation treatment

An experiment was performed to determine the efficacy of chlortetracycline in preventing the development of swine enzootic pneumonia lesions and in clearing inoculated pigs of Mycoplasma hyopneumoniae. Sixteen pigs approximately seven weeks of age were divided into three groups (A, B, and C) and housed in isolation units. All pigs were inoculated intranasally with four milliliters of a ten percent suspension of lung tissue containing Mycoplasma hyopneumoniae.

Seven days postinoculation, group A was placed on a ration containing four hundred grams chlortetracycline per ton. Twenty-eight days postinocu-

lation three pigs from this group were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination. The three remaining pigs were removed from the antibiotic treatment for twenty-one days and were then killed and examined as described above.

Fourteen days postinoculation, group B was placed on a ration containing four hundred grams chlortetracycline per ton. Thirty-five days postinoculation three pigs from this group were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination. The three remaining pigs were removed from the antibiotic treatment for twenty-one days and were then killed and examined as described above.

Group C constituted the inoculated, untreated control group. Twenty-one days after exposure to Mycoplasma hyopneumoniae all pigs in this group were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination.

Transmission prophylaxis

An experiment was performed to determine the efficacy of chlortetracycline in preventing the transmission of Mycoplasma hyopneumoniae-induced swine enzootic pneumonia from infected pigs on drug to susceptible, undrugged pigs.

Four pigs were placed in an isolation unit and were inoculated intranasally with six milliliters of a ten percent suspension of lung tissue

containing Mycoplasma hyopneumoniae. Twenty-eight days after inoculation an additional three pigs were added to the unit; the two groups of pigs were kept in contact for ten days. At this time the artificially exposed pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological study. The three naturally exposed pigs were observed for an additional twenty-two days; they were then killed and examined in the manner described above.

A second group of pigs was placed in an isolation unit and was artificially exposed to Mycoplasma hyopneumoniae as described previously. Twenty-one days after inoculation these pigs were placed on a ration containing four hundred grams chlortetracycline per ton.

When these pigs had been on drug for seven days, an additional four pigs were added to the isolation unit. These pigs were fed an antibiotic-free ration, but were in physical contact with the artificially exposed, drugged pigs. After ten days of contact, the artificially inoculated pigs were killed and examined for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological and microbiological examination. The four naturally exposed pigs were observed for an additional twenty-two days; they were then killed and examined in the manner described above.

Field Infections

An experiment was performed to determine: 1) the effect of chlortetracycline on the incidence of field cases of swine enzootic pneumonia, 2) the effect of chlortetracycline on lesion resolution in field cases of

swine enzootic pneumonia, 3) the effect of chlortetracycline on the average daily gain of pigs with field cases of swine enzootic pneumonia, 4) the effect of field cases of swine enzootic pneumonia on the average daily gain, and 5) the effect of field cases of swine enzootic pneumonia on carcass conformation.

Objectives extraneous to these but concerned with swine enzootic pneumonia were concomitantly pursued, utilizing the same experimental animals. Possible effects of the manipulations involved in these extraneous objectives will be explained later.

Experimental design

Source and disposition of swine Groups of eleven to twenty-six pigs were purchased from seven purebred Yorkshire breeders (herds 1-7) located in central Iowa, northern Iowa, and southern Minnesota. An eighth group of twenty animals (herd 8) was supplied from the research herd at the Veterinary Medical Research Institute. The number of animals per herd and the average weight at the initiation of the trial are listed in Table 1.

The pigs from each herd (1-7) were divided into two groups. One group was fed a ration containing no antibiotics[1]; the other group was fed a ration containing antibiotics[2]. The antibiotics and their amounts were Aureomycin (chlortetracycline, 100 gm./ton), sulfamethazine (100 gm./ton), and penicillin (50 gm./ton); of these three, only chlortetracycline has been reported as having an effect on swine enzootic pneumonia. The rations were fed ad libitum in self feeders; water was available from automatic

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[1]Growina--plain. Ralston Purina. St. Louis, Missouri.

[2]Growina--250. Ralston Purina. St. Louis, Missouri.

Table 1. Distribution and average weight of pigs at initiation of experiment

Herd number	Number of pigs	Average starting weight (lbs.)
1	26	44.5
2	13	74.0
3	12	49.0
4	14	59.0
5	12	47.0
6	11	48.0
7	12	56.0
8	20	87.0
Total	120	59.7

fountains.

Each herd (1-7) was initially composed of two groups, one on antibiotic feed and one on feed free of antibiotics. Early in the experiment, on the basis of a serological test (extraneous objective) each group was again divided into one composed of animals reacting positively in the test and another composed of animals reacting negatively to the test. Each herd, potentially, could be housed in four separate pens. Herd number eight was used to demonstrate the reality of in-contact transmission of swine enzootic pneumonia in this experiment. The twenty pigs were divided into five groups of four pigs each. Twenty-four days after initiation of the trial these groups (herd 8) were placed with pigs receiving the antibiotic-free ration; three groups were placed in pens with pigs reacting negatively to

the serological test and two groups were placed in pens with pigs reacting positively to the serological test.

The manipulations of the pigs during the trial consisted of the preliminary grouping movements and the periodic procurement of blood samples from all animals. The majority of the blood samples were collected through utilization of an orbital-sinus bleeding technique (Huhn et al., 1969). Sampling time per pig was about one minute; samples were drawn at ten day intervals.

Housing An area containing eighty individual, concrete-floored pens, each with an individual six by eight foot house was used. The pens for animals fed antibiotics were separated by approximately thirty yards of air space from the pens for animals fed no antibiotics. This distance was believed to be adequate to prevent aerosol spread of the enzootic pneumonia agent between the two areas. In addition, the pigs from each herd within a housing area were separated from other herds by an empty pen and two solid board fences.

Collection of information When the pigs reached market weight, they were marketed through a U.S.D.A. inspected packing plant. Arrangements were made to identify carcasses, to collect the lungs after inspection, and to obtain standard carcass measurements and quality evaluations. The lungs were returned to the Veterinary Medical Research Institute within one hour of collection and evaluated for gross lesions of swine enzootic pneumonia; the location and extent of lesions were recorded. Specimens were collected for histological examination.

Data analysis

The information obtained from the experiment was prepared for an analy-

sis of variance and a complete least squares constants analysis. The effects of herd of origin, feed type, sex, pneumonia, and marketed weight on performance and carcass characteristics were considered. Pneumonia was examined as both a dependent and an independent variable; the analysis was performed using the information gained from gross evaluation of pneumonic lesions.

A least squares constants analytical procedure was performed to determine the predicted effect of various degrees of pneumonia on rate of gain with the effect of all other variables removed.

Gross lesions of pneumonia were categorized for analysis as follows:

(0) = no pneumonia; no gross lesions.

(1) = very mild pneumonia; few lesions, less than five percent of any lobe.

(2) = mild pneumonia; multiple lobes involved but less than ten percent of any one lobe.

(3) = moderate pneumonia; up to thirty percent of one or two lobes involved, other lobes less involved.

(4) = moderate to severe pneumonia; over fifty percent of at least two lobes involved, other lobes less involved.

(5) = severe pneumonia; one hundred percent of at least two lobes involved, other lobes less involved but still extensive.

(6) = very severe pneumonia; one hundred percent of apical and cardiac lobes involved, up to forty percent of diaphragmatic lobes involved.

Microscopic lesions were categorized for analysis as follows:

(0) = no lesions.

(1) = lesions typical of swine enzootic pneumonia.

(2) = lesions typical of swine enzootic pneumonia plus bacterial infection.

(3) = atypical lesions.

(4) = slight peribronchiolar lymphoid hyperplasia and infiltration.

(5) = lesions of swine enzootic pneumonia plus scarring.

A small number of Hampshire pigs were included in the trial but were deleted from the statistical analysis.

RESULTS

Drug Sensitivity

Ether

The prophylactic effect of ether inhalation in pigs infected with Mycoplasma hyopneumoniae was determined. At necropsy all four infected, untreated pigs had gross lung lesions resembling swine enzootic pneumonia; from ten to thirty percent of the lungs were involved. The most commonly involved lobes were the apical, cardiac, and intermediate although portions of the diaphragmatic lobes also exhibited lesions. Microscopic examination revealed extensive lymphoid hyperplasia closely related to the bronchioles, resulting in obliteration of considerable areas of alveolar tissue. Neutrophils were present but were scarce. There was a considerable increase in the cellularity of the alveolar walls with septal cells being found in the lumen of the alveoli. These lesions were considered typical of swine enzootic pneumonia.

Nine of ten infected pigs that were treated with ether had gross lesions of pneumonia at necropsy, involving approximately five to forty percent of the lungs. Microscopic examination revealed lesions typical for swine enzootic pneumonia. The characteristic microscopic lesions observed in inoculated pigs are illustrated in Figures 2, 3, and 4.

All four pigs infected with lung material taken from the group of pigs infected on day zero and anesthetized on days six and eight had gross lesions of pneumonia at necropsy; from five to thirty percent of the lungs were involved. Microscopic examination revealed lesions typical of swine enzootic pneumonia. Cultures from these lungs were free of bacteria;

Figure 1. Lung from uninoculated control pig. Note absence of lesions in alveolar septa and in region of bronchioles. Hematoxylin and eosin stain. X100

Figure 2. Peribronchiolar lymphoid hyperplasia, septal cell proliferation, and neutrophil infiltration. Lesions from pig infected with lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X100

Figure 3. Peribronchiolar and perivascular lymphoid hyperplasia with septal cell proliferation and neutrophil infiltration. Lesions from pig anesthetized with ether 4 and 6 days after inoculation with a lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X35

Figure 4. Peribronchiolar lymphoid hyperplasia with septal cell proliferation and neutrophil infiltration. Lesions from pig anesthetized with ether 4 and 6 days after inoculation with a lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X100

Mycoplasma hyopneumoniae was recovered from three out of four cases.

None of the four uninoculated, untreated control animals had gross or microscopic lesions at necropsy. The appearance of lung tissue in an uninoculated control pig is illustrated in Figure 1.

Tylosin

The in vivo sensitivity of Mycoplasma hyopneumoniae to Tylosin tartrate was determined. The antibiotic was administered continuously in the drinking water. One of three infected untreated pigs had typical gross lesions of swine enzootic pneumonia at necropsy. The lungs of the other two pigs were edematous and reddened in the anterior and ventral lobes but consolidation and color typical of swine enzootic pneumonia were not seen. Microscopic examination revealed typical lesions of swine enzootic pneumonia in two of these three pigs.

At necropsy, two of four infected and treated pigs had gross lesions involving five to ten percent of the lungs. Two showed atypical pneumonic lesions. All four showed microscopic lesions typical of swine enzootic pneumonia. The characteristic appearance of the microscopic lesions is illustrated in Figures 5 and 6. The lungs of all seven pigs were free of Bordetella bronchiseptica, Pasteurella multocida, and Hemophilus spp. In every pig, Mycoplasma hyopneumoniae was recovered; the culture from pig 2442g grew exceptionally well and was kept for use in antigen studies. Colony morphology and the appearance of stained organisms grown in broth culture are illustrated in Figures 7 and 8.

