Chronological studies of respiratory disease in baby pigs

by

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INTRODUCTION

Gross income from swine production was over eight billion dollars in 1978 (1) and accounted for approximately ten percent of the income from livestock and poultry production. In the past five years, large scale confinement production has continued to increase in popularity (three-fourths of farms marketing over five-thousand hogs annually are confinement operations now, as compared to two-thirds in 1978 (2)), net capital investment per hundred weight of hogs marketed has increased from thirty-four to forty-six dollars (3) and the number of hogs marketed per year is now over eighty million (4). Disease, however, has become a major factor limiting expansion (5), and respiratory disease especially is a complaint among producers finishing hogs (6). Pneumonia and atrophic rhinitis decrease the pigs' growth potential, necessitating added financial investment in feed, antibiotics, and housing to ready the animals for market (7).

Complaints about respiratory disease while the young pigs are nursing are often overridden by concern about stillbirths, scours, and overlain pigs in the farrowing crate. However, facets of respiratory disease are very important during the nursing period. This is often when exposure first occurs, where early signs may first be noted, and where disease prophylaxis may be of use. Transmission of respiratory pathogens may easily occur among pigs in this age group. Pigs that have been infected early in life may develop clinical signs of

respiratory disease if stressed by congenital weakness, feeding irregularities, or enteritis (8). Many producers have attempted to prevent establishment of respiratory pathogens in the young pig by treating them with antibiotics several times during the first few weeks of life (9,10,11).

The research herein attacks a lack of understanding of respiratory disease by examining baby pigs from large confinement herds with a history of respiratory tract disease. The farms involved were having a severe enough problem to warrant consulting their respective veterinarians and the Iowa State University Veterinary Diagnostic Laboratory. In each case considered, coughing in baby pigs seemed to be associated with retarded growth and affected pigs' growth remained on a plateau two to three weeks longer than expected after weaning. Respiratory disease in one- to eight-week-old pigs was investigated on six such farms utilizing a three-fold approach: 1) characterization of the mycoplasmal and bacterial flora in nasal secretions, 2) determination of the prevalence of antibodies to selected respiratory pathogens, and 3) necropsy and microbiologic examination of selected pigs observed to be coughing. The upper respiratory tract flora was studied to identify the respiratory pathogens colonizing the nasal mucosa in belief that these organisms may invade the lower respiratory tract if the lung is compromised. Serologic tests were used to assess the occurrence and possible pathogenic significance of mycoplasmas and haemophili in respiratory disease. Necropsy of pigs with clinical signs allowed

examination of the entire respiratory tract for lesions and for organisms which might be etiologically involved. The data compiled have provided an insight to the respiratory problems that actually existed in these neonatal pigs.

EXPLANATION OF THESIS FORMAT

This thesis consists of an introduction, a literature review, two separate manuscripts, a conclusion, listing of literature cited, and acknowledgements. Both manuscripts are to be submitted to the American Journal of Veterinary Research. References cited in each manuscript are included with that manuscript. The listing of literature cited contains citations present in the introduction, literature review, and general discussion. The master's candidate, Barbara Ellen Kott, was the principal investigator for each of these studies and is the senior author of each manuscript.

LITERATURE REVIEW

The intensification of swine production in recent years has substantially increased the severity of certain disease problems. The respiratory component of these problems includes atrophic rhinitis and a variety of pneumonias. Management of pneumonias in swine requires an understanding of the microorganisms involved and the sequence of their occurrence in the respiratory tract. Large numbers of swine housed together in close confinement create an optimal environment for the rapid spread of pathogens by contact as well as by aerosol, with a high prevalence of chronic pneumonias being commonly recognized under such conditions (12).

The respiratory tract of the neonatal pig is especially susceptible to infections with respiratory pathogens. The immune system of the respiratory tract requires some time to fully develop. Immunoglobulin-containing cells do not reach numbers in respiratory tissues comparable to those in adult swine until the pig is three to four weeks old, and such cells are actually undetectable in pigs less than one week of age (13). Small size and poor ability to maintain body temperature may also predispose baby pigs to respiratory disease. The smaller the animal, the greater the overall retention of particles of any given size, thus there is an increased chance that particles greater than 2 microns (the approximate size of many aerosols bearing bacteria and mycoplasmas) will reach the alveoli (14,15). The neonatal animal is

disadvantaged when it comes to getting rid of these particles. Respiratory clearance of an E. coli aerosol was one-half as efficient in pigs one day of age as in those four weeks of age (16).

