INVESTIGATION OF VIRUS PIG PNEUMONIA AND OTHER PULMONARY LESIONS IN SPECIFIC PATHOGEN FREE REPOPULATION, COMMERCIAL, AND EXPERIMENTAL SWINE

by

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INTRODUCTION

Virus Pig Pneumonia (VPP),[1] enzootic pneumonia,[33] and transmissible pneumonia[12] are all names given to a common respiratory disease of swine that was first recognized in 1951.[12] This disease undoubtedly existed long before this period, but it was overlooked at necropsies either through careless examinations or inadvertently neglected. Research workers first reported on the disease in England, and now known cases have been reported from Australia,[24,29] Canada,[28] United States,[3,35] France,[8] Spain,[21] Austria,[7] Finland,[26] Sweden,[33] and Africa.[22] Reports[25] of 40 to 74% of all swine slaughtered in the United States show pneumonic lesions with VPP being the predominant cause.

Swine producers have experienced a great economic loss due to VPP because of depressed growth rate and reduced efficiency of feed utilization. Secondary complications of bacterial pneumonia are frequently observed in these infected herds. Because of the wide spread incidence of this disease, the swine producers have started a repopulation program by using Specific Pathogen Free (SPF) pigs. Since no test was available and no susceptible laboratory animal was known, this investigation was undertaken to obtain additional information to aid in the differential diagnosis of VPP and other respiratory infections in these repopulation herds if it should occur.

REVIEW OF THE LITERATURE

An infectious pneumonia of swine was described by Pullar[23]

in 1948 that had lesions similar to the lesions described for VPP by research workers. The gross lung lesion was pale pink to deep pink in color and involved the cardiac, apical and intermediate lobes of the infected lungs. The etiological agent was unknown, but a watery extract from the infected lungs would reproduce the disease in experimental swine.

While working with swine influenza (SI) virus, Gularjani and Beveridge[12] in 1951 reported that a sample of lung tissue received from Northern Ireland did not produce the same clinical symptoms and lesions in swine and laboratory animals that they had been producing with their SI virus. By experimental methods it was found that the new lung infection was caused by a filterable agent. The modified hemaglutination inhibition (HI) test gave good results for SI virus, but when this new virus was tested, the results were negative. In comparing the clinical symptoms of the two diseases, it was found that the SI virus gave no temperature rise until 48 hours and possibly a week elapsed before a temperature rise was noted.

Betts[1] in 1952 made further studies and confirmed the results of the first two English research workers.[12] His findings disclosed that the disease was caused by a rather large virus probably 200 mμ in diameter. In experimental cases the incubation period was from 5 to 12 days, and in field cases the incubation period varied from 10 to 16 days. The gross necropsy lesions were most generally found in the ventral portion of the lung. The apical and cardiac lobes were the ones found to be involved the most often, whereas the next most probable sights

were the intermediate and the anterior ventral diaphragmatic lobes. From his research work he proposed the term Virus Pig Pneumonia.

While working with pneumonia in swine in 1952, Lamount[14] agreed that this condition was caused by a filterable virus which was different than the SI virus. He was in agreement that the name should be VPP.

Wesslen and Lannek[33] in 1953 isolated the agent from pigs with enzootic pneumonia and observed the cytopathogenic effect using swine kidney and lung cells in monolayer. Their work was also carried out on bovine and human tissue cell monolayer. The pulmonary lymph glands were observed to be enlarged and edematous. No neutralizing antibodies were found in experimentally infected swine or in field cases of this disease. The agent did not agglutinate chicken red blood cells and did not cause pneumonia in laboratory mice.

Beveridge[5] in his report in 1953 states that the two cardiac lobes were the most commonly involved. The infected lung lobes at necropsy varied in gross appearance from a gray to a red color and were slightly depressed below the level of the surrounding tissue or level with the surrounding tissue but never above this tissue. There was always a sharp line of demarcation between the affected and unaffected tissue. The clinical symptoms of the disease observed in experimentally infected swine were a dry cough, especially in the morning, and very often a diarrhea for a few days.

Fulton et al.[9] in 1953 described two forms of VPP, an acute

form and a subacute form. In the acute form there was a high morbidity, and the disease spread very rapidly through a drove of swine that had no previous history of this condition; while the subacute form was found in herds that had a previous history of VPP and spread very slowly. The Pasteurella organism was often isolated in cases of VPP, but this bacterial organism alone did not produce pneumonia in swine.

In 1953 Betts[2] reported on pneumonic diseases caused by viruses. Variable degrees of fibrosis resulting in distortion of affected parts was found in old VPP lung lesions, and often active pneumonia infections were present in adjacent areas of the lung. Resolution from VPP lung infection occurred, but the tendency for resolution to occur was not marked. A pleuritis and pericarditis was also found occasionally. In an acute case when death had occurred from VPP alone, a frothy exudate was found in the trachea with an intense congestion and edema of the lungs. The lungs from swine dying with a swine influenza infection contained a lobular pneumonia which gave the lungs a mottled appearance.

A swine influenza study was made by Young and Underdahl[35] in 1955 using the HI test. Their immunological studies for SI virus indicated less than a 15% infection, but 50 to 70% of the lungs from the same animals contained gross lesions of current or past respiratory infections when they were examined at slaughter.

Betts et al.[4] reported in 1955 on a study of the effect of VPP upon the growth rate and the efficiency of feed utilization.

They found that the growth rate was depressed by 16% and food conversion by 22%.

Betts[3] in 1956 reported that a survey made for VPP at several of the meat-packing establishments in the United States during the past year indicated a 50% infection rate. He stated that the disease spread only by the airborne route and not by fomites. The young pigs usually become infected while still with the dam, or shortly after weaning when healthy and infected pigs were mixed together. The "secondary breakdown", another phenomenon of VPP, usually occurred when the pig was 19 to 26 weeks of age. This results from the presence of an old VPP lesion and the rapid multiplication of secondary bacterial organisms in the lungs.

A histological study was made by Pattison[19] in 1956 on lung tissue of swine, because the typical gross lesions of VPP exhibited such a wide variation in the histological picture. The gross lesions of experimentally infected animals was detected on or after the eleventh day postinoculate (PI). The lung lesions were usually plum colored, but sometimes were grey in color resembling lymphoid tissue. The microscopic lesions were detected on or after the seventh day PI. The outstanding early microscopic lesion observed was a peribronchiolar and a perivascular lymphoid hyperplasia. A later observation of lesions disclosed localized collapsed areas, a cellular reaction of lymphocytes and macrophages, and a hyperplastic bronchiolar epithelium. An old lesion may undergo almost complete resolution.

A comprehensive study was made by Schofield[28] in 1956 on

swine pneumonia. Clinically, VPP was described as a chronic disease lasting for possibly months, and the affected animals had a characteristic persistent cough. The gross lung lesions were found in the cardiac and adjacent diaphragmatic lobes which were firm to the touch and red or reddish brown in color. Emphysema was usually present in adjacent healthy lobules. The characteristic microscopic lesions disclosed a lymphocytic infiltration with proliferative changes around bronchioles and foci of interstitial pneumonia throughout the lobule. The alveoli contained either large numbers of cells or only a few cells. The cells were usually alveolar lining cells, lymphocytes, plasma cells, and a few histiocytes and neutrophils.

The oral use of wide spectrum antibiotics for the control of VPP was studied by Lannek and Börnfors[15] in 1956. An oral dose of 10, 15, and 20 mgs. per kg. of body weight completely or almost completely suppressed the development of VPP. Five mg. per kg. gave a 50% suppression, and one mg. per kg. gave no suppression of lesions.

Carter and Schroder[6] reported in 1956 that pleuropneumonia-like organism (PPLO) when given intranasally did not produce pneumonia in pigs. They were able to produce lung lesions similar to reported lesions of VPP when bacteria free filtrates of pneumonic lung tissue were given intranasally.

A classification of swine respiratory disorders was made by Whittlestone[34] in 1957, and VPP was placed categorically in the primary respiratory diseases of uncertain etiology. No immunity resulted in experimental pigs when suspensions of lung tissue

were given intraperitoneally, intrapleurally, and subcutaneously and later challenged.