Erythromycin

The in vivo sensitivity of Mycoplasma hyopneumoniae to erythromycin was determined. The antibiotic was administered continuously in the drink-

Figure 5. Peribronchiolar and perivascular lymphoid hyperplasia with septal cell proliferation and neutrophil infiltration. Note lack of functional alveoli. Lesions from Tylosin-treated pig infected with lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X35

Figure 6. Peribronchiolar lymphoid hyperplasia. Note marked lymphoid reaction in submucosa of bronchiole. Lesions from Tylosin-treated pig infected with lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X420

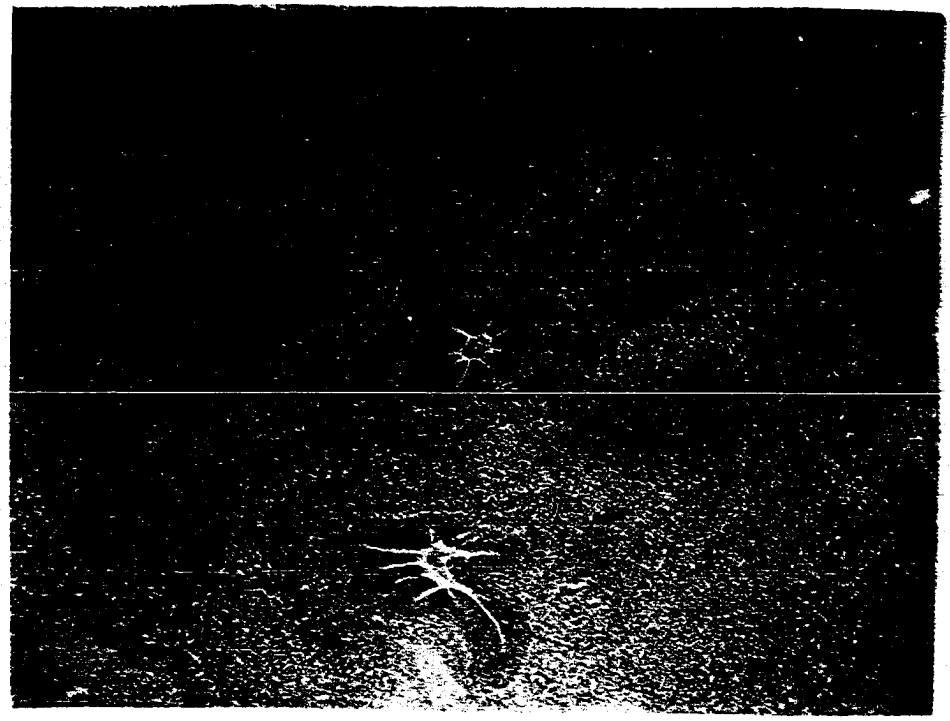
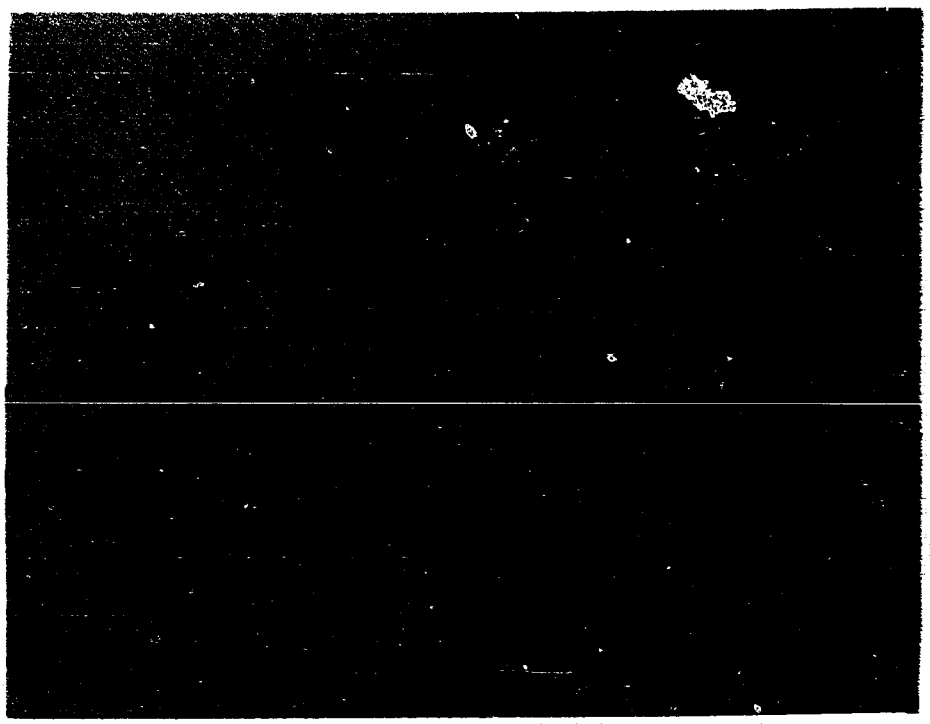


Figure 7. Colonies on agar surface inoculated with suspension of pneumonic lung 3 days previously. Note distinct central raised areas on larger colonies. X70

Figure 8. Mycoplasma hyopneumoniae, 31st passage in cell free medium. Clusters of coccoid to coccobacillary organisms with numerous ring forms. Giemsa stain. X970



ing water. At necropsy, all four infected untreated pigs had gross pneumonic lesions involving approximately ten to thirty percent of the lungs. Microscopic examination revealed lesions typical of swine enzootic pneumonia in all four pigs.

All four infected pigs which were treated with erythromycin had gross lesions resembling swine enzootic pneumonia. The extent of lesions varied from minute pneumonic areas at the tips of the cardiac lobes in one pig to an involvement of approximately forty percent of the lungs in the two most severely affected pigs. The characteristic gross lesions are illustrated in Figure 9. Microscopic examination revealed lesions typical of swine enzootic pneumonia in all four pigs. In both treated and untreated infected pigs, the apical and cardiac lobes and the intermediate lobe were the most frequent location of the gross lesions; lesions were not confined to these areas.

Three pigs in the uninoculated, untreated control group showed no gross lesions of the lungs. The appearance of the lungs of a control pig is illustrated in Figure 10. The fourth pig had a slight adhesion of the right cardiac lobe to the right diaphragmatic lobe but did not exhibit lesions typical of swine enzootic pneumonia. Microscopic examination did not reveal lesions typical of swine enzootic pneumonia in any of these pigs.

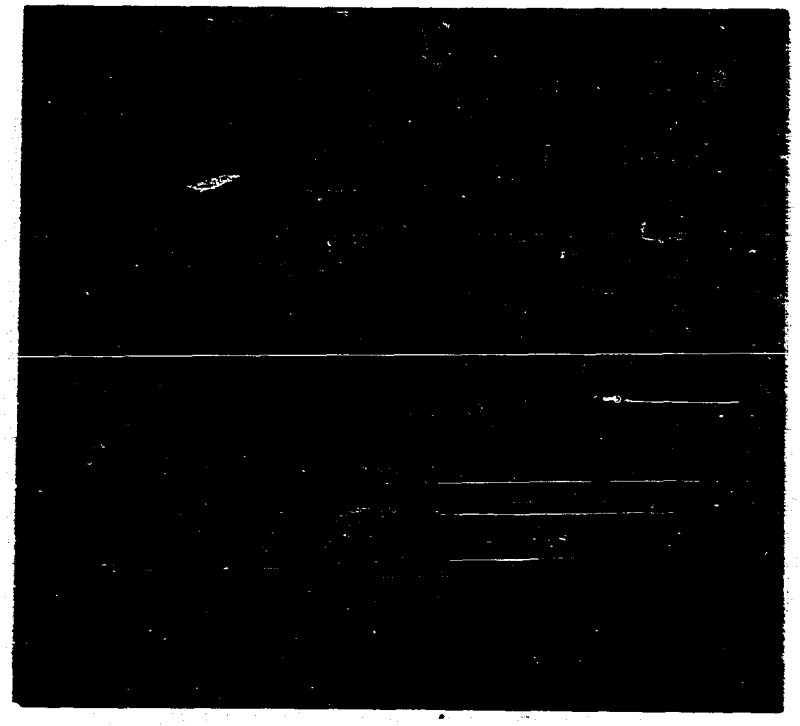
The lung tissues from these pigs were bacteriologically sterile. They were not cultured for Mycoplasma hyopneumoniae.

Chlortetracycline

The prophylactic effect of chlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. The antibiotic was administered continuously in the feed at several levels. The findings at necropsy and

Figure 9. Gross pneumonic lesions from pig infected with pneumonic lung suspension containing Mycoplasma hyopneumoniae

Figure 10. Normal lung tissue from uninoculated control pig



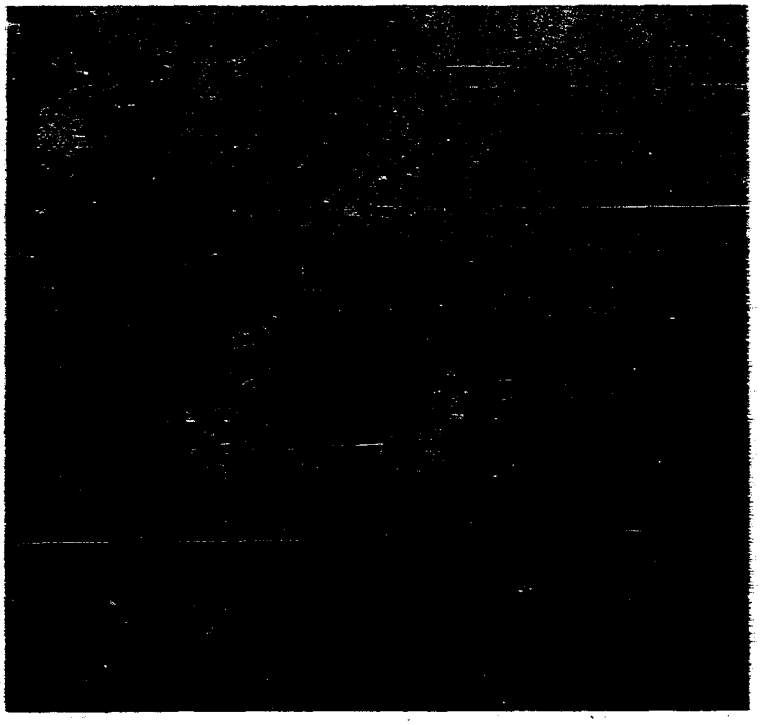


Table 2. Efficacy of chlortetracycline in preventing lesions of SEP

No. of pigs	Drug treatment	Day inoculated	Day killed	SEP lesions Gross	Micro.
4	200 gm/ton ctc	0	26	0/4	0/4
4	100 gm/ton ctc	0	26	0/4	0/4
4	50 gm/ton ctc	0	34	0/4	0/4
4	0 gm/ton ctc	0	34	4/4	4/4

the results of microscopic examination of tissue sections are presented in Table 2. Gross lesions involved three to twenty percent of the lungs in inoculated, untreated pigs. The absence of microscopic lesions in pigs receiving one hundred grams chlortetracycline per ton is illustrated in Figure 11. Characteristic microscopic lesions as found in the inoculated, untreated pigs are illustrated in Figure 12. Both gross and microscopic lesions were typical of swine enzootic pneumonia. The lungs of the pigs used in this trial were not cultured.