Mortality data may serve to illustrate further the disparity in disease susceptibility between nursing and growing pigs. A 1982 compilation of data from Iowa swine suggests that birth to weaning mortality of liveborn pigs is over seventeen percent, whereas weaning to market mortality decreases to six percent (3). Over seventy percent of pre-weaning losses occur within the first few weeks of life, and over forty percent of these are attributed to disease causes (17). Relatively few of these deaths are due to pneumonia, even though many pigs become infected with respiratory pathogens during the pre-weaning period (17,18,19,20).

Pneumonia in baby pigs has been blamed on poor environmental conditions. Fluctuations in temperature and high humidity, common in confinement housing, may chill the baby pig, and ineffective pulmonary clearance may result (21,22). When pigs one day of age were exposed to an aerosol of non-pathogenic E. coli, those housed at 6°C had bacterial concentrations in the lungs five-fold higher than that of pigs housed at 32°C (16). Pigs four weeks of age were able to clear the material as well at 6°C as at 32°C. It has been postulated that mucus secretions in the baby pig are modified by chilling (23). The same author suggested that extremes in relative humidity, whether high or low, also may exert unfavorable effects on the respiratory tract.

Ammonia and dust have been implicated as causes of sneezing and coughing in pigs (15,24,25). Ammonia levels in farrowing houses usually range from 30 ppm to 50 ppm, and the dust usually has 100,000 to 700,000 particles per cubic foot in the lung-depositable size (<5 microns) (15,21,26,27). In a study done in 1971, pigs one week of age were exposed to 100 ppm NH_3 and about 1.5 million particles (<5 microns) per cubic foot of organic dust for up to six weeks (25). Very mild signs of conjunctivitis were seen during the first week, but the pigs adjusted to the irritants, and the clinical signs abated. Neither coughing nor sneezing was apparent in these pigs, even when the ammonia level was raised to 150 ppm. However, there were microscopic changes in the respiratory tract. Tracheal and nasal epithelia were thickened by the second week of ammonia exposure and there were decreased numbers of goblet cells. There were a few neutrophils scattered in the lamina propria and epithelium of the trachea. Though the damage was slight, it could possibly compromise the respiratory tissues enough to allow establishment of microbial infection. It can be stated that pulmonary clearance and physiological resistance to environmental changes that reduce clearance in swine improve with age (13,16,17,21,28).

Organic feedstuffs are the cause of small granulomata in the lung (14,29). These firm, dark areas seem to be reactions to inhaled wood or starch particles. Most are not extensive and seem not to be associated with bacterial infection; rather, they are usually an incidental finding.

The bulk of pneumonias in swine are ascribed to infectious agents, whether as primary causes or secondary invaders in a compromised lung. Often a number of microbes cooperate to produce a severe disease state. As lung lesions develop, the number of different organisms may increase with the extent of the lesions. By the time an infected animal shows clinical signs of pneumonia many organisms may be present in the lung, complicating disease as well as diagnosis of the primary pathogen.

Mycoplasma

Mycoplasma hyopneumoniae

Mycoplasma hyopneumoniae is believed to be a primary agent in pneumonia of growing swine, and is considered to increase the susceptibility of pigs to other pneumonia-causing organisms. In the naturally-occurring infection, a persistent non-productive cough progresses through acute, subacute, and chronic stages. All of the hogs exposed will usually become infected, yet many of them will never show signs of pneumonia. Rather than coughing, the chronic disease is more likely evidenced by reduced growth rate, decreased feed conversion, and, often, general debility (30,31).

The incubation period for M. hyopneumoniae disease is generally considered to be two to three weeks (32). Because clinical signs of mycoplasmal pneumonia may be sporadic (33), the presence of the disease in a herd may not be suspected until lesions are noted when animals

are slaughtered (34). Early lesions consist of tan to purple areas of consolidation with the consistency of atelectatic lung, and are found primarily in the cranioventral portions of the lung (33). Histologically, these are areas of increased cellularity of the alveolar walls with extensive perivascular and peribronchiolar lymphohistiocytic cell accumulations and infiltration (33,35). Chronic lesions, evidenced by lymphocytic nodules that may constrict bronchioles, may be present in pigs as late as two to three months after infection (35,36).