A study of the influence of the swine ascarid larvae on the lungs of disease-free pigs was reported by Underdahl and Kelley[31] in 1957. Their study consisted of ascarid larvae alone, VPP virus along, and ascarid larvae followed by VPP virus five days later. They found that the lesions produced by ascarid larvae migration and pneumonia virus together were 10 times more extensive than when either were used alone. The lung lesions were comparable to field cases of VPP.

The experimental work of Pattison et al.[20] in 1957 disclosed that the swine fever virus would cause lung lesions like the VPP lung lesions when inoculated intratracheally. The pathogenic action of the virus was blocked by the use of crystal violet vaccine, a commercial lapinized virus vaccine, and swine fever hyperimmune serum. The microscopic appearance of the lung sections varied from a complete occlusion of the alveoli to only a partial collapse of the alveolar spaces with the presence of numerous histiocytes. Hyperplastic bronchiolar epithelium was frequently noted, and in some areas of the lung section, neutrophils were frequently found in the bronchial and bronchiolar exudate. The occasional cuffing of blood vessels with small round cells was also observed.

The agent causing enzootic pneumonia[33] was reported by Rockburn[27] in 1957 to range from 500 to 800 mμ in size, which was much larger in size than the one reported for VPP.[5] This enzootic agent also produced an agglutination titre in rabbits of

1 to 8, and positive complement fixation results were obtained in a serum dilution of 1 to 128. The immunization of rabbits also produced neutralizing antibodies, but the attempts to immunize pigs failed to produce a measurable titre.

A comparative histopathological study of swine influenza and VPP was made by Urman et al.[32] in 1958. Their observation disclosed a very close similarity in the two diseases. In both diseases a small and large round cell infiltration occurs both peribronchiolar and perivascular, but in VPP the reaction was more severe. A bronchopneumonia with polymorphomuclear leukocytes and bronchiolar epithelium necrosis occurred early in swine influenza, while in VPP there was an alveolar cell pneumonia and a partial detachment of bronchiolar epithelium due to infiltration of large numbers of round cells but no necrosis.

The relationship of atropic rhinitis and VPP on growth rate in market swine was investigated by Young et al.[37] in 1959. In the group of swine that were studied, they found 68% of the animals with lesions of atropic rhinitis and 57% with lesions of VPP. It was also found that it required a month longer feeding period for pigs infected with atropic rhinitis and VPP to reach the market weight when they were compared with a similar group that were not infected with the two diseases. Incidence and severity of atropic rhinitis had little effect on growth rate, but the rate of gain was retarded when a VPP infection was present.

A report on a microbiologic survey of pneumonia lung and normal lungs of swine by L'Ecuyer et al.[17] in 1961 disclosed that

Mycoplasma hyorhinis was recovered from 51% of pneumonic lungs and 6% of the normal lungs. In pneumonic lungs the two principal bacteria recovered were Pasteurella multocida and Streptococcus spp. The isolation of swine influenza virus failed, and no rickettsia were found. The same size filters that allowed the passage of the VPP agent also allowed the passage of Mycoplasma hyorhinis. The two antibiotics, penicillin and streptomycin, had no effect on the PPLO organism.

L'Ecuyer and Switzer[16] in 1963 reported that they had successfully propagated two different strains of the agent causing VPP in primary swine kidney and human cervical (HeLa) cell monolayer cultures. They confirmed their results by producing gross and microscopic lesions of VPP in pigs that were inoculated with cell future fluids. The third passage cell culture fluid produced gross and microscopic lung lesions in experimental pigs.

Some methods that have been used for swine repopulation programs were reported by Snowdon,[29] Pullar,[23] Goodwin and Whittlestone,[10] Young and Underdahl,[36] and O'Brien.[18]

MATERIALS AND METHODS

The first 48 samples of swine lung tissue were collected from commercial swine on April 17, 1962, at a slaughtering establishment in Kansas City, Kansas.* The tissue samples were selected from what appeared as gross lesions of VPP as described

*Armour & Company, Kansas City, Kansas.

by Beveridge.[5] Immediately upon collection they were placed in a glass container and a 10% buffered formalin solution was added.

From June 7, 1962, to April 8, 1963, samples of swine lung tissue were collected at slaughtering establishments in Arkansas City, Kansas,* and Great Bend, Kansas,** whenever a SPF check was held. Five hundred and sixty-five swine lung tissue samples were collected from this source. Sections of all lungs were taken irregardless of the appearance. These lung samples were treated the same as mentioned above.

Another 48 swine lung samples were collected on April 8, 1963, at a slaughtering establishment in Arkansas City, Kansas,* from the commercial swine received that day excluding the pigs presented from SPF check in the repopulation program. Sections were taken only from lungs showing gross lesions of VPP. After collection the tissues were treated as stated above.

When samples of lung tissue were collected from what appeared as gross lesions of VPP, the tissue sections were taken at the margin of the lesions and both normal and abnormal tissue secured. The sample of lung tissue from the SPF swine was taken from one of the apical or cardiac lobes or from any gross lesion that was present.

After the tissue was fixed for 48 hours, it was then sectioned and processed. The tissue sections were routinely cut at six microns in thickness and stained with hematoxylin-eosin

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*Maurer Neuer Meat Packers, Arkansas City, Kansas.

**Thies Packing Company, Inc., Great Bend, Kansas.

(H&E). When a more extensive study was desired, one of the special stains[11] was used, Wilder's reticulum stain, trichrome stain, Mallory's phosphotungstic acid hematoxylin (PTAH) stain, Shorrs, Schliefstein, Machiavello, Grocott, acid fast and periodic acid Schiff (PAS).

Preliminary attempts to produce a bacterial pneumonia were tried beginning in Oct. 1962. Several methods were tried using different bacterial cultures, different sources of bacteria, and the application of different stressors. The swine used for this work were purchased in two groups from a local swine producer, and the pigs had no previous history of VPP. The bacterial cultures used in all experiments except the first one had been isolated by the Diagnostic Laboratory, Pathology Dept., K.S.U. All bacterial cultures were grown for 6 hours at 37C in a nutrient broth with a pH 7.0 to 7.2 and given intratracheally. One pig was selected at random from this group and necropsied before the start of the experimental work. Gross pathological and microscopic histopathological examinations were done on the lungs from this animal.

Five pigs 8 weeks of age were used in experiment I. All inocula were given by the intratracheal route, using double needle and syringe. Each pig was held in a dorsal recumbent position while the intratracheal injection was being given into the trachea at a point approximately midway between the pharynx and the anterior thoracic inlet. The temperature of each pig was taken the day of inoculation and for 13 days PI. Total red blood cell (RBC) and white blood cell (WBC) counts were made for

each animal on the day of inoculation and on the fourth and ninth day PI. The Pasteurella sp. was a stock culture secured from the Pathology Department, K.S.U., and the Streptococcus sp. was hemolytic and obtained from the Diagnostic Laboratory. Pig 1 received nothing and served as a control; pig 2 received 3 milliliters (ml.) of sterile nutrient broth; pig 3 was given 3 ml. of the Streptococcus sp. broth culture; pig 4 was given 3 ml. of the Pasteurella sp. broth culture; and pig 5 was given 1.5 ml. of the above Pasteurella sp. and 1.5 ml. of the above Streptococcus sp. in a broth culture.

In experiment II one pig was used. The same bacteria were used that were used in experiment I. The pig was placed in a cooler at 4C for 4 hours prior to inoculation. When the animal was removed from the cooler, it was given 4 ml. of the Streptococcus sp. and 4 ml. of the Pasteurella sp. broth cultures with a modified tracheal tube while being restrained in a lateral recumbent position. Feed and water were withheld for 12 hours before and 12 hours after the inocula were administered. The animal was placed for the first night in a stall with very little bedding. Temperatures were taken for 25 consecutive days.

One pig was used in experiment III. The Pasteurella sp. was obtained from the lungs of a pig that was presented for necropsy at the Pathology Laboratory, K.S.U. The bacteria culture was given intraperitoneally to mice which succumbed in 24 hours, and the bacteria were reisolated. This isolate was grown in nutrient broth, and this culture was then given intraperitoneally to a rabbit which died in 18 hours, and the culture was again reisolated.