Age Susceptibility

Inoculation at three days

The susceptibility of young piglets to infection by Mycoplasma hyopneumoniae was determined. Seven pigs were inoculated intranasally with Mycoplasma hyopneumoniae at three days of age. At necropsy twenty-eight days later, all seven pigs had gross lesions of pneumonia involving five to fifty percent of the lungs. Characteristic gross lesions are illustrated in Figures 13 and 14. Microscopic examination revealed lesions typical of

Figure 11. Lung from pig inoculated with pneumonic lung suspension containing Mycoplasma hyopneumoniae. Pig was treated with 100 gm. chlortetracycline per ton of feed. Note absence of lesions in alveolar septa and in region of bronchioles. Hematoxylin and eosin stain. X100

Figure 12. Peribronchiolar lymphoid infiltration, septal cell proliferation, alveolar intersitial thickening, and neutrophil infiltration. Lesions from untreated pig inoculated with pneumonic lung suspension containing Mycoplasma hyopneumoniae. Hematoxylin and eosin stain. X100

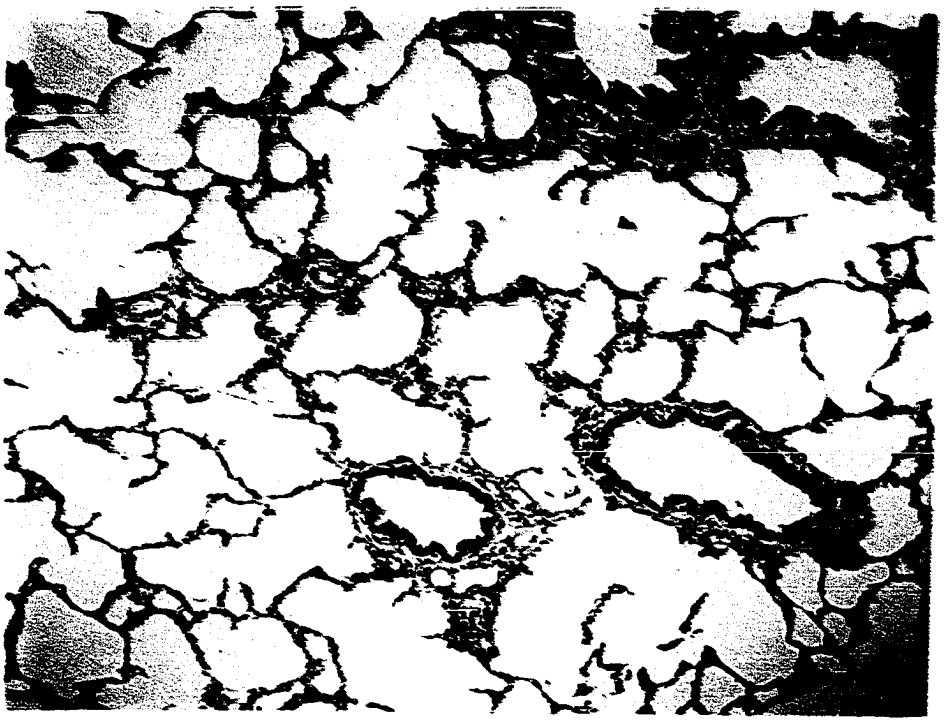
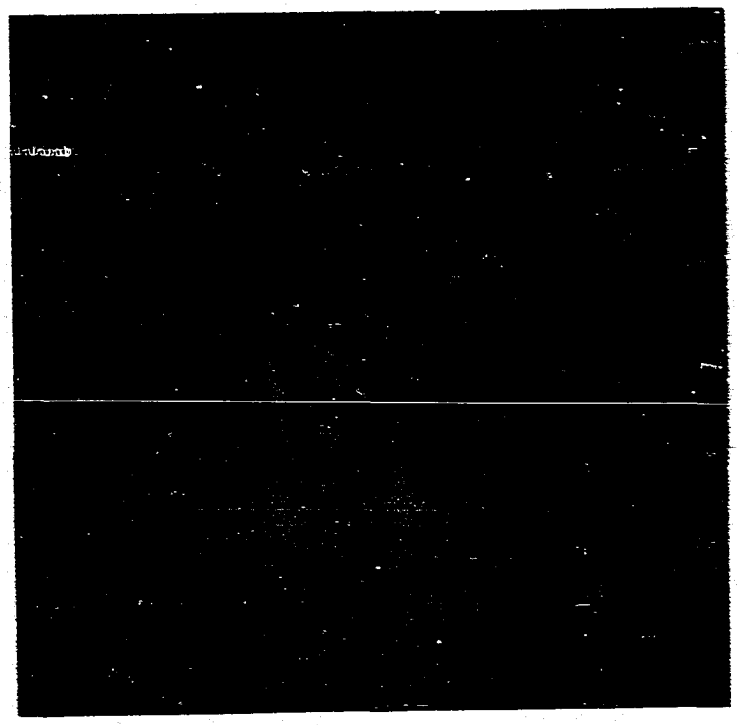
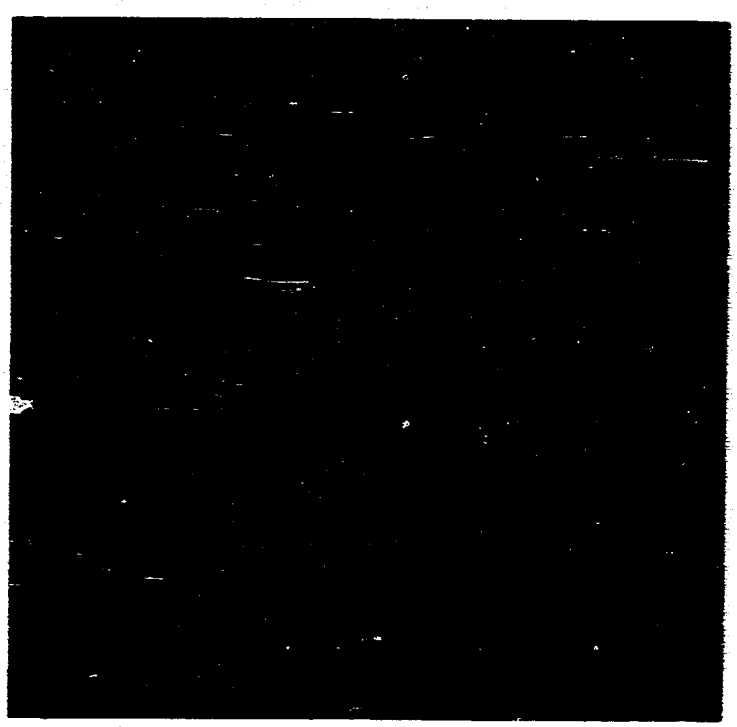


Figure 13. Gross pneumonic lesions from pig inoculated at 3 days of age with pneumonic lung suspenion containing Mycoplasma hyopneumoniae. Dorsal aspect; note extensive lesions in apical and cardiac lobes and involvement of anterior aspect of diaphragmatic lobes

Figure 14. Gross pneumonic lesions from pig inoculated at 3 days of age with pneumonic lung suspension containing Mycoplasma hyopneumoniae. Ventral aspect; note extensive lesions in apical and cardiac lobes, and intermediate lobe with involvement of portions of the diaphragmatic lobes





swine enzootic pneumonia in all cases. The lungs from all seven pigs were free of bacteria, Mycoplasma hyorhinis, and Mycoplasma granularum. The medium used to culture the specimens for Mycoplasma hyopneumoniae was contaminated, preventing detection of this organism.

Inoculation at sixteen hours

The susceptibility of young piglets to infection by Mycoplasma hyopneumoniae was determined. Eight piglets were inoculated intranasally with Mycoplasma hyopneumoniae when sixteen hours old. At necropsy twenty-one days later, seven pigs showed gross lesions typical of swine enzootic pneumonia. Involvement of the lungs varied from one to two percent up to sixty percent. Microscopic examination revealed lesions typical of swine enzootic pneumonia in those cases where gross lesions were observed. One pig exhibited neither gross nor microscopic lesions. The lungs of all pigs were free of bacteria, Mycoplasma hyorhinis, and Mycoplasma granularum; the medium used for isolation of Mycoplasma hyopneumoniae was contaminated with bacteria preventing the detection of this organism.

Treatment Schemata

Demethylchlortetracycline without milk

The prophylactic effect of demethylchlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. The antibiotic was administered orally; no milk was fed after treatment for four hours. At necropsy, both untreated infected pigs had gross lesions of pneumonia involving approximately thirty percent of the lungs. Microscopic examination revealed lesions typical of swine enzootic pneumonia. Mycoplasma hyopneumoniae was isolated in pure culture from both pigs.

None of four infected pigs treated with demethylchlortetracycline had gross lesions of pneumonia at necropsy. Microscopic examination revealed no lesions typical of swine enzootic pneumonia. Mycoplasma hyopneumoniae was not recovered from any of these animals.

Chlortetracycline without milk

Experiment one The prophylactic effect of chlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. No milk was given to the drugged animals within four hours of each daily dosing. The presence of Mycoplasma hyopneumoniae was determined by culturing in artificial media and by challenging susceptible pigs with suspensions of lungs taken at necropsy. The results of this experiment are presented in Table 3.

Chlortetracycline appears to be effective in preventing the development of lesions in pigs exposed to Mycoplasma hyopneumoniae. The clearance of the organism from lungs of treated pigs is not complete; this is indicated by the development of lesions in some pigs challenged with lung material taken from treated pigs that were previously challenged with Mycoplasma hyopneumoniae.

Experiment two The efficacy of chlortetracycline in preventing development of lesions and in clearing the pigs of the organism when infected with Mycoplasma hyopneumoniae was determined. No milk was given to pigs within four hours of treatment. At necropsy three weeks after inoculation, one of two infected untreated pigs had gross lesions of pneumonia involving approximately fifteen percent of the lungs. Microscopic examination revealed lesions typical of swine enzootic pneumonia to be present only in the pig which had gross lesions.

Neither of two treated, infected pigs had gross or microscopic lesions

Table 3. Chlortetracycline without milk. Efficacy of chlortetracycline in lesion prophylaxis and clearance of Mycoplasma hyopneumoniae from pig lungs

Number of pigs	2	2	2	2	2	2	2	2	2	1	2	2	3	2	4
Postinoculation necropsy day	3	3	7	7	10	10	14	14	17	17	21	21	--[1]	--[2]	--[3]
CTC[4] treatment	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-
SEP lesions:															
gross	0/2	0/2	0/2	1/2	0/2	0/2	0/2	2/2	0/2	1/1	0/2	2/2	0/3	0/2	0/4
histological	0/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2	0/2	1/1	0/2	2/2	0/3	0/2	0/4
M. hyopneumoniae recovery	0/2	0/2	1/2	1/2	0/2	0/2	0/2	2/2	0/2	1/1	0/2	0/2	0/3	0/2	0/4
SEP lesions in reinfected pigs:															
gross	N.D.[5]	2/4	5/7	7/7	0/4	0/4	3/5	4/4	0/4	4/4	1/4	4/4	N.D.	N.D.	N.D.
histological	N.D.	0/4	4/7	7/7	0/4	1/4	3/5	4/4	0/4	4/4	1/4	4/4	N.D.	N.D.	N.D.

[1]Treated controls, not challenged. Necropsied after 21 days on chlortetracycline treatment.

[2]Untreated unchallenged controls. Necropsied after 21 days in isolation unit.

[3]Unchallenged control pigs necropsied at four days of age.

[4]CTC - chlortetracycline.

[5]Not done.

of pneumonia at necropsy. The absence of microscopic lesions is illustrated in Figure 15.

Two of the four infected and treated pigs that were maintained without treatment for three weeks after five weeks treatment had gross lesions of pneumonia at necropsy involving from less than one percent to approximately twenty percent of the lungs. Microscopic examination revealed lesions typical of swine enzootic pneumonia in two of four pigs. These characteristic lesions are illustrated in Figure 16.