Experimentally, pigs as young as four hours old have been shown to be susceptible to M. hyopneumoniae (37). In pigs inoculated with M. hyopneumoniae at 6 to 9 weeks of age, gross lesions of active mycoplasmal pneumonia developed within 10 to 28 days (35,37). Sows in chronically infected herds are suspected of harboring the organism in their respiratory tracts and it is presumed that such sows serve to infect young pigs. There have been a few reports of naturally-occurring mycoplasmal pneumonia in pigs two to five weeks of age (detected by direct immunofluorescent testing) (38) and eight to twelve weeks of age (detected by isolation of M. hyopneumoniae) (34). It may be possible for spread of the disease to be pronounced when pigs with mycoplasmal pneumonia are co-mingled at weaning time (35,38). Older swine not previously exposed to M. hyopneumoniae are susceptible to infection (39). The peak prevalence of mycoplasmal pneumonia in infected herds is in swine 16 to 19 weeks of age (8).

Considering the extended duration of the disease in swine, it is no wonder that mycoplasmal pneumonia is a major concern in modern swine confinement operations. In the presence of secondary organisms such as Pasteurella multocida, Bordetella bronchiseptica, Haemophilus spp., or other mycoplasmas, M. hyopneumoniae disease may be quite severe (40,41).

The direct fluorescent antibody technique is used successfully to diagnose mycoplasmal pneumonia. M. hyopneumoniae can be detected on the bronchial epithelium (29,42). The organism has been shown to possess a tropism for the ciliated epithelial cells of the respiratory tract (43).

Mycoplasma hyorhinis

Mycoplasma hyorhinis is often isolated from the nasal cavities and lungs of swine. In two Iowa studies (44,45), thirty percent and forty-two percent of the herds examined had pigs with M. hyorhinis in their nasal cavities. In a study of herds in Switzerland, thirty-two percent had pigs with this organism (46). M. hyorhinis is commonly isolated from pneumonic lungs of market hogs (greater than fifty percent) (34,47,48), and it has been implicated as a major secondary invader in pneumonia caused by M. hyopneumoniae (31,37,49). M. hyorhinis is considered to produce a primary disease, most notably an acute arthritis and polyserositis in pigs three to ten weeks of age (50), with a morbidity of two to five percent and a low mortality (49).

Pneumonia is often associated with mycoplasmal pleurisy and arthritis (51,52,53,54). It has been suggested that some strains of M. hyorhinis are pneumotropic and do not produce pleurisy and arthritis (53).

M. hyorhinis has been considered a primary respiratory pathogen by many authors (12,52,53,54,55,56,57). Naturally-born pigs, however, seem to have an innate resistance to respiratory disease caused by M. hyorhinis when the organism is given by the respiratory route. Evidence supporting the concept that M. hyorhinis causes pneumonia was obtained by inoculation of young gnotobiotic pigs. Three- to ten-day-old gnotobiotic pigs inoculated intranasally with M. hyorhinis developed a catarrhal bronchopneumonia within two to three weeks (53,55). Clinical signs in the experimental disease were usually limited to occasional coughing and anorexia, but in more severe cases, lethargy and lameness occurred (53,57). When M. hyorhinis was inoculated into gnotobiotic pigs four to ten weeks of age, rhinitis and sneezing followed by a short, dry cough of several weeks duration were the only signs (54,56). Gross lesions at post mortem included scattered foci of pneumonia, usually limited to the cardiac and apical lobes of the lung (53,54,56,57); gross lesions of pneumonia were absent in some pigs (52,55,57). Histologically, the lesions of pneumonia were characterized by perivascular, peribronchiolar, and interstitial lymphohistiocytic infiltrates (53,54,55,56). These lesions are similar to, but not as extensive as, those produced by M. hyopneumoniae. More extensive lesions have been produced after repeated inoculation with

M. hyorhinis (54). A possible explanation for the absence of lesions in some older pigs may be that M. hyorhinis causes a mild, transient pneumonia; thus, results obtained with gnotobiotic pigs suggest that the organism may predispose the pig to further infection with other agents which cause pneumonia (58).

M. hyorhinis disease may be diagnosed by detection of the organism in typical serofibrinous lesions. M. hyorhinis may be isolated from the lung, but its role as a primary pathogen in the etiology of pneumonia is unclear due to its common isolation from lesions attributed to M. hyopenumoniae.