The Pasteurella sp. for this experiment was secured from this source. The Staphylococcus aureus and Streptococcus sp. were obtained from the diagnostic laboratory. The Staphylococcus aureus was coagulase positive, and the Streptococcus sp. was hemolytic. An injection of 9 ml. of India ink was given intravenously via the ear vein 4 hours prior to giving the intratracheal inocula which consisted of 3 ml. of each of the mentioned bacteria. The pig was held in a lateral recumbent position while the bacterial cultures were given intratracheally with a modified tracheal tube. The temperature was taken for 6 consecutive days, and a total WBC count was made on the first and sixth day.

One pig was used in experiment IV. The Pasteurella sp. and Staphylococcus aureus that were used were recovered from the pig used in experiment III. The Streptococcus sp. was a different culture secured from the diagnostic laboratory. The animal was subjected to a small flow of live steam for 30 minutes and 3 ml. of each of the bacterial cultures were given intratracheally with a modified tracheal tube while the animal was held in an upright position. Feed and water were withheld for 12 hours before and 12 hours after the administration of the inocula. No bedding was placed in the pen for 48 hours. Temperatures were taken the day of inoculation and for 11 days PI.

In experiment V two pigs were used. The stressor applied was ultrasound. The instrument* used was the same instrument used in the clinic for the treatment of sprains and interverte-

*Medco Sonlator MS-2, Medco Products Co., Tulsa, Okla.

bral disk syndromes. The hair coat of each animal was clipped over the thoracic and anterior lateral abdominal regions on both the right and left sides. A film of mineral oil was applied to the skin before each treatment. Each animal was given identical treatments for 3 consecutive days that consisted of 20 watts for 3 minutes per side over the clipped area. Following the third treatment, a 5 ml. dose of a bacterial culture consisting of Pasteurella sp., Staphylococcus aureus, and Streptococcus sp. in equal parts was given intratracheally with a modified tracheal tube with the animal restrained in a lateral recumbent position. The Pasteurella sp. was the same one as used in experiment IV, and the Staphylococcus aureus and Streptococcus sp. were isolated in the diagnostic laboratory. The temperature of each animal was recorded for 13 consecutive days. A WBC count was taken the first, second, and third days of ultrasound treatment and fifth day post-treatment. The number 1 animal was necropsied on the thirteenth day post-treatment and a microscopic study was made of the lung tissue.

In experiment VI attempts were made to produce a bacterial pneumonia and a combination virus and bacterial pneumonia. Three pigs were used in which the remaining pig in experiment V was the number 1 pig, and 2 other litter mates were designated number 2 and number 3. A frozen specimen of lung tissue that was suspected grossly of having lesions of VPP present was obtained from the diagnostic laboratory.

A suspension of lung tissue was prepared from this VPP tissue by mincing the tissue into fine pieces in a sterile pyrex

100 ml. beaker. It was then quick frozen and thawed 4 times. This lung suspension was suspended in a phosphate buffered saline solution with a pH of 7.2 to give an approximate dilution of 1 to 1000. The supernatant was removed with a sterile 10 ml. pipette, and one thousand units of penicillin and 1 mg. streptomycin were added for each ml. of suspension. The suspension was placed in a refrigerator for 2 hours at 4C before inoculation. The Pasteurella sp., Staphylococcus aureus, and Streptococcus sp. were the ones used in experiment V.

All 3 experimental animals were given 10 ml. of the lung extract intratracheally with a modified tracheal tube and with the animal restrained in a lateral recumbent position. On the fifth day PI the number 3 animal was given 9 ml. of the bacterial cultures in equal parts using the same method of administration as used for the lung suspension. The temperature of each experimental animal was taken for seventeen consecutive days beginning with the day of the lung suspension inoculation. All 3 animals were necropsied on the sixteenth day PI.

RESULTS

First Collection of Commercial Swine Lungs

The first outstanding gross lesion observed in lungs collected from slaughtering establishments was the enormous amount of congestion and hemorrhage present in the lungs of some of the slaughtered swine (Plate I, Fig. 1). The color varied from a dark red to a bright orange red and then to a light pink of the

normal lung (Plate I, Fig. 2).

The first group of lungs collected was from commercial slaughtered swine. The gross VPP lung lesions (Plate II, Fig. 3) were purple in color; and they were found in the ventral part of either the apical, cardiac, intermediate, and diaphragmatic lobes or in any combination of lobes. The gross lesion disclosed that the apical lobes, either the right or left or both, were involved 87% of the time; whereas the cardiac lobes, either the right or left or both, were involved 58% of the time. The diaphragmatic lobes, either the right or left or both, were involved only 22% of the time; and the intermediate lobe was involved 25% of the time.

The microscopic study for VPP lung lesions revealed that 77% of these lungs were positive, 8% were suspicious, and 14% were negative. Two of the negative microscopic results were from separate cases where the right diaphragmatic lobe was the only grossly involved lobe. Severe congestion and hemorrhage were observed, but no VPP lung lesions were found microscopically in 6% or 3 lungs that displayed gross lung lesions. The lungs classified as suspicious were possibly the result of resolution from old lung lesions. They contained only a very minimal amount of round cell infiltration.

The varieties of tissue reaction found accompanying the VPP lesions were of three major types. The most common type was an acute or subacute suppurative bronchial pneumonia in which neutrophils were the predominant cells with varying numbers of alveolar epithelial cells, lymphocytes, plasma cells, and

fibroblasts (Plate II, Fig. 4). The other most commonly found tissue reaction along with the VPP lesions was a nonsuppurative pneumonia in which the alveolar epithelial cells were the predominant cells in the alveoli, bronchi, and bronchioles, along with some lymphocytes and plasma cells in a serous fluid (Plate III, Fig. 5). The other tissue change was one that had very little cellular exudate. In this type the deviation from the normal tissue was a thicker alveolar wall, resembling the tissue in (Plate III, Fig. 6), that contained numerous alveolar epithelial cells, a few lymphocytes, plasma cells and sometimes fibroblasts; but the lumen of the alveoli, bronchioles, and bronchi were free, or almost free, of a cellular exudate with only an occasional detached alveolar cell and lymphocyte found present.

Varying degrees of atelectasis occurred in all three types of tissue changes. Congestion, hemorrhage, edema and emphysema were also found in varying amounts. There was a pleuritis in some cases and a fibrosis with a thickening of the interlobular connective tissue in some tissue sections.

The typical VPP lung lesion displayed a varying amount of small and large round cell infiltration. This aggregation of round cells was found near or completely surrounding bronchi, bronchioles, and blood vessels (Plate IV, Fig. 7). The cells varied in size and consisted of cells resembling lymphocytes of various size, plasma cells, and histocytes. Some lung tissue sections contained round cell accumulations that resembled a lymph gland in which several germinal centers were observed in the tissue surrounding one bronchiole (Plate IV, Fig. 7).

As the numbers of round cells increased, atelectasis occurred in the surrounding lung parenchyma. Also, as the numbers of round cells increased, there appeared to be a separation of the smooth muscle fibers surrounding the bronchus or bronchiole, and the round cells migrated through the separation collecting in the propria mucosca between the band of smooth muscle and the bronchiolar epithelium (Plate V, Fig. 8). As the increase of cells continued the epithelium was pushed into the lumen of the bronchus or bronchiole, resulting in a narrowing of the air passage (Plate IV, Fig. 7). In lesions containing large numbers of round cells it was often found that the muscle tissue had completely atrophied in some areas surrounding the bronchus or bronchiole (Plate V, Figs. 8 and 9). There appeared to be some increase in the interlobular connective tissue, which also contained numerous lymphocytes.

No necrosis of bronchus or bronchiolar epithelium was noticed, but there was an occasional detachment of epithelium in some of the severe reactions.

When the round cell accumulation was found to involve the vessels, the lumen was often constricted until only a small amount of blood was allowed to pass. Some arteries were compressed into an oblong vein-like structure with a thick smooth muscle wall.

VPP was often accompanied by an acute or subacute suppurative bronchiolar pneumonia, which probably was caused by bacteria resulting in the so-called "secondary break". Often the accumulation of neutrophils was so great in the alveolar sacs, bronchi and bronchioles, that under scanning lens the round cell accumu-

lation was hard to detect. Found associated with the neutrophils were the detached alveolar cells and lymphocytes. Depending on the stage of inflammation if subacute or chronic, there were fewer neutrophils and more alveolar cells and lymphocytes, as well as some fibrous connective tissue in some instances.