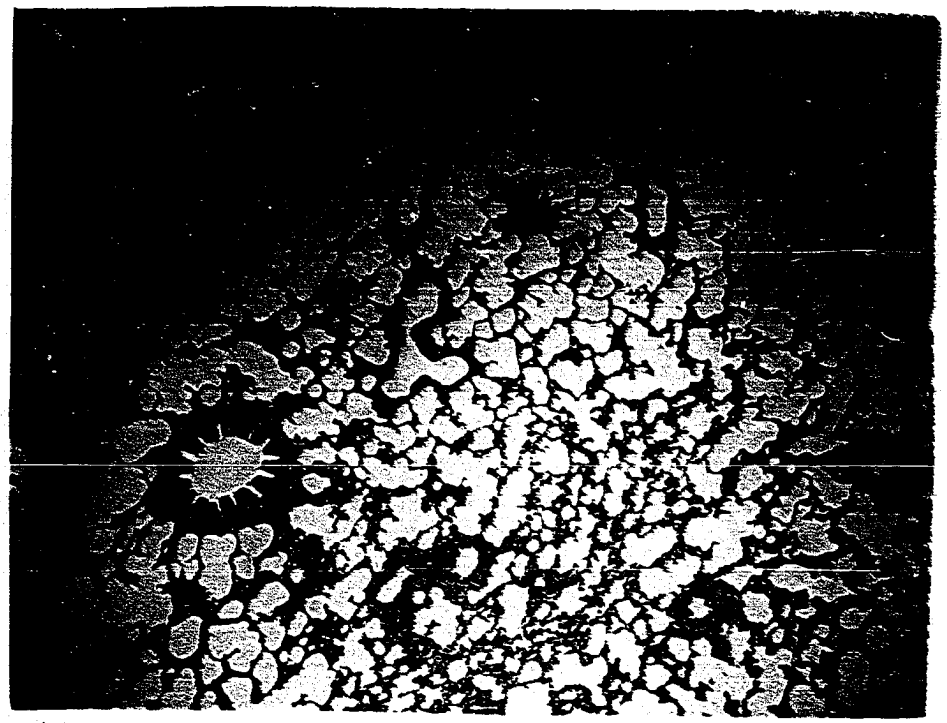
Chlortetracycline with milk

Experiment one The prophylactic effect of chlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. The daily oral treatment with drug was followed by a feeding of milk ad libitum. At necropsy all four infected untreated pigs had gross lesions of pneumonia involving thirty to fifty percent of the lungs. The pigs were amazingly free of clinical signs of pneumonia even though large portions of the lungs were involved. The apical, cardiac, and intermediate lobes were very severely affected; the diaphragmatic lobes were also involved with up to thirty-five percent of these lobes being affected. Microscopic examination revealed lesions typical of swine enzootic pneumonia in all cases.

Two of four infected and treated pigs had gross lesions of pneumonia at necropsy. One of these pigs had only ten percent of the right apical lobe involved while the other pig had approximately thirty percent of the right apical and cardiac lobes and fifty percent of the intermediate lobe involved. This constituted a marked reduction in the extent of lesions when compared to the infected, untreated animals. Two of four infected and treated pigs showed no gross lesions at necropsy. Microscopic examination

Figure 15. Lung from pig inoculated with pneumonic lung suspension containing Mycoplasma hyopneumoniae. Pig was treated daily with chlortetracycline. Note absence of lesions in area of bronchioles. Thickened septa may be artifactual. Hematoxylin and eosin stain. X35

Figure 16. Peribronchiolar lymphoid hyperplasia, septal cell proliferation and neutrophil infiltration. Note lack of functional alveoli. Lesions from pig infected with pneumonic lung suspension containing Mycoplasma hyopneumoniae. Pig was treated daily for 5 weeks, then removed from treatment for 3 weeks. Hematoxylin and eosin stain. X35



revealed lesions typical of swine enzootic pneumonia in the two pigs that showed gross lesions.

Experiment two The prophylactic effect of chlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. The drug was administered orally in daily doses to pigs nursing their dam. At necropsy, all three infected untreated pigs had extensive gross lesions of pneumonia involving approximately fifty to seventy percent of the lungs. Microscopic examination revealed lesions typical of swine enzootic pneumonia. Mycoplasma hyopneumoniae was isolated from two of three pigs. Lung material from these pigs was frozen for challenge use in future experiments.

One of four infected pigs treated with chlortetracycline had gross lesions of pneumonia at necropsy involving about five percent of the lungs. The lesions were typical of swine enzootic pneumonia but were not well developed. Microscopic examination revealed lesions typical of swine enzootic pneumonia only in the pig which showed gross lesions.

Postinoculation treatment

The prophylactic effect of chlortetracycline in pigs infected with Mycoplasma hyopneumoniae was determined. The antibiotic was administered in the feed; therapy was begun at one and two weeks postinoculation. The results of the trial are presented in Table 4.

The results of this trial indicate that lesion prophylaxis cannot be equated with clearance of Mycoplasma hyopneumoniae.

Transmission prophylaxis

The efficacy of chlortetracycline in preventing transmission of Mycoplasma hyopneumoniae-induced swine enzootic pneumonia from infected pigs on drug to susceptible, nondrugged pigs was determined. The results are pre-

Table 4. Efficacy of delayed treatment induction, using chlortetracycline, in preventing swine enzootic pneumonia lesions and in clearing swine of Mycoplasma hyopneumoniae

Number of pigs	Group	Day of Challenge	Day of Drug induction	Day of Drug termination	Day of Necropsy	SEP lesions Gross	SEP lesions Micro.	Culture Mycoplasma hyopneumoniae
3	A	0	7	28	28	1/3+	1/3+	0/3+
3	A	0	7	28	49	3/3+	3/3+	0/3+
3	B	0	14	35	35	0/3+	0/3+	0/3+
2	B	0	14	35	56	1/2+	2/2+	2/3+
5	C	0	--	--	21	5/5+	5/5+	5/5+

Table 5. Efficacy of chlortetracycline in preventing transmission of SEP

Number of pigs	Exposure day Artificial	Exposure day Natural	Day on drug	Day necropsied	SEP lesions Gross	SEP lesions Micro.	Culture Mycoplasma hyopneumoniae
4A	0	--[a]	21	38	3/4+	4/4+	3/4+
4B	-	28[a]	--	60	2/4+	3/4+	1/4+
4C	0	--[b]	--	38	4/4+	4/4+	1/4+
3D	-	28[b]	--	60	2/3+	3/3+	2/3+

[a]Exposed to group 4A for 10 days.

[b]Exposed to group 4C for 10 days.

sented in Table 5.

The results of this trial indicate that chlortetracycline is ineffective in preventing the transmission of swine enzootic pneumonia from drugged animals to susceptible, undrugged animals.

Field Infections

Disposition of pigs

One hundred one of the original one hundred twenty pigs reached a marketable weight by the end of the trial. Twenty-eight pigs were deleted from the complete statistical analysis for the following reasons:

1. One pig died on the truck on the way to market.
2. Two pigs were destroyed because of broken legs.
3. Four pigs died from the effects of gastric ulcers.
4. Four pigs died from sequelae of castration or blood collection procedures.
5. Three pigs died from pneumonia.
6. Five pigs were too small to market and were necropsied. Four of these had severe cases of pneumonia.
7. Nine Hampshire pigs were deleted from the analysis to remove breed differences.

The information from ninety-two pigs was used in the complete statistical analysis; data obtained from these animals is summarized in Table 6.

Feed conversion and analysis

Two feeds were used in this experiment. One feed was devoid of antibiotics; the other feed contained chlortetracycline (100 gm./ton), sulfamethazine (100 gm./ton), and penicillin (50 gm./ton). With the exception

Table 6. Summary data on trial. Original figures

Category	Number of pigs	Ave. live wt.	Ave. daily gain in lbs.	Ave. dressing percent
All pigs	92	205.6	1.42	71
Herd 1	21	207.6	1.50	72
Herd 2	9	214.3	1.33	71
Herd 3	8	202.6	1.29	71
Herd 4	11	195.0	1.19	71
Herd 5	10	192.0	1.25	72
Herd 6	8	203.1	1.40	71
Herd 7	11	219.5	1.55	72
Herd 8	14	207.1	1.63	69
All males	55	209.4	1.49	71
All females	37	200.0	1.31	71
Antibiotic feed	41	207.9	1.44	72
Plain feed	51	203.7	1.40	71
Pneumonia score 0	46	212.4	1.54	71
Pneumonia score 1	17	204.9	1.39	72
Pneumonia score 2	8	203.1	1.37	71
Pneumonia score 3	10	208.0	1.31	71
Pneumonia score 4	6	179.1	1.16	71
Pneumonia score 5	2	180.5	1.01	71
Pneumonia score 6	3	173.3	1.06	70

Table 6 (Continued)

Category	Ave. length in inches	Ave. backfat in inches	Ave. loin eye in sq. inches	Ave. ham wt. in lbs.
All pigs	30.16	1.34	4.68	34.49
Herd 1	29.93	1.48	4.84	34.59
Herd 2	31.14	1.25	4.95	36.89
Herd 3	30.17	1.28	4.73	35.35
Herd 4	30.50	1.11	4.77	34.22
Herd 5	30.14	1.34	4.39	28.55
Herd 6	29.59	1.43	4.36	33.87
Herd 7	30.14	1.49	4.70	38.06
Herd 8	29.98	1.25	4.58	34.31
All males	30.16	1.43	4.55	34.15
All females	30.16	1.22	4.88	35.01
Antibiotic feed	30.17	1.40	4.84	34.65
Plain feed	30.16	1.29	4.56	34.36
Pneumonia score 0	30.21	1.38	4.77	35.56
Pneumonia score 1	30.23	1.36	4.76	35.52
Pneumonia score 2	30.54	1.41	4.53	34.24
Pneumonia score 3	30.76	1.29	4.61	31.89
Pneumonia score 4	28.63	1.25	4.26	30.72
Pneumonia score 5	30.00	1.15	4.28	31.25
Pneumonia score 6	29.17	0.95	4.77	31.17

Table 6 (Continued)

Category	Percent ham & loin	Ave. loin wt. in lbs.	Ham & loin wt. in lbs.	Quality score one
All pigs	41.28	25.68	60.17	2.76
Herd 1	40.76	26.30	60.90	2.48
Herd 2	42.38	27.85	64.74	3.67
Herd 3	42.82	26.27	61.62	2.63
Herd 4	43.10	25.11	59.33	2.36
Herd 5	37.60	22.36	50.91	2.70
Herd 6	40.49	24.53	58.41	3.00
Herd 7	42.10	27.92	65.98	2.27
Herd 8	41.50	24.76	59.07	3.29
All males	40.39	25.68	59.83	2.75
All females	42.62	25.69	60.69	2.78
Antibiotic feed	40.97	26.14	60.79	2.54
Plain feed	41.54	25.32	54.68	2.94
Pneumonia score 0	41.27	26.43	62.00	2.63
Pneumonia score 1	41.79	26.16	61.69	3.12
Pneumonia score 2	41.75	25.75	59.99	3.63
Pneumonia score 3	38.06	24.03	55.92	2.50
Pneumonia score 4	42.03	22.53	53.25	2.17
Pneumonia score 5	45.20	26.61	57.86	4.00
Pneumonia score 6	44.07	22.65	53.82	1.67

Table 6 (Continued)

Category	Quality score two	PSE[1]
All pigs	2.26	1.09
Herd 1	1.95	1.05
Herd 2	2.56	1.00
Herd 3	2.75	1.25
Herd 4	2.36	1.18
Herd 5	2.10	1.20
Herd 6	2.75	1.12
Herd 7	1.91	1.00
Herd 8	2.29	1.00
All males	2.42	1.04
All females	2.03	1.16
Antibiotic feed	2.17	1.12
Plain feed	2.33	1.06
Pneumonia score 0	2.09	1.04
Pneumonia score 1	2.35	1.12
Pneumonia score 2	3.00	1.00
Pneumonia score 3	2.20	1.10
Pneumonia score 4	2.17	1.17
Pneumonia score 5	4.50	1.50
Pneumonia score 6	1.33	1.33

[1]Measured here was pale-soft-exudative pork (PSE). The scale used was: 1) no presence of PSE, 2) presence of PSE.

of antibiotics, the feeds were of the same formula. The data gained on feed conversion is presented in Table 7.

Animals on the ration containing antibiotics were more efficient in converting feed into live weight than were animals fed antibiotic-free feed.

Two samples of feed were checked for antibiotic levels[1]; this analysis was performed on feed that had remained in the feeders at termination of the trial. The analysis results are presented in Table 8. A reduction in the activity of all three antibiotics was noted.

Lesion analysis and incidence

The presence or absence of pneumonia was determined by gross visual appraisal and by microscopic evaluation of tissue sections. Figure 17 illustrates a lesion-free lung; Figures 18, 19, and 20 illustrate lesions typical of swine enzootic pneumonia. Information on the lungs from one hundred sixteen pigs is presented in Table 9. The gross and microscopic evaluations agreed in ninety-one and thirty-eight hundredths percent of the cases. They differed in eight and sixty-two hundredths percent of the cases. Within the group where evaluations differed, there is an indication that more animals were considered positive with the microscopic technique and negative on gross visual evaluation than the other way around. The best estimate of the percentage of cases where the two scores will not agree is eight and six-tenths; the standard error of this estimate is two and eight-tenths percent. The ninety-five percent confidence interval is approximately from three and one-tenth to fourteen and one-tenth percent

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[1]Performed by American Cyanamid Company, Pearl River, New York.