Mycoplasma hyosynoviae

Mycoplasma hyosynoviae probably does not play a part in respiratory tract disease in swine, but in animals over five weeks of age it is a frequent isolate from tonsillar and pharyngeal secretions (49,59,60). Upper respiratory tract infections with M. hyosynoviae could be a source of infection for the lower tract, and it is not uncommon to isolate the organism from pneumonic lungs of market hogs, probably secondary to M. hyopneumoniae and other causes of pneumonia (12,61,62). When young gnotobiotic pigs were inoculated intranasally with M. hyosynoviae, no lesions were detected in the respiratory tract; however, the organism was recovered from oral secretions (12). In swine three to six months of age, M. hyosynoviae may cause an uncomplicated serofibrinous polyarthritis (49,60).

Other mycoplasmas

Other mycoplasmas isolated from the respiratory tracts of swine are generally considered to be non-pathogenic. Mycoplasma arginini has been isolated from the nasopharynx and tonsils of normal appearing swine, and from pneumonic lungs of market hogs (49). Attempts to produce disease by inoculating gnotobiotic pigs with M. arginini resulted in no clinical signs and few gross lesions (12,56,63). Microscopically, there was slight peribronchial lymphohistiocytic proliferation in a few lungs (12,56).

Mycoplasma flocculare has an affinity for the respiratory tracts of swine (64), and has been isolated from lungs of market hogs with gross lesions of pneumonia (65). Gnotobiotic pigs inoculated intranasally with the organism "developed small mononuclear cell accumulations in the nasal epithelium and around some bronchioli" (66).

Mycoplasma salivarium and Mycoplasma buccale are organisms commonly isolated from the human upper respiratory tract. They have been isolated occasionally from the nasal cavities of market hogs (49).

Several Acholeplasma spp. have been isolated from the respiratory tracts of swine (47,49). In young pigs, A. granularum is often isolated from nasal secretions (44,45). Fewer isolates have been from market hogs, where A. laidlawii has also been isolated (47). Pigs inoculated with A. axanthum were reported to develop catarrhal pneumonia (67).

Bacteria

Haemophilus pleuropneumoniae

Haemophilus pleuropneumoniae is a cause of primary pneumonia and pleurisy with fever, anorexia, and dyspnea in growing and finishing swine. All exposed pigs may be affected, with a mortality of about twenty percent (51,68). Clinical signs of acute pleuropneumonia may occur within 24 hours after infection (68,69,70), and at post mortem areas of fibrinohemorrhagic pneumonia and serofibrinous pleurisy may be present in all lobes of the lung and are unusually common in the diaphragmatic lobes (51,70,71). When the disease becomes chronic, areas of consolidation in the lung often develop into abscesses (68, 69,72). Pneumonia caused by H. pleuropneumoniae is histologically distinct, with large, discrete areas of necrosis, granulation, and whorling of fibroblasts (69,72). In many lesions fibrinous vasculitis with thrombosis may be noted (71). Swine convalescent from pleuropneumonia are often severely retarded in growth (51,68,69). Persistent colonization of the tonsil and lung may leave recovered swine as potential carriers (68,69,73).

Although typical lesions of pleuropneumonia are usually seen in three- to four-month-old swine, H. pleuropneumoniae has been isolated occasionally from pneumonia in younger pigs[a] (34). Pigs five weeks of

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[a] H. pleuropneumoniae has been isolated from young pigs with typical lesions of pleuropneumonia by R. A. Schultz, Avoca, Iowa, and in a few cases examined by the Iowa State University Veterinary Diagnostic Laboratory.

age inoculated intratracheally with H. pleuropneumoniae died peracutely with respiratory distress or had a protracted illness characterized by fever, anorexia, and coughing (72). Convalescent young swine may infect older penmates during the growing and finishing period (68). Also, isolation of the organism from nasal secretions of young swine without disease has been reported (74).

Specific diagnosis of H. pleuropneumoniae disease is based on demonstration of typical lesions and the isolation of the organism. Seven serotypes are recognized (75,76,77,78), with serotypes 1, 2, 4, and 5 producing the characteristic fibrinous pleuropneumonia (73,79). Pasteurella multocida, a frequent secondary invader in pleuropneumonia, may inhibit in vitro growth of H. pleuropneumoniae (80). Isolation of H. pleuropneumoniae was achieved by dilution of lung homogenates prior to inoculation on agar media (81).

Haemophilus parasuis

Haemophilus parasuis[b] has been associated with a polyserositis syndrome known as Glässer's disease (87,88), but has been isolated more frequently from the nasal cavities of clinically normal young pigs.