The nonsuppurative bronchiolar pneumonias found associated with VPP had from a few to a large number of alveolar cells present in the alveolar sacs, bronchi, and bronchioles. Usually a few lymphocytes and neutrophils were present also in the cellular exudate. In some cases the alveolar walls were lined with closely arranged swollen alveolar cells with only a few detached alveolar cells free in the lumen (Plate III, Fig. 6).

In microscopic positive cases of VPP, three special stains, Shorrs, Schliefstein and Machiavello stains, were used for the identification of inclusion bodies. A careful study was made of each tissue section, and no inclusion bodies were observed.

Second Collection of Commercial Swine Lungs

A record of the location of VPP lung lesions was not made in the second collection of commercial swine lungs because of the speed in the slaughtering operation; but during the collection of the lung lesions, a check was made to determine the percentage of infection. The lung collections were made at different intervals to reduce the possibility of the swine originating from one swine producer. A 31% infection rate was found by using gross lung lesions as a criterion.

The tissue sections from this same group of commercial swine

lungs were studied by staining with H & E only. The results of the microscopic tissue section study disclosed that 83% were positive for VPP, 8% were suspicious for VPP, and 8% were negative for VPP. The lung sample was recut in a different part on four separate cases that were previously diagnosed either negative or suspicious from the first examination. The diagnoses were unchanged in all except one case that was suspicious the first and second times and then diagnosed positive after a more intensive study of both sections. This was probably an old lesion that was undergoing resolution.

The microscopic lesions found in the lung sections other than those characteristic of VPP were acute and subacute suppurative bronchial pneumonia, nonsuppurative bronchial pneumonia, mixed suppurative and nonsuppurative bronchial pneumonia, and a lesion of alveolar epithelial cell hyperplasia. Atelectasis, emphysema, congestion or hyperemia, edema, and hemorrhage were also present in a varying amount in the tissue sections. A fibrinous pleuritis was present in some sections.

There were 27% of the lung sections that had an acute to subacute suppurative bronchial pneumonia present. A predominance of neutrophils was present in the alveoli and the bronchiolar lumens. Depending on the stage of infection, other cells, lymphocytes, detached alveolar epithelial cells, plasma cells and a few fibroblasts were also present. In a limited number of sections, some of the alveoli and bronchiolar lumens contained eosinophils with the remaining alveoli and bronchiolar lumens filled with neutrophils. This was an indication probably of a

verminous involvement, although no lung worms were observed in the sections. Some edema and fibrin was usually associated with these suppurative conditions. Atelectasis varied depending on the severity of the infection, although it was always a common feature in the areas of round cell hyperplasia. Emphysema was more often observed in the lobules adjacent to the severely involved lobules, and generally these adjacent lobules contained the greatest amount of hemorrhage.

The nonsuppurative bronchial pneumonias which were associated with the VPP lesions constituted 42% of the findings during the microscopic study. Here, over 50% of the cells found in bronchiolar lumens and alveoli were detached alveolar epithelial cells, and the remaining cells were lymphocytes, plasma cells, and an occasional neutrophil. The alveolar walls were generally thickened and contained a large number of attached swollen alveolar epithelial cells and usually numerous lymphocytes, plasma cells, and a few fibroblast and neutrophils.

Atelectasis was most always present from a moderate amount to a very extensive amount. Some of the atlectasis was mechanical in origin, which was caused by the methods of tissue preparation and collection. Atelectasis was always a constant finding in the area surrounding round cell hyperplasia. Some edema and fibrin were present mixed with the cellular exudate, and hemorrhage varied from section to section. This condition could be resolution following an acute suppurative bronchiolar pneumonia, or it could be the lung tissue response to a virus infection.

The mixed suppurative and nonsuppurative bronchial pneumonias represented 19% of the findings that accompanied the round cell hyperplasia. In this condition the cellular exudate was mixed between neutrophils and detached alveolar epithelial cells, lymphocytes and plasma cells, or within a lobule there were foci that had a predominance of one or the other of the cells. The alveolar wall was thickened in parts of the tissue section due to an attached alveolar epithelial cell hyperplasia and an increase in lymphocytes, plasma cells, fibroblasts, and neutrophils. This tissue response in all probability was a resolution of an acute infection changing to a chronic infection or back to normal tissue. The extent of atelectasis was never severe except in the tissue where round cell hyperplasia was present, and hemorrhage and edema were variable in the amounts found present in the tissue sections.

A tissue reaction of only alveolar epithelial cell hyperplasia, as previously described (Plate III, Fig. 6), with no cellular exudate or only an occasional detached alveolar epithelial cell and lymphocyte, was found in 11% of the lung tissue sections. The attached alveolar epithelial cells were very hyperplastic, swollen, and often lined the complete alveoli. From only a few lymphocytes to a moderate number of lymphocytes were detected in the alveolar walls.

In this condition atelectasis was not an extensive lesion and found usually involving the lung tissue near the areas of round cell hyperplasia. The amount of emphysema present was dependent on the amount of round cell hyperplasia resulting in

bronchiolar pressure atrophy. No edema was present, and the amount of hemorrhage varied and was not always found present.

The lesions of VPP, peribronchiolar and perivascular round cell hyperplasia, were found as described in the first group of lung tissue sections studied. No new or different VPP lesions were observed.

Results of Repopulation Herd Investigation

One of the requirements for a swine producer to maintain a SPF herd for the purpose of swine repopulation was that the producer must present for examination at the time of slaughter a minimum of seven animals from each farrowing. The repopulation herds must also originate from primary or secondary SPF stock and also meet other standards that are required by the Swine Repopulation Association. This investigation deals only with the lung examination.

All lungs were examined grossly at the slaughtering establishments for VPP lesions. If lung lesions were found that resembled the gross lesions previously mentioned, a section was secured from this involved area; and if no gross lesions were found, a section of one of the apical or cardiac lobes was always taken from each animal.

During the ten-month period of this investigation, 502 swine lung samples from 29 repopulation swine herds, and 63 lung samples from 7 nonrepopulation swine herds were collected and examined grossly and microscopically. Four of the repopulation swine herds were classified as suspicious for VPP, and five of

the herds were classified as positively infected with VPP. In the seven nonrepopulation swine herds examined, one herd was classified as being suspicious for VPP, and three were classified positively infected with VPP. Subsequent swine lung examinations have either cleared some of the suspicious herds, or they were diagnosed positive for VPP.

In contrast, this investigation did not disclose the large amount of bronchial pneumonia that accompanied the VPP lung lesions in the normal run of slaughtered swine. An eosinophilic bronchial pneumonia was found to accompany the VPP lesions in two herds. All that was observed in the unaffected VPP swine lungs of the repopulation herds was varying amounts of alveolar epithelial cell hyperplasia, atelectasis, emphysema, hemorrhage, and edema.

The VPP lesions found in the lungs of the repopulation swine herds were similar to the lesions found in the lungs of swine during examination of the normal run of slaughtered swine which were discussed previously. The round cells were found to involve one side or completely surround bronchioles and vessels. The round cell accumulation in the propria mucosa of the bronchioles was often so marked that the lumen was almost obstructed (Plate V, Fig. 9). The epithelium lining the bronchioles was usually intact and firmly attached. Only when the lung tissue was not properly prepared or sectioned was the epithelium detached or broken. The hyperplastic round cells found closely associated with the bronchioles and vessels resembled lymphocytes, plasma cells and histiocytes, with the predominant cells appearing

to be lymphocytes (Plate VI, Fig. 10).

As the hyperplastic round cells accumulated and the area around vessels and bronchioles became more involved, the round cell tissue sometimes resembled lymph nodules with germinal centers as previously stated (Plate IV, Fig. 7). The smooth muscle that surrounds the bronchioles was only separated, or there was partial or complete atrophy of the muscle layer as previously described (Plate V, Figs. 8 and 9). As the size of the nodule of round cells increased, the more extensive was the atelectasis present in the surrounding tissue (Plate IV, Fig. 7).