Table 7. Feed conversion as related to feed type

Feed	Number of animals	Pounds gained	Pounds of feed used	Conversion ratio lbs. feed::1bs. gained
1. Plain	68	8,662	30,650	3.54::1
2. With antibiotics	49	7,199	23,800	3.31::1
Total	117	15,861	54,450	3.44::1

Table 8. Analysis of the antibiotic-containing ration

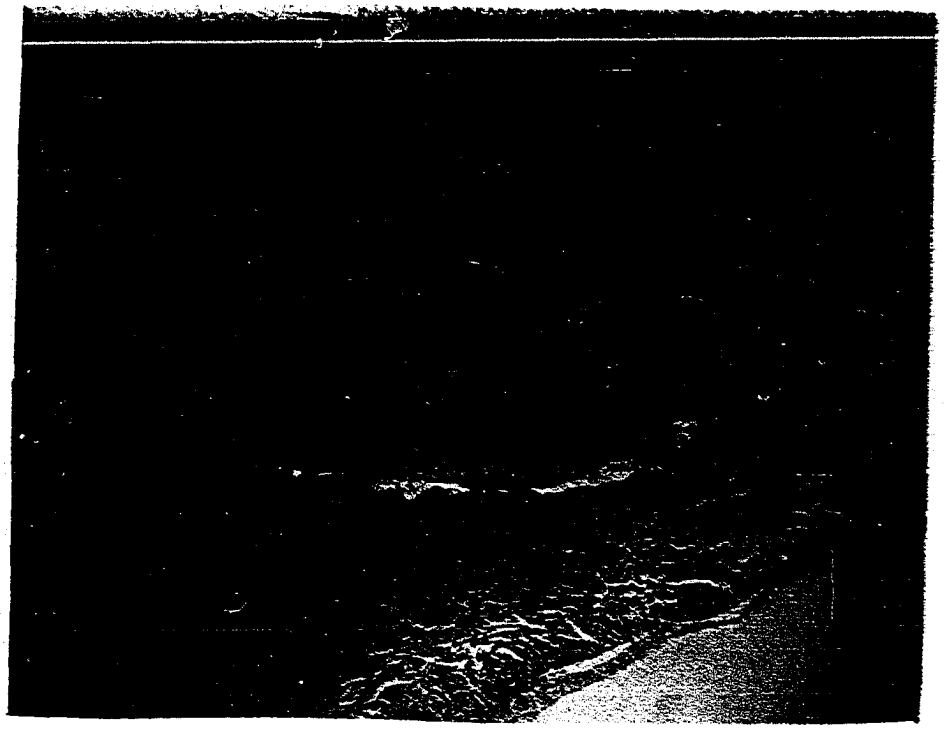
	Grams/ton of feed					
	Chlortetracycline		Sulfamethazine		Penicillin	
Sample	Actual	Expected	Actual	Expected	Actual	Expected
1.	56	100	65	100	39	50
2.	77	100	73	100	47	50
Average	66.5	100	69	100	43	50

Figure 17. Lung from pig classified as free of swine enzootic pneumonia. Note absence of lesions in alveolar septa and in region of bronchiole. Hematoxylin and eosin stain. X100

Figure 18. Peribronchiolar lymphoid hyperplasia and alveolar interstitial thickening. Lesions from pig showing mild gross lesions of swine enzootic pneumonia. Hematoxylin and eosin stain. X100

Figure 19. Pronounced peribronchiolar and perivascular lymphoid hyperplasia and alveolar interstitial thickening with septal cell proliferation. Note occlusion of alveolar spaces. Tissue from pig with extensive gross lesions of swine enzootic pneumonia. Hematoxylin and eosin stain. X35

Figure 20. Pronounced peribronchiolar lymphoid hyperplasia, alveolar interstitial thickening, septal cell proliferation and neutrophil infiltration. Tissue from pig with extensive gross lesions of swine enzootic pneumonia. Hematoxylin and eosin stain. X100



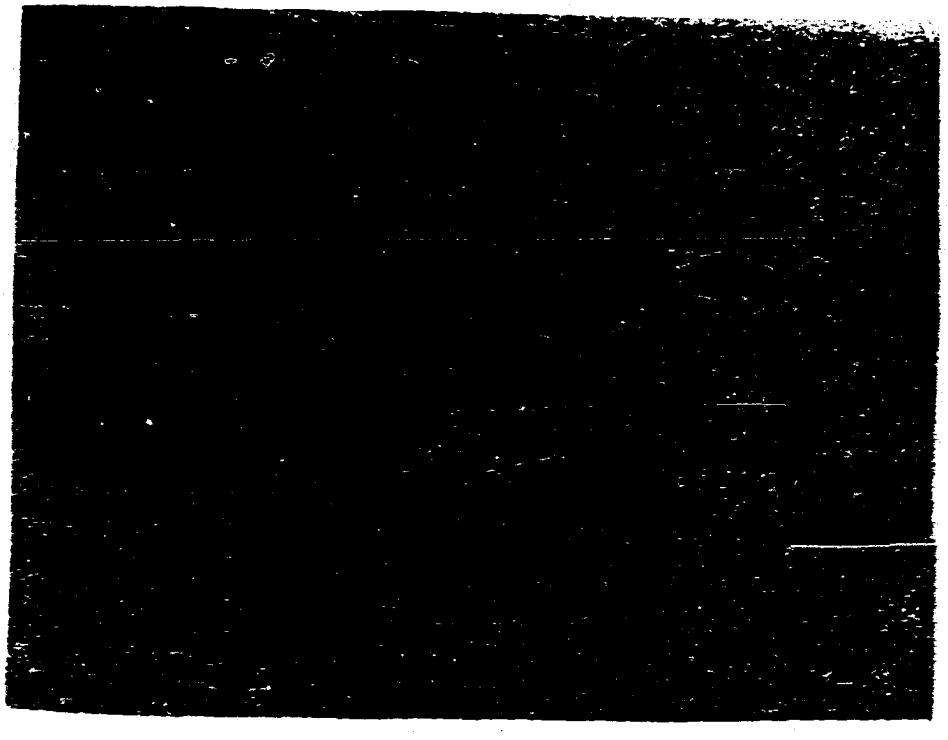


Table 9. Relationship between gross and microscopic appraisal of lungs

Gross lesions[1]	Microscopic lesions	Number of animals	Percent
0	0	44	37.93
0	+	7	6.03
+	0	3	2.59
+	+	62	53.45
Total		116	100.00

[1] + = lesions considered present; 0 = lesions not considered present.

for this value.

Natural transmission

Twenty pigs from the Veterinary Medical Research Institute's respiratory disease-free herd were placed in several pens with pigs from various herds of unknown disease status. Information was gained on nineteen of these animals. Six of the nineteen naturally exposed animals developed gross lesions representative of swine enzootic pneumonia; nine of the nineteen pigs had microscopic lesions resembling those of swine enzootic pneumonia.

Effects of certain variables on pneumonia

Herd of origin and sex The effect of herd of origin and sex on the incidence and severity of pneumonia was determined. Data obtained on the incidence and severity of pneumonia as related to herd of origin and sex is presented in Table 10. Some herds appeared to have a higher incidence of pneumonia than others; the severity of pneumonia also varied with the herd of origin. Males appeared to have more severe cases of pneumonia than did

Table 10. Distribution of pneumonia by herd and sex

Herd	No. of[2] pigs	Pneumonia score[1] 0	1	2	3	4	5	6
1	21	13/5[3]	1/1	0/0	1/0	0/0	0/0	0/0
2	9	1/3	0/2	0/2	1/0	0/0	0/0	0/0
3	8	0/0	1/1	0/2	1/0	0/1	1/0	1/0
4	11	1/2	1/1	0/0	1/1	1/0	1/0	1/1
5	10	1/0	2/0	1/1	2/0	0/3	0/0	0/0
6	8	1/0	1/2	1/0	2/0	1/0	0/0	0/0
7	11	5/2	2/1	0/0	0/1	0/0	0/0	0/0
8	14	8/4	1/0	0/1	0/0	0/0	0/0	0/0
Total	92	30/16	9/8	2/6	8/2	2/4	2/0	2/1

[1]Pneumonia score 0 = no pneumonia; 1 to 6 represent increasing degrees of pneumonia with 6 being very severe.

[2]Included are animals used in statistical analysis.

[3]Figures represent males/females in each category.

Table 11. Analysis of variance summary. Pneumonia score a dependent variable. Pneumonia score as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	75.027	10.718	7.43**
Sex	1	3.357	3.357	2.33 N.S.[a]
Feed	1	0.681	0.681	0.47 N.S.
Reg. on L.W.	1	24.326	24.326	16.86**
Error	8	116.839	1.44	

[a]N.S. = not significant at $p \leq .05$ level.

*Significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

females. An examination of the analysis of variance (Table 11) reveals that the herd of origin was a highly significant source of variation in the incidence and severity of pneumonia but that sex was not significant at the five percent level.

Chlortetracycline (feed type) The effect of chlortetracycline given in the feed on the incidence and severity of pneumonia was determined. Table 12 contains the data obtained. Pigs fed feed containing chlortetracycline appeared to have a reduced incidence of pneumonia as compared to pigs fed antibiotic-free feed; the severity of the pneumonia cases appeared to be similar regardless of feed type. An examination of the analysis of variance (Table 11) reveals that the variation in incidence and severity of pneumonia as related to feed type was not significant at the five percent

Table 12. Pneumonia and feed type: incidence and severity

Feed type	Pigs with Pneumonia score[1] 0	1 & 2	3 & 4	5 & 6	Total pigs	% 0 score	% 1 & 2 score	% 3 & 4 score	% 5 & 6 score
Plain feed	24	17	9	3	53	45.3	31.9	17.1	5.7
Antibiotic feed	22	8	7	2	39	56.4	20.5	18.0	5.1
Totals	46	25	16	5	92	50.0	27.2	17.4	5.4

[1]Pneumonia score 0 = no pneumonia; 1 & 2 = mild pneumonia; 3 & 4 = moderate pneumonia; 5 & 6 = severe pneumonia.

level.

Marketed weight It was necessary to market some of the slow gaining pigs at light weights in order to evacuate the facilities used in the trial. The slow gaining pigs consistently had lesions of swine enzootic pneumonia. An examination of the analysis of variance (Table 11) reveals that marketed weight (Reg. on L.W.) was a highly significant source of variation in the incidence and severity of pneumonia.

Pigs not analyzed For the several reasons stated in the section treating the disposition of pigs, some animals were not included in the complete statistical analysis; of the twenty-eight animals excluded, information on the disease status of the lungs was obtained for twenty-four pigs. This information is presented in Table 13. The incidence of pneumonia was higher than in the group analyzed statistically (seventy-nine percent versus fifty percent); also a greater percentage of the excluded animals had severe or very severe pneumonic lesions than did those analyzed statistically (forty-two percent versus five and one-half percent).