Alveolar epithelial cell hyperplasia, similar to that found in (Plate III, Fig. 6), was almost always a constant finding along with the atelectasis, but this hyperplastic alveolar epithelial cell condition was also seen in swine lung sections in which VPP lesions were absent. When the alveolar epithelial cells were found detached and were the predominant cells or the only cells present in the cellular exudate in the alveoli and bronchioles, the condition was considered a nonsuppurative bronchial pneumonia, and VPP lesions may or may not be present with this condition. Ephysema in varying amounts was often observed in the lung section in the areas some distance from the VPP involved bronchioles.

Since there was always a sharp line of demarcation between VPP involved and noninvolved lung tissue, the lung sample was secured in such a way that infected and noninfected tissue was always taken. The VPP infection always followed a lobular pattern, and it was found that a normal lobule adjacent to an

infected lobule contained no abnormal amount of hyperplastic round cell tissue. Although if a so-called "secondary break" were occurring, the suppurative bronchial pneumonia lesions were found in both the VPP infected lobules as well as the VPP non-infected lobules.

Small nodules of round cells were sometimes found in the VPP negative lung sections (Plate VI, Fig. 11, and Plate 7, Fig. 12). They were located near bronchi and bronchioles or between two closely arranged bronchioles, and occasionally they were observed just beneath the pleura of the lung in the parenchyma of the lung tissue. There were usually only one and sometimes two nodules per lung section in VPP negative sections, and the bronchioles were not involved in any way by the round cells. Atelectasis resulting from the presence of the round cells was minimal. The smooth muscle present surrounding the bronchioles and vessels was intact and appeared to be uninvolved.

In the commercial swine herds examined during this same period of time by the same methods used in examination of the repopulation SPF herds, the three swine herds positive for VPP also had accompanying pneumonias of nonsuppurative bronchial pneumonia, subacute suppurative bronchial pneumonia, and eosinophilic bronchial pneumonia. A varying amount of atelectasis was always present in the positive cases of VPP. The VPP lesions present in the positive cases were extensive enough that numerous bronchioles were involved. The lesions were typical of VPP with a peribronchiolar and perivascular round cell hyperplasia and an extensive accumulation of round cells between the remaining

smooth muscle layer and bronchiolar epithelium. The cellular exudate was present in the alveoli and bronchioles, unless the lumen of the bronchioles was compressed by round cell hyperplasia until the lumen was closed or almost closed.

A careful microscopic study of each lung section must always be made. Usually by a quick scanning lens examination, the presence or absence of hyperplastic round cell nodules can be determined. When a nodule or several nodules of cells were detected by scanning lens examination, a higher power microscopic examination of each nodule was made. In all the sections of lung tissue studied, one section in the commercial herd examination disclosed a mass of cells that resembled a VPP lesion using a scanning lens examination; however, what had appeared as germinal centers on first examination was found by higher magnification study (Plate VII, Fig. 13) to be a large number of alveoli filled with cells, and no bronchioles were found to be involved. A negative VPP diagnosis was given and special stains, reticulum, acid fast, Grocott and PAS were used to make a diagnosis. The results from the special stains were all negative, and more stains and study will be necessary for a diagnosis.

Results of Preliminary Attempts to Produce Pneumonia in Swine

This investigation deals entirely with the experimentation in the production of a bacterial pneumonia and a virus pneumonia in commercial swine. In the first group of swine purchased, one pig was picked at random and necropsied prior to the start of the

experimental work. There were no lung lesions found grossly or microscopically that indicated the presence of a bacterial or viral pneumonia.

Experiment I. In this experiment five pigs were used. Each individual animal was identified by an ear notch (EN) (Table 1).

Table 1--Identification and Dosage Given Each Animal

Identification	Quantity	Material given intratracheally
No notch	0	----------
1 EN right (R)	3 ml.	Sterile nutrient broth
1 EN left (L)	3 ml.	Streptococcus sp. broth culture
2 ENR	3 ml.	Pasteurella sp. broth culture
2 ENL	3 ml.	Streptococcus & Pasteurella spp. broth culture

The temperatures of all animals (Table 2) were taken for 21 consecutive days PI. At the end of this period, pig 2 ENL was euthanatized and necropsied. The temperature of all animals varied one to two degrees from day to day; and at no time was the temperature elevated sufficiently to suspect a severe inflammatory reaction. Twelve days PI an investigation into the kind of feed disclosed that it contained a low level antibiotic protein supplement which was removed immediately from the feeding ration. On the next day there was a slight elevation in the temperature of all animals except one, and for the remainder of the experiment, in no animal was there a temperature elevation that would indicate an inflammatory reaction was taking place.

No organisms were recovered from the lungs of 2 ENR or 2 ENL when the lungs of both animals were cultured on blood agar following necropsy. No pneumonic lesions were observed grossly. Five sections were prepared from the lungs of 2 ENL, and all sections were negative microscopically for VPP and bronchial pneumonia. The lung sections contained some atelectasis and an alveolar epithelial cell hyperplasia. The alveolar walls were thicker than normal containing swollen alveolar epithelial cells, lymphocytes, plasma cells, fibroblasts and neutrophils, and only a few cells were found free in the alveoli and bronchioles.

The four lung sections from the animal numbered 2 ENR were similar microscopically to the lung section from the animal numbered 2 ENL, except there was more hemorrhage and congestion present. This animal, 2 ENR, was found dead the morning of the twenty-first day PI. At necropsy an intussusception was found involving approximately the last 16 inches of the ileum which had

Experiment II. One pig was used in this experiment with the inoculum containing a Pasteurella sp. and a Streptococcus sp. The animal was subjected to a temperature of 4 C for four hours prior to the intratracheal inoculation of organisms. The daily rectal temperature was recorded for 25 consecutive days, and the temperature remained within the normal range throughout this period. The greatest variation in temperature occurred on the day of inoculation when the animal's temperature increased 2 degrees during the four hour period spent in the cooler at 4 C.

The animal was not necropsied at the end of the experimental period, because no elevated temperature had occurred that would indicate an infection.

Experiment III. The pig used in this experiment was given an intravenous injection of India ink four hours prior to the intratracheal injection of three bacterial organisms, Pasteurella sp., Staphylococcus aureus, and Streptococcus sp. The rectal temperature (Table 2) was the highest on the second day PI followed by a gradual drop in temperature for the next three days. The WBC count was elevated to 36,500 on the fifth day PI (Table 2).

Table 2--Temperature and WBC Count for Pig Given India Ink

	Day of inoculation	Days post inoculate				
Days		1	2	3	4	5
Temperature	103.6	103.4	106.8	106.0	105.6	104.8
Total WBC count	16050	---	---	---	---	36500

The animal was euthanatized, followed by a necropsy on the fifth day PI. Gross examination of the lungs revealed them to be grey in color and somewhat atelectatic. Lung tissue was inoculated on to blood agar medium, and a Pasteurella and Staphylococcus spp. were recovered. Six sections of lung tissue were prepared for microscopic study.

The microscopy study of the lung sections disclosed some atelectasis throughout all sections with some congestion also present. The alveolar walls were thicker than normal and contained many swollen alveolar epithelial cells, lymphocytes,

neutrophils, plasma cells, and fibroblasts. Some of the alveolar epithelial cells were found free in the alveoli. Small particles to clumps of India ink were found in the alveolar walls and appeared to be phagocytized by the alveolar epithelial cells. Only a small number of particles was observed in the neutrophils. Some of the alveoli contained clumps of black material that resembled the material in the alveolar walls. Besides the alveolar epithelial cell hyperplasia, there was some pleuritis present. The pleura was thicker than normal and contained an increased number of lymphocytes, fibroblasts, and neutrophils. A few fibrin strands were noticed extending outward from the pleural surface.

Experiment IV. The stressor applied in this experiment was live steam for a period of 30 minutes. The pig's rectal temperature was taken on the day of inoculation and for 11 days PI. On the seventh day PI the animal's temperature was 104.4, which was the highest that it reached for the eleven experimental days. On the seventh day PI pelleted antibiotic feed was found in the ration. The animal's temperature gradually dropped till on the eleventh day PI the temperature was 102.6, and at this time the experiment was stopped. The animal was released from the experiment, and there was no necropsy performed.

Experiment V. Ultra-sound treatment was applied to two pigs for three days in this experiment. After the ultra-sound treatment on the third day identical bacterial cultures were given to both animals. Respiratory rales were heard in both pigs for four days following the bacterial culture inoculation, and by the eighth day, pig 1 was displaying noticeable dyspnea, but pig 2

did not have this clinical symptom. The rectal temperatures (Table 3) were taken for 13 consecutive days beginning with the first day of ultra-sound treatment.