Effect of certain variables on average daily gain and carcass measurements

Average daily gain The effect of herd of origin, sex, feed type, and pneumonia on average daily gain was determined. The information obtained on average daily gain is presented in Table 6. Considerable variation in average daily gain was found between herds. Males outgained females by about two-tenths pound per day. Animals on antibiotics outgained those on plain feed by four hundredths pounds per day. An increase in the extent of pneumonic lesions appeared to adversely influence average daily gain. An examination of the analysis of variance (Table 14) reveals that herd of origin, feed type, and pneumonia all significantly influenced rate of gain;

Table 13. Distribution of pneumonia by herd and sex of pigs not analyzed statistically

Herd	No. of pigs	Pneumonia score[1]						
		0	1	2	3	4	5	6
1	5	1/0[2]	0/0	0/0	0/0	1/0	0/0	2/1
2	4	0/1	0/0	1/0	0/0	0/1	0/1	0/0
3	3	1/0	0/0	0/1	0/0	0/0	0/0	1/0
4	3	1/0	0/0	0/0	1/0	0/0	0/0	1/0
5	2	0/0	0/0	1/0	0/0	1/0	0/0	0/0
6	2	0/0	0/0	0/0	0/0	0/0	0/0	2/0
7	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
8	5	1/0	1/0	0/0	0/0	1/0	1/0	1/0
Total	24	4/1	1/0	2/1	1/0	3/1	1/1	7/1

[1]Pneumonia score 0 = no pneumonia. 1 to 6 represent increasing degrees of pneumonia with 6 being very severe.

[2]Figures represent males/females in each category.

Table 14. Analysis of variance summary. Average daily gain as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.722	0.103	2.15*
Sex	1	0.501	0.501	10.42**
Feed	1	0.271	0.271	5.64*
Pneumonia	6	0.683	0.114	2.37*
Error	76	3.653	0.048	

*Significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 15. Analysis of variance summary. Dressing percent as influenced by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.007	0.001	1.39 N.S.[a]
Sex	1	0.0004	0.0004	0.56 N.S.
Feed	1	0.0005	0.0005	0.67 N.S.
Pneumonia	6	0.002	0.0003	0.49 N.S.
Reg. on L.W.	1	0.0001	0.0001	0.20 N.S.
Error	75	0.052	0.0007	

[a]N.S. = not significant at $p \leq .05$ level.

sex appeared to be highly significant in affecting rate of gain.

Dressing percent The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on dressing percent was determined. The information obtained on dressing percent is presented in Table 6. Very little variation occurred in any category. An examination of the analysis of variance (Table 15) reveals that none of the variables tested influenced dressing percent significantly.

Carcass length The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on carcass length was determined. The information obtained on carcass length is presented in Table 6. Considerable variation existed in some categories. An examination of the analysis of variance (Table 16) reveals that herd of origin and marketed weight were highly significant in their influence on carcass length; pneumonia also had a significant influence on carcass length in that severely pneumonic pigs were shorter regardless of marketed weight. Feed type and sex did not significantly affect carcass length.

Average backfat The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on average backfat was determined. The information obtained on average backfat is presented in Table 6. An examination of the analysis of variance (Table 17) reveals that herd of origin, sex, and marketed weight (Reg. on L.W.) were highly significant sources of variation in average backfat; pigs marketed at light weights had less backfat than pigs marketed at heavy weights. Females had less backfat than males. Feed type and pneumonia were significant but not highly significant as sources of variation in backfat. Antibiotics in the feed tended to produce fatter pigs; an increase in severity of pneumonia produced

Table 16. Analysis of variance summary. Carcass length as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	16.250	2.321	5.35**
Sex	1	1.378	1.377	3.18 N.S.[a]
Feed	1	1.163	1.162	2.68 N.S.
Pneumonia	6	1.002	1.002	2.31*
Reg. on L.W.	1	40.303	40.302	92.91**
Error	75	35.533	0.434	

[a]N.S. = not significant at $p \leq .05$ level.

*Significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 17. Analysis of variance summary. Average backfat as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.721	0.103	4.23**
Sex	1	0.393	0.393	16.17**
Feed	1	0.145	0.145	5.99*
Pneumonia	6	0.415	0.069	2.84*
Reg. on L.W.	1	0.820	0.820	33.69**
Error	75	1.826	0.024	

*Significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

no consistent trend in backfat thickness.

Average loin eye area The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on average loin eye area was determined. The information obtained on average loin eye area is presented in Table 6. Little variation occurred in the pneumonia or herd of origin categories. Females had larger (.33 square inches) loin eyes than males. Animals on antibiotics had larger loin eyes (.28 square inches) than those on plain feed. Light animals had smaller loin eyes than heavy animals. An examination of the analysis of variance (Table 18) reveals that no significant amount of the variation in average loin eye area was explained by herd of origin or pneumonia. Sex was highly significant in influencing average loin eye area; feed type and marketed weight were significant sources of the variation found in average loin eye area.

Average ham weight The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on average ham weight was determined. The information obtained on average ham weight is presented in Table 6. An examination of the analysis of variance (Table 19) reveals that the weight marketed explained a highly significant amount of the variation in ham weight. Herd of origin, sex, feed type, or pneumonia did not significantly influence average ham weight.

Percent ham and loin The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on the percent ham and loin was determined. The information obtained on percent ham and loin is presented in Table 6. Little variation is seen in these categories. An examination of the analysis of variance (Table 20) reveals that none of the sources of variation tested had a significant influence on the percent ham

Table 18. Analysis of variance summary. Average loin eye area as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	1.004	0.143	0.55 N.S.[a]
Sex	1	3.399	3.399	13.03**
Feed	1	1.291	1.291	4.95*
Pneumonia	6	1.112	0.185	0.71 N.S.
Reg. on L.W.	1	1.470	1.470	5.64*
Error	75	19.560	0.260	

[a]N.S. = not significant at $p \leq .05$ level.

*Significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 19. Analysis of variance summary. Average ham weight as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	223.195	31.885	1.89 N.S.[a]
Sex	1	28.030	28.030	1.66 N.S.
Feed	1	3.423	3.423	0.20 N.S.
Pneumonia	6	69.804	11.634	0.69 N.S.
Reg. on L.W.	1	336.523	336.523	19.98**
Error	75	1262.987	16.839	

[a]N.S. = not significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 20. Analysis of variance summary. Percent ham and loin as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	224.615	32.087	1.60 N.S.[a]
Sex	1	19.329	19.329	0.96 N.S.
Feed	1	5.088	5.088	0.25 N.S.
Pneumonia	6	88.411	14.735	0.73 N.S.
Reg. on L.W.	1	74.061	74.061	3.69 N.S.
Error	75	1504.812	20.064	

[a]N.S. = not significant at $p \leq .05$ level.

Table 21. Analysis of variance summary. Average loin weight as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	65.572	9.367	0.75 N.S.[a]
Sex	1	6.848	6.848	0.55 N.S.
Feed	1	0.001	0.001	0.00 N.S.
Pneumonia	6	53.735	8.955	0.72 N.S.
Reg. on L.W.	1	268.334	268.334	21.60**
Error	75	931.718	12.422	

[a]N.S. = not significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

and loin yield.

Average loin weight The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on average loin weight was determined. The information obtained on average loin weight is presented in Table 6. Little variation was observed. An examination of the analysis of variance (Table 21) reveals that a highly significant portion of the variation in average loin weight was attributable to the weight at market time. Light weight pigs had fewer pounds of loin than did heavy pigs.

Average ham and loin weight The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on the combined ham and loin weight was determined. The information obtained on the combined ham and loin weight is presented in Table 6. An analysis of variance (Table 22) reveals that the marketed weight had a highly significant effect on the combined ham and loin weight, with the heavier pigs having more total pounds of ham and loin. The other variables tested were not significant.

Quality score one (color) The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on quality score one (color) was determined. The information obtained on quality score one is presented in Table 6. Considerable variation in quality score one was found to occur among the pneumonia categories and among the various herds. An examination of the analysis of variance (Table 23) reveals that herd of origin and pneumonia were statistically significant sources of variation in quality score one (color). It must be noticed that several of the classes under pneumonia score had very few pigs.

Quality score two (consistency and marbling) The effect of herd of

Table 22. Analysis of variance summary. Average combined ham and loin weight as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	490.078	70.011	1.28 N.S.[a]
Sex	1	62.591	62.591	1.15 N.S.
Feed	1	3.546	3.546	0.06 N.S.
Pneumonia	6	206.634	34.439	0.63 N.S.
Reg. on L.W.	1	1205.912	1205.912	22.08**
Error	75	4096.634	54.621	

[a]N.S. = not significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 23. Analysis of variance summary. Quality score one (color) as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	15.204	2.172	2.35*
Sex	1	0.541	0.54	0.59 N.S.
Feed	1	0.505	0.505	0.55 N.S.
Pneumonia	6	15.122	2.520	2.73*
Reg. on L.W.	1	0.124	0.124	0.13 N.S.
Error	75	69.295	0.923	

[a]N.S. = not significant at $p \leq .05$ level.

*Significant at $p \leq .05$ level.

origin, sex, feed type, pneumonia, and marketed weight on quality score two (consistency and marbling) was determined. The information obtained on carcass quality score two is presented in Table 6. Observation of the values in Table 6 shows that considerable variation in quality score two occurred among the various classes of pneumonia. An examination of the analysis of variance (Table 24) reveals that sex as well as pneumonia was highly significant in explaining the variation in quality scores. It should be noted that the actual difference in quality score two between males and females is not large (four-tenths of a point); second, the number of pigs in the pneumonia classes showing marked variation is quite small.

Pale, soft, and exudative pork (PSE) The effect of herd of origin, sex, feed type, pneumonia, and marketed weight (Reg. on L.W.) on the presence of pale, soft, and exudative pork (PSE) was determined. The information obtained on this category is presented in Table 6. Cursory observation of this table reveals little variation in the incidence of PSE. An examination of the analysis of variance (Table 25) reveals that sex was a highly significant source of the variation in the incidence of PSE. None of the other variables tested were significant.

Least squares constants analysis

A complete least squares constants analysis was performed on the data obtained from the field infection experiment. Least squares estimates of the effect of several variables on average daily gain were made.

Herd effect The greatest least squares estimate of the mean difference in average daily gain between two herds was twenty-five hundredths of a pound per day. That is, with the effect of other measured variables removed, one would not have expected any two herds to vary in rate of gain

Table 24. Analysis of variance summary. Quality score two (consistency and marbling) as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	6.463	0.923	1.40 N.S.[a]
Sex	1	4.661	4.661	7.07**
Feed	1	0.462	0.462	0.70 N.S.
Pneumonia	6	15.497	2.582	3.92**
Reg. on L.W.	1	0.355	0.355	0.54 N.S.
Error	75	49.424	0.658	

[a]N.S. = not significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

Table 25. Analysis of variance summary. Pale, soft and exudative pork (P.S.E.) as affected by several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.709	0.101	1.42 N.S.[a]
Sex	1	0.643	0.643	9.04**
Feed	1	0.073	0.073	1.03 N.S.
Pneumonia	6	0.764	0.127	1.79 N.S.
Reg. on L.W.	1	0.009	0.009	0.13 N.S.
Error	75	5.341	0.071	

[a]N.S. = not significant at $p \leq .05$ level.

**Significant at $p \leq .01$ level.

performance by more than this amount.

Sex effect The least squares estimate of the mean difference in rate of gain between sexes was eight-hundredths of a pound per day. One would have expected males to outgain females by this much if the effects of all other measured variables were removed.

Feed effect The least squares estimate of the mean difference in rate of gain between pigs on the feed containing antibiotics and pigs on antibiotic-free feed was eight-hundredths of a pound per day. One would have expected an animal on the antibiotic-containing ration to gain this much more than a pig on the antibiotic-free ration.

An examination of the analysis of variance for herd of origin, sex, and feed effects (Table 26) reveals that sex and feed type had significant effects on average daily gain while herd of origin had no significant effect.

Pneumonia effect The least squares estimate of the mean average daily gain for the various degrees of pneumonia is compared with the arithmetic means in Table 27. The least squares estimate reveals that the rate of gain dropped in proportion to the degree of pneumonia present.