Table 3--Temperature Range and WBC Count of Ultra-Sound Treated Pigs

	Animal identification numbers			
	1		2	
Days	Temperature	Total WBC count	Temperature	Total WBC count
1*	104.0	10465	104.2	7012
2	105.6	9250	104.2	11400
3**	105.0	12649	104.6	8544
4	105.6		105.0	
5	106.0		104.2	
6	106.2	12253	104.2	17605
7	105.4		103.4	
8	104.2		103.0	
9	104.6		103.0	
10	105.0		103.6	
11	105.8		103.0	
12	106.0		103.0	
13	106.4	Blood clotted	103.2	

*Beginning day of ultra-sound treatment

**Day of bacteria culture inoculation

A total WBC count (Table 3) for each animal was made for the three days of ultra-sound treatment and also on the third day PI. On

the thirteenth day of the experiment the number one animal was euthanatized and necropsied. The gross pathology found at necropsy was a pleuritis of all the lobes of the lung with extensive adhesions of both the right and left diphragmatic lobes. There was also a pericarditis with adhesions between the epicardium and pericardium. No bacterial growth occurred when blood agar plates were inoculated with material from the pericardial sac, but bacterial growths resembling Pasteurella sp. and staphylococcus sp. by Gram's method of staining did occur when blood agar plates were inoculated with material from lung tissue.

Six sections of lung tissue were prepared for microscopic study. The pleura on all lung sections was thickened, varying from only a slight amount to an extensive amount of thickening in some areas. There were large numbers of lymphocytes, macrophages, and fibroblasts located in the fibrinous stroma. Atelectasis and some emphysema were present in all sections. The alveolar walls were thickened containing swollen alveolar epithelial cells, lymphocytes, neutrophils, and fibroblasts. A thin band of fibrinous material was observed along the edge of the alveolar wall. Some of the bronchioles and alveoli contained a cellular exudate of detached alveolar epithelial cells and neutrophils.

Experiment VI. Three pigs were used in this experiment in an attempt to produce a viral pneumonia and a mixed, viral and bacterial, pneumonia. Pig 1 in this experiment was used previously in Experiment V. The rectal temperature of each animal (Table 4) was taken for 17 consecutive days except the fourth

day PI when no temperatures were taken.

Table 4--Temperatures of Animals Used in Experiment VI

	Animal identification numbers		
	1	2	3
Day	Temperature	Temperature	Temperature
0*	103.8	103.2	103.0
1	105.4	102.8	104.0
2	104.4	105.0	104.2
3	104.4	103.6	103.8
4	No temperature taken		
5	104.4	103.4	103.8**
6	103.4	104.0	104.8
7	103.6	103.8	106.2
8	103.4	103.2	106.0
9	103.6	104.0	105.0
10	104.2	104.0	104.0
11	105.6	104.0	104.0
12	104.8	104.2	104.0
13	104.8	103.6	103.2
14	104.4	103.6	103.0
15	105.0	104.0	102.8
16	102.8	102.6	103.2

*The day all three pigs were given viral lung suspension inoculum.

**The number 3 pig given second inoculum consisting of bacterial culture.

The temperature of each animal was elevated one and two days PI following the intratracheal administration of the suspected viral lung suspension. The temperature of the number 3 animal was only a high normal at this time, but two days after administering the bacterial culture inoculum, the temperature was elevated to 106.2 with the temperature only slightly lower on the following day and gradually dropping to 102.8 by the fifteenth day PI. The highest temperature recorded for pig 1 was on the eleventh PI day. The temperature of this animal remained in this range until the sixteenth PI day when it returned to normal. The temperature of pig 2 was the highest on the second PI day, and the temperature for the remaining days of the experiment was within normal limits.

All three pigs were euthanatized and necropsied on the sixteenth day PI. The gross lesions observed in pig 1 included dark red to purple streaks in the right apical and cardiac lobes of the lungs. The same gross lesions were found in pig 2, and the same lobes were involved. A similar lesion was found in pig 3, but the lobes involved were the left apical and cardiac, the left anterior ventral diaphragmatic, and the intermediate lobe.

Nine lung tissue sections were studied microscopically from each of animals 1 and 2, and fifteen lung sections were studied from pig 3. Six sections were stained with special stains, including reticulum, trichrome, and PTAH.

The microscopic study of the lung sections from pig 1 disclosed a pleuritis present in some of the sections. There were varying amounts of atelectasis and emphysema. The alveolar walls

in all sections were thicker than normal and contained swollen alveolar epithelial cells, lymphocytes and some fibroblasts. In one section the alveoli contained a cellular exudate consisting of numerous eosinophils and detached alveolar epithelial cells. A minimal number of round cells were found near or around the bronchioles in one lung tissue section. There were no nodules of round cells present in any of the tissue sections.

The lesions found in the sections of lung tissue from pig 2 when studied microscopically resembled the lesions observed in the lung sections of pig 1, except there were only a few eosinophils found in the alveoli. The number of round cells near or around bronchioles were few in number and not in distinct nodules.

The microscopic lesions found in the lung sections of pig 3 were similar to the lesions that were found in the other two animals. There were three sections from this animal that were more extensively affected by atelectasis than were found in the sections from the other two experimental animals. Alveolar epithelial cell hyperplasia was commonly found in all sections. The number of round cells present near bronchioles and vessels was minimal. There were no lung sections from the three animals that had lesions characteristic of VPP.

DISCUSSION

The results of this investigation indicated that there was a high percentage (over 30%) of VPP present in commercial swine going to slaughter. Diagnosis of VPP by the gross lesions alone was achieved in over 80% of the cases. Errors in the diagnosis

of VPP at necropsy occurred when a large amount of congestion and hemorrhage was present in the lungs, in early cases with the absence of typical gross lung lesions, possibly in cases of early acute suppurative bronchial pneumonia, and the presence of a hemorrhagic infarct. Two gross lung lesions that were found only in the diaphragmatic lobes of the lung were negative for VPP when they were examined microscopically.

The microscopic examination of the lungs from commercial swine indicated that the lesions of bronchial pneumonia often were present with the lesions of VPP. The pneumonias were either acute or subacute suppurative, or nonsuppurative bronchial pneumonias. Atelectasis and emphysema were common lesions associated with VPP, but they were also found in other types of pneumonia. The amount of atelectasis present in the immediate area of the VPP lesion depended on the amount of round cell hyperplasia; when the nodule of round cells was large, the amount of atelectasis was extensive. The amount of atelectasis and emphysema present in a lobule was greater when the lumen of the bronchiole was compressed by the presence of large numbers of round cells between the layer of smooth muscle and the bronchial epithelium. An alveolar epithelial cell hyperplasia was present in almost all lung sections collected from the commercial swine, but the areas of acute suppurative inflammation contained only a small amount, while the bordering areas were more involved.

Special stains of VPP positive lung sections were studied since some diseases caused by viral agents were characterized by the presence of intranuclear or cytoplasmic inclusion bodies. No

inclusion bodies were observed when VPP positive lung sections were stained with special stains and examined microscopically. The large cells, histiocyte, found in the round cell nodules contained a large body which was not differentially stained by the special stains.

The alveoli, bronchioles, and bronchi of some of the commercial swine lung sections contained large numbers of eosinophils with no lung parasites accompanying the cellular exudate. There was the possibility that the lung parasite was present in other areas of the lung, but also other foreign agents, such as other migrating parasites, dust, feed, and plant aromatics, could possibly cause the condition. The commercial swine would be more likely to be infected with lung parasites than the SPF swine, although the SPF swine could be infected if allowed access to parasitic contaminated feed lots. The eosinophilic condition was found in a few of the SPF lung samples.