A comparison of the average daily gain of pigs having pneumonia score 0 (no pneumonia) with pigs whose scores were 1-6 (all degrees of gross pneumonia) was performed. The least squares estimate of the mean difference in average daily gain between pigs in these two categories was one-tenth pound per day; this is considerably less than the arithmetic difference (twenty-four hundredths pound per day) between these categories. With the effects of other measured variables removed, one would have expected a pig without gross pneumonia lesions to gain one-tenth pound per day more

Table 26. Average daily gain. Influence of several variables

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.770	0.110	3.72 N.S.[a]
Sex	1	0.123	0.123	4.14*
Feed	1	0.138	0.139	4.69*
Pneumonia	6	0.138	0.023	0.78
Reg. on L.W.	1	1.434	1.434	48.44
Error	75	2.220	0.030	

[a]N.S. = not significant at $p \leq .05$ level.

*Significant at $p \leq .05$ level.

Table 27. Least squares estimation of average daily gain for various degrees of pneumonia as compared to arithmetic means

Pneumonia status	No. of pigs	Average daily gain (in pounds) Arithmetic mean	L.S.C.[1] mean
0 - no pneumonia	46	1.54	1.60
1 - very mild pneumonia	17	1.39	1.57
2 - mild pneumonia	8	1.37	1.57
3 - moderate pneumonia	10	1.31	1.45
4 - moderate to severe pneumonia	6	1.16	1.39
5 - severe pneumonia	2	1.01	1.13
6 - very severe pneumonia	3	1.06	1.23

[1]L.S.C. = least squares constants mean estimation.

Table 28. Analysis of variance summary. Least squares estimate of average daily gain

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	1.033	0.147	
Sex	1	0.384	0.384	
Feed	1	0.186	0.186	
Score 0 Vs. 1-6	1	0.124	0.124	2.39 N.S.[a]
Error	81	4.212	0.052	

[a]N.S. = not significant at $p \leq .05$ level.

Table 29. Analysis of variance summary. Least squares estimate of average daily gain

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Herd	7	0.958	0.137	
Sex	1	0.512	0.513	
Feed	1	0.300	0.300	
Score 0, 1, 2 Vs. 3, 4, 5, 6	1	0.461	0.461	9.64**
Error	81	3.875	0.047	

**Significant at $p \leq .01$ level.

than a pig with gross pneumonia lesions, taken as an average. An examination of the analysis of variance (Table 28) reveals that this difference in average daily gain was not significant at the five percent level.

A comparison of the average daily gain of pigs having pneumonia scores of 0, 1, 2 (no pneumonia to mild pneumonia) with pigs whose scores were 3, 4, 5, 6 (moderate to very severe pneumonia) was performed. The least squares estimate of the mean difference in average daily gain between pigs in these two groups was two-tenths pound per day. This compared with an arithmetic difference of twenty-eight hundredths pound per day. An examination of the analysis of variance (Table 29) reveals that this least squares estimate was highly significant.

DISCUSSION

The discovery and characterization of a mycoplasma, Mycoplasma hyopneumoniae, as the causative agent of swine enzootic pneumonia by Mare' and Switzer (1965, 1966a, 1966b and 1966c) contradicted the virus etiology previously suggested by other workers; this finding suggested that control or eradication of the disease through chemoprophylaxis and/or chemotherapy might be feasible.

Several in vivo sensitivity tests were performed in the present work to determine if certain of the common antibiotics could be used to control or eradicate swine enzootic pneumonia. The prophylactic effect of ether inhalation in pigs infected with Mycoplasma hyopneumoniae was also determined. Donovan (1967 and 1968) has shown that ether inhalation therapy was effective in treating cases of canine distemper which were caused by an ether-sensitive virus. Although Mare' and Switzer (1966a) have reported Mycoplasma hyopneumoniae to be ether-sensitive in vitro, no in vivo effect of ether on the organism could be demonstrated. Nine of ten infected pigs that received the ether inhalation treatment developed gross and microscopic lesions of swine enzootic pneumonia. Susceptible pigs inoculated with pneumonic lung material taken from infected, ether-treated pigs developed lesions of swine enzootic pneumonia from which Mycoplasma hyopneumoniae was recovered in three of four cases. The reason for the failure of ether via inhalation to inactivate Mycoplasma hyopneumoniae remains unexplained. This approach appears to have no value in freeing a pig from Mycoplasma hyopneumoniae or in preventing the development of swine enzootic pneumonia lesions.

Tylosin tartrate did not have any effect on Mycoplasma hyopneumoniae in the in vivo sensitivity trial performed; two possible explanations for this result exist. Mycoplasma hyopneumoniae might be quite resistant to the action of Tylosin tartrate or the drug may not have reached the organism in amounts sufficient to inhibit or kill it. Mare' (1965) found that Mycoplasma hyopneumoniae was quite resistant to the action of Tylosin tartrate in vitro. This suggests that even though Tylosin tartrate might have been in contact with the organism in vivo, its action on the organism would not have been effective. The suggestion by Mare' (1965) that tylosin might be useful in developing media which could support growth of Mycoplasma hyopneumoniae but not of Mycoplasma hyorhinis or Mycoplasma granularum both of which are sensitive to the drug appeared to have merit in theory, but Switzer[1] could not practically implement this idea.

Erythromycin had no effective in vivo action on Mycoplasma hyopneumoniae; this finding is interesting in that this drug is widely used to treat chronic respiratory disease in poultry which is caused by Mycoplasma gallisepticum. The possibility that the drug did not reach Mycoplasma hyopneumoniae in sufficient concentration must be considered. The water soluble form of erythromycin used in the trial had a bitter taste which may have caused the pigs to ingest suboptimal amounts.

Mycoplasma hyopneumoniae was sensitive to the in vivo action of chlortetracycline; this finding is in agreement with the report of Mare' and Switzer (1966a) and with reports of several investigators working on swine enzootic pneumonia in several parts of the world. The prevention of swine

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[1]Switzer, W. P., Veterinary Medical Research Institute, Ames, Iowa. Data from laboratory investigations. Private Communication. 1969.

enzootic pneumonia lesions with low levels of chlortetracycline suggests that commercially available antibiotic supplement preparations might be used in schemes aimed at controlling or eradicating swine enzootic pneumonia.

The determination of age susceptibility to infection by Mycoplasma hyopneumoniae revealed that pigs could not only be infected when several hours old but would develop quite extensive lesions. Since young pigs are susceptible to and are usually exposed to Mycoplasma hyopneumoniae at an early age, prophylaxis would have to be initiated at this time.

The treatment of infected pigs with chlortetracycline or a deirvative, demethylchlortetracycline, under optimal conditions appears to be very effective in preventing the development of swine enzootic pneumonia lesions; however, two of four pigs that were infected and treated subsequently developed lesions of enzootic pneumonia when the drug treatment was stopped. In several cases, lung tissue taken from infected and treated pigs was infective for previously unexposed pigs; the pigs from which the tissues were primarily taken were being treated to the time of necropsy. Laboratory recoveries of Mycoplasma hyopneumoniae were more frequently made from tissues removed from infected, untreated pigs than from infected, treated pigs, suggesting that greater numbers of organisms were present in the lung tissue of untreated animals than in treated pigs.

There were several cases where laboratory cultures of lung tissue from infected, treated pigs were negative for Mycoplasma hyopneumoniae; however, pigs which were inoculated with suspensions of these tissues developed lesions of swine enzootic pneumonia. These findings suggest that the susceptible pig is a more sensitive indicator system for detecting infected

lung tissue than are laboratory culturing techniques.

The results of the drug treatment trials indicate that a pig may be infected without developing lesions; this suggests that a critical number of organisms may be necessary for lesion development--a number considerably greater than that necessary for infection alone. The action of chlortetracycline appears to prevent this critical number from developing, yet does not eliminate the infection or prevent infected animals from developing numbers of organisms sufficient to infect other pigs both naturally and artificially. Pigs infected with Mycoplasma hyopneumoniae were capable of transmitting the infection to susceptible pigs naturally; similarly, infected pigs that were treated with chlortetracycline were capable of transmitting the infection to susceptible pigs. The idea that infection of young pigs could be prevented by treating the infected dam with chlortetracycline is invalidated by these results.

The inability of Betts and Campbell (1956) and Mare' and Switzer (1966a) to demonstrate the swine enzootic pneumonia agent in tissues of pigs treated with antibiotics may have been because of recovery techniques inappropriate for the task. The inability of Goodwin et al. (1968) to recover the swine enzootic pneumonia agent from field cases of swine enzootic pneumonia may have been because too few organisms were in the tissues examined; a titer of organisms in infected respiratory tissues sufficient for detection using laboratory techniques cannot be assumed.

The phenomenon of antibiotic-suppressed expression of Mycoplasma hyopneumoniae infection has an apparent counterpart as reported by Slotkin et al. (1967) and Larin et al. (1967). These workers reported a chlortetracycline-induced delay of Mycoplasma pneumoniae colonization in vivo and

in vitro where complete killing of organisms was not seen.

Milk or milk replacer, if given at the time of oral chlortetracycline administration to young infected pigs appeared to reduce the effectiveness of the drug. Infected pigs manipulated in this way developed gross and microscopic lesions of swine enzootic pneumonia; these lesions were much reduced in extent and severity as compared to infected, untreated animals. This suggests that the drug had a depressant effect on lesion formation, but was inefficient when compared to a treatment scheme where milk was not given for several hours after each treatment. Dearborn et al. (1957) and MacDonald et al. (1964) concluded that calcium ions interfere with the absorption of tetracycline drugs. Interference by the calcium ions in the milk and milk replacer would explain the incomplete inhibition of lesion formation by chlortetracycline in cases where these substances were given with the drug.

Lesion formation was suppressed (but incompletely so) in pigs where chlortetracycline treatment was initiated seven or more days after infection with Mycoplasma hyopneumoniae. In the majority of cases where therapy was terminated several weeks before necropsy, lesions of swine enzootic pneumonia developed and Mycoplasma hyopneumoniae was recovered from the lesions. These findings tend to substantiate the role of chlortetracycline in keeping the number of organisms below a level critical for lesion development.

The observations in this study suggest an aspect of swine enzootic pneumonia that has not been previously considered. A group of young weaned pigs could be exposed to swine enzootic pneumonia while receiving an antibiotic ration capable of suppressing lesion development. Some of these animals could become carriers of the causative agent; if these pigs are fed

an adequate level of the appropriate antibiotic until marketed, they would show no clinical signs or gross lesions of swine enzootic pneumonia and would pass slaughter inspection for accreditation in a Specific Pathogen Free (SPF) herd health program. Replacement stock saved from such a group would usually be maintained on a ration free of antibiotics; this would allow the development of pneumonia. Such replacement stock could then infect their offspring with the swine enzootic pneumonia agent.

One of the current problems with reliance on inspection at slaughter time in the SPF program is that detection of pneumonia usually occurs a few months after the actual outbreak. The findings presented in this study suggest that detection of swine enzootic pneumonia by slaughter inspection could be an entire generation after the actual exposure and even then might be only partially successful. This possibility constitutes a major weakness in the current SPF inspection program.

A high correlation was found between gross visual and microscopic evaluation of swine enzootic pneumonia lesions occurring in naturally infected swine. That some animals were scored negative for gross pneumonic lesions and positive for microscopic lesions of swine enzootic pneumonia may have been due to the microscopic evaluation being more sensitive; the reverse cases may have been due to improper selection of tissues for microscopic evaluation. Since the two modes of evaluation were highly correlated, the trial was analyzed utilizing the gross visual evaluation of lungs because this method alone considered severity of lesions in addition to lesion type, allowing the effect of several variables on the severity of lesions to be tested. Conversely, the effect of severity of lesions on several variables could also be tested.

The effects of several variables on the incidence and severity of swine enzootic pneumonia were determined. The highly significant effect of herd of origin on the incidence and severity of pneumonia suggests that variation in the virulence of the swine enzootic pneumonia agent might have occurred; also possible was a genetically-related susceptibility to the SEP agent. A third factor that might have been partially responsible for the variation in incidence and severity of pneumonia among herds is the management practices carried out in rearing the pigs prior to their procurement for the trial. A poorly managed group of pigs might have had a higher frequency of pneumonia than a well managed group.