Investigation in the repopulation herds revealed that only a small number of lung sections were considered nearly normal; and the complete section was never completely normal. Congestion, hemorrhage, edema, swollen alveolar epithelial cells, atelectasis, and emphysema, all of which may be present in only a slight amount, constituted conditions that were deviations from the normal lung tissue. The question of how much lymphoid tissue was normal for the lung of swine probably passes through the mind of many pathologists, especially if a section of lung tissue was being studied that was suspicious for VPP lesions. Trautmann and Fiebiger[30] described interstitial lung tissue as tissue that contained

leukocytes and, especially in swine, lymph nodules. Some of the lung tissue sections from VPP negative SPF swine herds (Plate VI, Fig. 11, and Plate VII, Fig. 12) contained small nodule of round cells along bronchi and between bronchioles and vessels; and in contrast, the VPP lung displayed lesions in which the smooth muscle fibers of the bronchi and bronchioles were either separated or atrophy of the smooth muscle had occurred.

The bronchial pneumonia lesions were not found as an extensive lung involvement along with the VPP lesions in the SPF repopulation swine lung sections. This was probably due to better care and management of the swine herds; although with this better management VPP was found in some of the SPF repopulation swine herds. Probably with improved animal husbandry, bacterial infections were reduced, resulting in a reduced amount of suppurative pneumonias. The VPP lung lesions were found in some SPF repopulation herds. A correlation of herd history, management practice, and lung pathology indicated that only small errors in methods of caring for the animals resulted in VPP infections. In some cases infected SPF swine were purchased. One herd of swine was found infected because the owner had failed to first dispose of all the infected animals on his farm.

The criteria for the development of a bacterial pneumonia were investigated using commercial swine. Different stressors were used, and different bacterial cultures and combinations of bacterial cultures were given to the animals. The results of two of the experiments were affected when antibiotic feed was added to the ration of the animals.

The Pasteurella sp. used in one experiment was from uncertain origin, and if it were from an animal species other than the one being used experimentally, this could have been the cause for the negative findings. An acute suppurative pneumonia was not found in any of the lung sections from the experimental swine. The use of different stressors, some of which often occur in the normal handling of swine, had no apparent influence on the development of lung infections.

Many factors must be involved in the development of a typical bacterial lung infection. Factors such as, parasitic infections, inadequate nutrition, fatigue, exposure to inclement weather, lowering of body resistance by other infectious agents, and probably many more are involved. Undoubtedly, the presence of VPP lesions has an influence that lowers the animal's resistance or makes the lung tissue more susceptible to a bacterial infection. Possibly a virus that has no lesion producing ability itself aids in lowering the resistance of the lung tissue. Also a bacterial virus, bacteriophage, could possibly be a factor involved. The administration of too many or too few bacterial organisms or non-pathogenic organisms could be factors that influenced the experimental results.

India ink was used as a method of retarding phagocytosis of intratracheal inoculation of bacteria with the theory in mind that a resulting bacterial lung infection could occur. Two of the three organisms were recovered following necropsy of the animal, but no bronchial pneumonia was found when the tissue was studied microscopically.

CONCLUSIONS

This investigation indicates that VPP was still an important disease in commercial swine. Acute and subacute suppurative bronchial pneumonia or a nonsuppurative bronchial pneumonia often accompanies VPP pneumonia. However, VPP was found when only alveolar epithelial cell hyperplasia was present. In the microscopic study of lung sections, at least one and usually two or more of these lung conditions were always found in the tissue section along with the VPP lesions.

The lung lesions of VPP were found present in some of the SPF repopulation herds. This indicates that all precautions, such as, sanitation, herd management, farm visitor regulations, and many more, must be closely scrutinized. There was a low percentage of the SPF repopulation swine herds found infected with VPP, and a high percentage of the lungs from the repopulation swine herds was found free of pneumonic lesions. It was demonstrated that swine producer by careful management practice can produce VPP free swine, which in turn, will result in healthier animals, reducing the possibility of secondary bacterial infections occurring and result in a more economical swine production operation.

The following methods are recommended for the positive diagnosis of VPP:

1. When a representative group of lung sections from a herd of swine is examined, gross and microscopic lung lesions of VPP should be present.

2. If gross and microscopic lung lesions are found and a suspicious or positive diagnosis of VPP is made, a special study on the farm of clinical symptoms and herd history should be made of all swine.

3. Following a herd study, lung tissue from the lungs of VPP positive or suspicious animals should be prepared and injected into VPP free swine, preferably primary SPF swine, with the production of gross and microscopic VPP lesions.

Preliminary experimental trials were used in an effort to produce a bacterial bronchial pneumonia. The results were inconclusive, and lesions of bronchial pneumonias were not produced. The stressors and bacterial organisms that were used had only minor effect on the lung tissue. Other bacterial organisms, sources and methods of administering bacterial organisms, along with the application of the different stressors, must be considered when the production of swine pneumonias are considered.

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APPENDIX

EXPLANATION OF PLATE I

Fig. 1. The gross appearance of some of the lungs of slaughtered swine, a lung displaying massive congestion and hemorrhage which could mask lesions of VPP.

Fig. 2. The gross appearance of lungs from slaughtered swine with only slight or complete absence of congestion and hemorrhage.

PLATE I

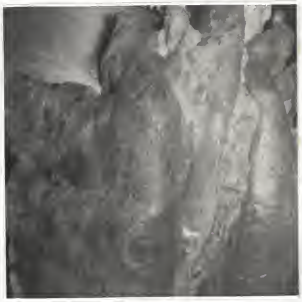

Fig. 1.

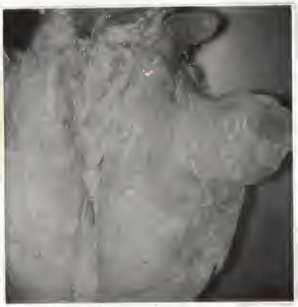

Fig. 2.

EXPLANATION OF PLATE II

Fig. 3. Gross appearance of VPP lung lesions found present in the ventral part of the left cardiac lobe with sharp line of demarcation (arrow) between infected and noninfected tissue.

Fig. 4. Bronchiole containing a serofibrinous material; cellular exudate of neutrophils 2, plasma cells 3, lymphocytes 4, and bronchiolar epithelium 5. x 500.

PLATE II

Fig. 3.

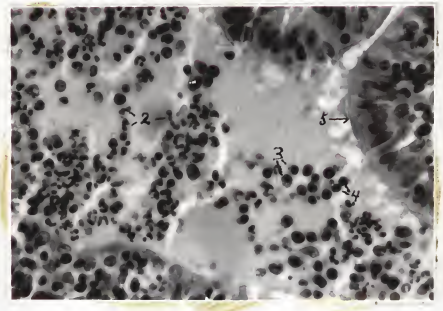

Fig. 4.

EXPLANATION OF PLATE III

Fig. 5. Alveoli of the lung containing a serofibrinous material 1, detached alveolar epithelial cells 2, plasma cells 3, fibroblasts along alveolar wall 4, lymphocytes 5, and RBC 6. x 500.

Fig. 6. Thickened alveolar walls of the lung containing swollen alveolar epithelial cells 1, lymphocytes 2, and detached alveolar epithelial cells 3. x 500.

PLATE III

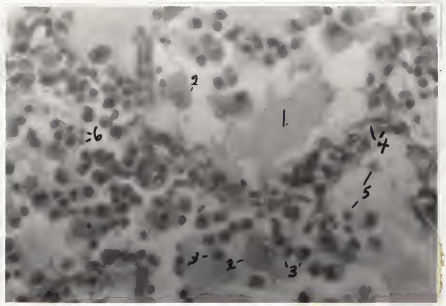

Fig. 5.

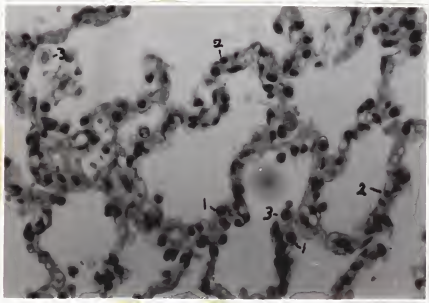

Fig. 6.

EXPLANATION OF PLATE IV

Fig. 7. Typical VPP lung lesions that are found microscopically, numerous bronchioles with peribronchiolar round cell hyperplasia, tissue with germinal centers resembling lymphoid tissue 1, lumen of bronchioles small and compressed 2, atelectasis observed in the tissue surrounding the areas of hyperplastic round cell accumulation 3. x 18.

PLATE IV

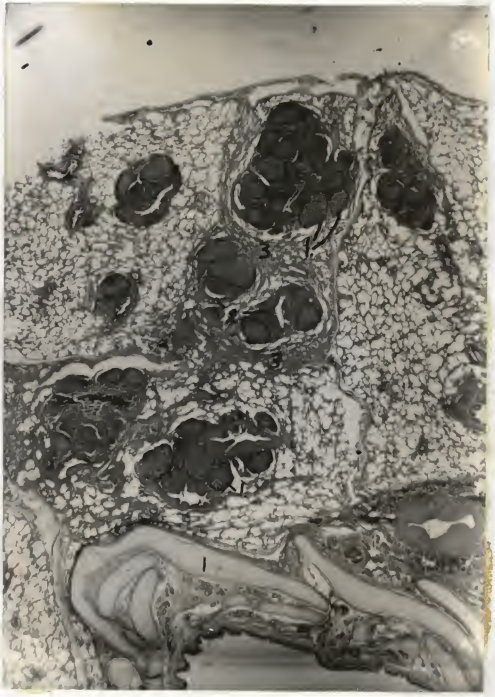

Fig. 7.

EXPLANATION OF PLATE V

Fig. 8. Round cell hyperplasia found in a VPP lesion involving a bronchiole, resulting in a narrowing of bronchiolar lumen, and a separation of smooth muscle fibers lower left and atrophy of smooth muscle upper center. Note large numbers of round cells between bronchiolar epithelium and smooth muscle. x 125.

Fig. 9. Three bronchioles displaying round cell hyperplasia. Center bronchiole with an elongated and compressed lumen, also atrophy of smooth muscle. Observe cellular exudate in alveoli. x 50.

PLATE V

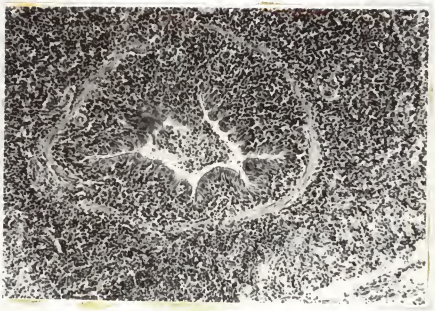

Fig. 8.

Fig. 9.

EXPLANATION OF PLATE VI

Fig. 10. Cells that are found in round cell masses near bronchiole and vessels, lymphocytes 1, histiocytes 2. x 500.

Fig. 11. Longitudinal section of bronchi with small follicle of round cells present, arrow. x 50.

PLATE VI

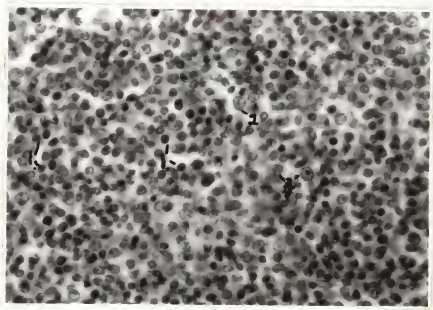

Fig. 10.

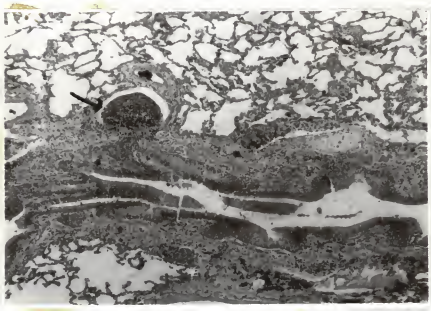

Fig. 11.

EXPLANATION OF PLATE VII

Fig. 12. Small follicle of round cells located between two bronchioles and a vessel. The bronchioles and vessel display no pathological changes. x 50.

Fig. 13. Mass of tissue of undetermined origin and not found near a bronchus or bronchiole. The cellular tissue appears to fill alveolar sacs. x 125.

PLATE VII

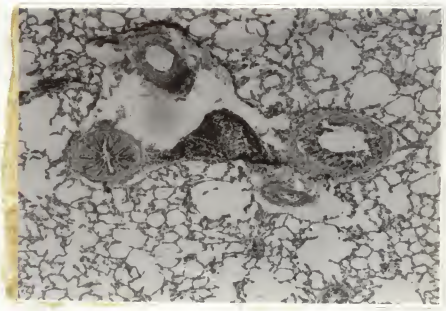

Fig. 12.

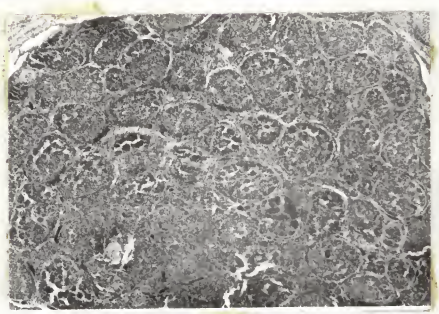

Fig. 13.

INVESTIGATION OF VIRUS PIG PNEUMONIA AND OTHER PULMONARY LESIONS IN SPECIFIC PATHOGEN FREE REPOPULATION, COMMERCIAL, AND EXPERIMENTAL SWINE

by

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B. S., and D. V. M., Kansas State University, 1953

AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

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Great economic losses have been encountered by swine producers from animal deaths and reduced efficiency of feed utilization due to Virus Pig Pneumonia (VPP) which was often complicated by bacterial pneumonia. To aid in overcoming this economic loss, some producers have started to develop Specific Pathogen Free (SPF) repopulation swine herds. This investigation was made to help in the differential diagnosis of VPP from other respiratory diseases when they were encountered in these repopulation swine herds and also other commercial herds.

Most of the research workers have concluded that VPP was caused by a filterable agent which was between 200 and 300 m in size. Reports indicated that lungs having lesions of VPP were often further complicated by a bronchial pneumonia. The results of this investigation were in agreement, especially in the lungs of commercial swine.

The gross lung lesions of VPP, which were reported by other research workers and found by this investigation to be diagnostic in over 80% of the cases, were plum colored and located in the ventral part of the cardiac and apical lobes, anterior ventral part of the diaphragmatic lobes, and the intermediate lobe of the lungs. The results further indicate that the right or left apical and cardiac lobes of the lung were the lobes generally involved, although one lobe or any combination of lobes may be involved. If lesions were found only in diaphragmatic lobes, a diagnosis of VPP was questionable.

The microscopic lesions of VPP were similar to the lesions described by other research workers. A perivascular and peri-

bronchiolar round cell hyperplasia was found with a large number of round cells present between the smooth muscle or remnant of smooth muscle and the bronchiolar epithelium resulting in a narrowing of the lumen. Often the round cell nodule was extensive, which resulted not only in the separation of smooth muscle fibers but also an atrophy of the muscle fibers. Numerous bronchioles and vessels were involved with the VPP lesions.

The bronchial pneumonia that was found by microscopic examination associated with the VPP lesions was also found in VPP negative lung sections. The microscopic lesions varied from an acute suppurative bronchial pneumonia to a nonsuppurative pneumonia. In the acute suppurative pneumonias large numbers of neutrophils and a serous exudate were found in the bronchi, bronchioles, and alveoli. The nonsuppurative bronchial pneumonia was characterized by the bronchi, bronchioles and alveoli containing only detached alveolar epithelial cells.

Other microscopic lesions that were found in lung sections were hemorrhage, congestion, edema, atelectasis, emphysema, fibrosis and alveolar epithelial cell hyperplasia. These lesions, all or in part, were present in both pneumonic lung sections and nonpneumonic lung tissue.

Five hundred two lung sections from SPF repopulation herds were examined. The lesions of VPP were found in a minimal number of herds. A study of herd history and management practices in the VPP infected herds disclosed that careful herd management must be observed at all times.

A preliminary experimental investigation was conducted in an

effort to study the microscopic lesions of a bacterial pneumonia and a combination bacterial and viral pneumonia. Bacterial organisms and an extract from swine lung tissue were injected intratracheally following the use of different stressors. Reports of research workers indicated that the Pasteurella organism was often recovered from pneumonic lesions. In this investigation all experiments conducted included the Pasteurella organism. Lung lesions but no bronchial pneumonias were produced, and no characteristic VPP lung lesions were found. It was concluded that other bacteria, stressors, and certain conditions must be present to produce a pneumonia in swine.