Animals of each sex appeared to be equally susceptible to swine enzootic pneumonia; this was as expected.

Efficiency of feed conversion varied little between feed types. This is difficult to interpret in light of a number of variables in the trial which may have been affecting feed conversion in a manner that could not be determined.

The reduction in the levels of antibiotics from expected levels in the one ration may have been because the antibiotic analysis was conducted on feed that was several months old. Fresh feed as used in the trial probably contained antibiotics in the expected concentrations.

Variation in the incidence and severity of pneumonia as related to feed type was not significant. This suggests that chlortetracycline has little to no therapeutic effect on swine enzootic pneumonia. This finding agrees with that of Betts and Campbell (1956).

The results of other experiments presented in this study indicate that the formation of swine enzootic pneumonia lesions is prevented if chlor-

tetracycline is given before or at the time of exposure; this fact in combination with the lack of therapeutic effect due to chlortetracycline indicates that the pattern of infection may have been established in the groups of pigs at quite a young age (before initiation of the trial). This may mean that some of the lesion-free pigs were resistant to infection either naturally or by development of immunity through exposure to the causative agent. The detection and selection of such animals would greatly enhance the control and eradication of swine enzootic pneumonia.

The association of marketed weight with pneumonia is actually an expression of the effect of pneumonia in reducing average daily gains. Several slow-gaining animals used in the trial were marketed at light weights to evacuate the facilities; these same animals had a high incidence of pneumonia. This created an artificial correlation of pneumonia with marketed weight.

Although several pigs were not included in the complete statistical analysis, these animals were important to the trial. Animals not analyzed statistically had a much higher incidence of pneumonia and had more severe cases of pneumonia than did those animals analyzed statistically. They represented a considerable economic loss to the project as they would to a commercial producer. The death loss due to pneumonia and losses due to the extremely slow-gaining pigs might have been reduced if special treatment had been introduced; such treatment would in itself have represented an increased cost of production due to swine enzootic pneumonia.

Herd of origin, sex, feed type, and pneumonia all significantly affected average daily gain; this suggests that each of these variables should be considered when selecting pigs on the basis of weight gain per-

formance. The present lack of a suitable diagnostic test for swine enzootic pneumonia precludes adequate evaluation of this variable in swine selection procedures.

A significant amount of the variation in carcass length, backfat and quality scores one and two was explained by pneumonia. Carcass length was definitely shorter in pigs with moderate to severe, severe, and very severe degrees of pneumonia; this suggests that pneumonia has a stunting effect on carcass length in addition to reducing average daily gain. The data shows that the small number of pigs in some of the categories of pneumonia severity had abnormal backfat measurements which created a statistically significant test where no corresponding biological significance existed. The statistical significance found in the quality score variation as affected by pneumonia should probably be similarly interpreted. To correctly interpret the effect of pneumonia on carcass measurements one should ideally have a more even number of pigs in each classification of pneumonia severity.

The herd of origin accounted for a highly significant amount of the variation in carcass length and a significant amount of the variation in backfat and carcass color (quality score one). It has been demonstrated that both backfat and carcass length are heritable characteristics that vary between herds. The significance of herd of origin in relation to carcass color remains in question. This evaluation is qualitative; the difference in scoring, while statistically significant does not necessarily indicate a significant variation in the actual carcass characteristic.

The variation in backfat thickness, average loin eye area, consistency and marbling of the carcass (quality score two), and the presence of pale

soft and exudative pork (PSE) between males and females was highly significant. The difference in backfat thickness (males fatter) and loin eye area (females larger) is well documented in swine genetics. The statistical significance of quality score two (consistency and marbling) and of the presence of pale, soft, and exudative pork is probably not related to any real significance as far as differences between males and females are concerned.

The variation in backfat thickness and in average loin eye area between animals on different feed types was significant at the five percent level; animals on feed containing antibiotics had more backfat (0.12 inches $\pm$ .04 inches) and more loin eye area (0.30 square inches $\pm$ 0.12 square inches) than did animals on an antibiotic-free ration. The animals on antibiotics were in general more active during the trial than were animals fed antibiotic free feed; this activity may account for the increased muscling. The reason for the relationship between feed type and backfat thickness remains in question.

The marketed weight was highly significant in explaining variation in carcass length, backfat thickness, ham weight, loin weight and combined ham and loin weight, and significant in explaining the variation in loin eye area. Of the variables tested, marketed weight is probably the most easily understood; variation in the quantitative carcass measurements was directly proportional to the weight marketed as would be expected.

The effect of herd of origin, sex, feed type, and pneumonia on average daily gain was estimated by using a least squares constants analysis of the data gained in the trial involving field infections of swine enzootic pneumonia. Using this analysis, estimation of the effect of each tested variable on this trait with the effects of all other tested variables removed

was performed; this procedure allowed the most accurate estimations to be made, correcting distortions present in arithmetic values due to an imbalance in the number of pigs within each variable tested.

Variation in the significance of an estimated difference in average daily gain was noted in that feed type and sex effects on average daily gain were considered significant with an eight-hundredths pound per day differential while one of the pneumonia score comparisons (0 vs. 1-6) was not considered significant with a one-tenth pound per day estimated differential. This phenomenon may be explained as follows: to test a difference between A and B the difference is divided by its standard error.

$$t = \frac{\bar{A} - \bar{B}}{S_{\bar{A}-\bar{B}}}$$

The standard error ($S_{\bar{A}-\bar{B}}$) is calculated by taking the square root of the sum of the variances ($S^2_{\bar{A}} + S^2_{\bar{B}}$). When the population was divided on the pneumonia score, the variance of one group (the pigs with pneumonia) was very high. This gave a high standard error and required a larger mean difference to be significant than when the population was divided on the basis of sex or feed type where the variability in the groups was less. It should be remembered also that statistical tests are not the final determinator of the importance of information.

The least squares estimated reduction in average daily gain due to pneumonia (all degrees of severity considered) was one-tenth pound per day or a seven percent reduction; this estimate is considerably less than the arithmetic difference between nonpneumonic and pneumonic pigs of twenty-four hundredths pounds per day or fifteen percent. Betts (1952), Betts and

Beveridge (1953), and Betts et al. (1955) reported reductions in average daily gain due to swine enzootic pneumonia as varying between five and twenty-five percent. Such a wide range in these estimations might be because the trials were performed under varied climatic conditions. Eikmeier and Mayer (1965), Björklund and Henricson (1965), and Englert and Eisenack (1964) believed swine enzootic pneumonia to be insignificant in its effect on average daily gain; their conclusions were couched in the statement that pneumonia might have been significant had the management conditions been of lower quality. This point of the effect of management is well taken for Eernstman (1963), Kosztolich (1966) and Gordon (1963b) all reported that environmental variation could meaningfully affect the incidence and severity of pneumonia. Mackensie (1963) and Underdahl and Kelley (1957) also showed that micro-environment significantly affected the severity of swine enzootic pneumonia.

Such variations in estimates of the effect of swine enzootic pneumonia on average daily gain suggest that the effect of pneumonia_1 does not equal the effect of pneumonia_2, that is, the reduction in average daily gain may be proportional to the severity of lesions. This was shown to be the case in this study when the least squares estimate of the effect of severity of pneumonia on average daily gain was performed. Other workers have not taken this phenomenon into account when assessing the effect of the disease on daily gains.

The noted effect of environment on the incidence and severity of swine enzootic pneumonia in coordination with the finding that reduction in daily gain corresponds to the degree of lesion severity suggests that, in lieu of total eradication, pig producers might eliminate much of the economic effect

of swine enzootic pneumonia through management procedures. The benefit that might be realized through utilization of good management as compared to poor management was estimated by comparing pigs with no pneumonia to mild pneumonia with pigs having moderate to very severe pneumonia (pneumonia scores 0, 1, 2 vs. 3, 4, 5, 6). The least squares estimate suggests that a pig producer might expect a two-tenths pound per day increase in daily gain (fourteen percent) by reducing in severity or eliminating swine enzootic pneumonia lesions through management procedures.

The estimation of the effect of swine enzootic pneumonia in this study was biased toward moderation. The estimates were made on the basis of information obtained from the marketed animals. Two and one-half percent of the animals in the trial died as a result of pneumonia. Another four percent did not reach market weight during the trial because of slow gains attributed to severe pneumonia; these animals would have taken two to three extra months to reach a marketable weight. These losses must be added to those caused by a general reduction in daily gain. Increased housing and feed costs due to the prolonged production time required by pneumonic pigs must also be added to the cost of the disease. A report by Betts et al. (1955) suggests that a reduced feed efficiency is associated with swine enzootic pneumonia. The design of this study did not allow the determination of this factor.

Many pigs may be quite severely infected with swine enzootic pneumonia without showing clinical signs. This constitutes a hidden source of variation in performance which makes necessary larger numbers of pigs in research trials where performance is measured. Also swine enzootic pneumonia effects will mask genetic potential, creating a problem in selection of breeding

stock.

The results of these studies outline the seriousness of swine enzootic pneumonia. The chemotherapeutic and chemoprophylactic materials used in this study were not very efficacious; the only drug found to be of value, chlortetracycline, can at best be used as a pressure device to keep the number of organisms down. Chlortetracycline cannot be counted on to eradicate swine enzootic pneumonia. Other antimicrobial substances should be investigated for their effect on Mycoplasma hyopneumoniae.

In view of the seriousness of the disease and the lack of a successful and generally applicable treatment scheme, the development of improved diagnostic procedures for use in the identification of Mycoplasma hyopneumoniae-induced swine enzootic pneumoniae should receive high priority, since this would facilitate control and eradication programs greatly.

Methods of attenuation or inactivation of Mycoplasma hyopneumoniae should also be developed for use in prophylactic and possibly therapeutic immunization, since chemoprophylaxis and chemotherapeusis are not at present fruitful approaches.

SUMMARY

The susceptibility of Mycoplasma hyopneumoniae to the action of several chemotherapeutic substances was determined through the use of swine in an in vivo sensitivity detection system. In vivo this organism was found to be resistant to the action of ether, Tylosin tartrate, and erythromycin and was susceptible to the action of chlortetracycline and demethylchlortetracycline. Chlortetracycline was effective in preventing the development of swine enzootic pneumonia lesions in pigs infected with Mycoplasma hyopneumoniae but was not effective in eliminating the organism from these animals, for lesion development occurred if the drug was removed; Mycoplasma hyopneumoniae was then reisolated.

In treatment schemes involving young piglets, chlortetracycline was capable of completely suppressing the formation of swine enzootic pneumonia lesions in infected pigs if no milk was given within four hours of the daily oral administration of the drug; however, if milk was given ad libitum, incomplete suppression of lesion formation was observed.

An unsuccessful attempt was made to prevent transmission of swine enzootic pneumonia from infected pigs to susceptible pigs by treating the infected pigs with chlortetracycline while they were in contact with the susceptible pigs.

Chlortetracycline was found to be incapable of significantly altering the course of developed lesions, precluding its use as a therapeutic agent.

The effect of swine enzootic pneumonia on rate of gain and on certain carcass measurements was determined using naturally infected pigs from commercial sources. The incidence and severity of pneumonia was found to vary

significantly among herds; carcass length was found to be reduced in the more severe cases of pneumonia.

The correlation of average daily gain and carcass measurements with herd of origin, sex, feed type, and marketed weight was determined and discussed.

A least squares constants prediction of the average daily gain expected with varied degrees of pneumonia was performed. Average daily gain was found to vary inversely with the severity of pneumonia. The effect of pneumonia on rate of gain performance was more marked than the effect of sex or the addition of antibiotics to the ration.

The inefficacy of chemoprophylactic or chemotherapeutic measures with regard to swine enzootic pneumonia when considered in the light of the economic loss due to the disease, suggests that priority in an eradication or control program be given to the development of an accurate and sensitive diagnostic test. Strong consideration should also be given to the investigation of immunologic procedures in coping with this disease. The search for effective antimicrobial agents should be continued.

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