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[b] H. parasuis synthesizes porphyrin from δ - aminolevulinic acid (i.e., does not require the X, or hemin, factor for growth); requires V factor (nicotinamide adenine dinucleotide); ferments glucose and sucrose; and fails to ferment lactose, xylose, and mannitol. This is identical to H. suis, except for the absence of X factor requirement. The overwhelming majority of strains in culture collections previously regarded as H. suis have not required the X factor for growth and have been capable of forming hemin in the absence of preformed iron porphyrins. Therefore, the name H. parasuis will be used here to solely represent the classical use of the name H. suis (82,83,84,85,86).

Herd surveys involving apparently healthy pigs six to ten weeks of age suggest that haemophili may be part of the nasal flora in almost all pigs at some time (44,45,46).

When young pigs somehow become susceptible to H. parasuis disease (either due to stress or another infectious process), H. parasuis septicemia develops and the organism localizes in the serous membrane-lined body cavities (72). In outbreaks of Glässer's disease, up to thirty percent of nursery-age pigs may be affected (41). At necropsy there is a diffuse serofibrinous pleuritis that may be accompanied by peritonitis, pericarditis, meningitis, and arthritis (45,72,89,90). There have been occasional reports of pneumonia in young pigs associated with H. parasuis. In one report there was sudden death of pigs one to two weeks of age with no previous signs of disease. An increased cellularity of alveolar walls was noted, along with focal necrosis in the liver and spleen (91). In another report, pigs six to eight weeks of age had mild peribronchitis and interstitial pneumonia, and two- to six-month-old pigs had severe rhinitis along with mild peribronchitis and chronic pleurisy (92).

When H. parasuis was inoculated into gnotobiotic or colostrum-deprived pigs in attempts to produce pneumonia, severe cases of Glässer's disease developed (12,72,93,94). Attempts to produce the disease by inoculation of H. parasuis into conventionally-reared pigs have generally failed. It has recently been reported that apparently healthy pigs with spontaneous nasal infections with H. parasuis were predisposed to establishment of Pasteurella multocida and thus developed atrophic

rhinitis (93). It is possible that separate serotypes may produce varying forms of clinical disease, or that special conditions may allow the organism to invade the lower respiratory tract. H. parasuis was isolated from the lungs of 1.7% of swine with respiratory disease in one abattoir study (95).

Diagnosis of Glässer's disease is usually based on observation of typical gross lesions at post mortem and isolation of H. parasuis from the tissues involved.

Bordetella bronchiseptica

Bordetella bronchiseptica is important in herds where it is present as an etiologic agent in atrophic rhinitis, and it is also known to invade the lower respiratory tract and cause bronchopneumonia. With infection of the nasal mucosa of pigs three to eight weeks of age, B. bronchiseptica may induce osteoclastic activity with resorption of the bony trabeculae and atrophy of the nasal turbinates (36). Clinical signs of atrophic rhinitis may occur in most infected young pigs, but mortality is low. However, mortality may reach sixty percent in cases where B. bronchiseptica infects the lower respiratory tract (19). Coughing and high fever are noted in pigs with pneumonia, and at necropsy there is marked congestion of the lung associated with hemorrhage and edema (19). The disease can persist and reduce growth rate, potentially increasing production costs (51,96).

When B. bronchiseptica was inoculated into young gnotobiotic pigs,

a severe purulent pneumonia involving proliferation of connective tissue developed. Sneezing or coughing were not observed in such pigs, but some were depressed and had reduced appetites (96,97). In a study where gnotobiotic pigs were inoculated with B. bronchiseptica at three to five days of age, all pigs coughed and sneezed from time to time (12). Fourteen days post infection these pigs were necropsied revealing only slight lesions of pneumonia and nasal mucosa that were severely swollen and covered with mucus.

B. bronchiseptica is a common isolate from the upper respiratory tracts of apparently normal young pigs (44,45,98). To diagnose atrophic rhinitis involving B. bronchiseptica, the snout is transected to examine the ventral turbinates for atrophy and presence of the organism (96). In pigs with severe B. bronchiseptica pneumonia, a pure culture of the organism may be isolated from the pneumonic lung (19,96). B. bronchiseptica also may be found occasionally in chronic pneumonias of older swine, probably as a secondary invader (36).

Streptococci

Streptococci are numerous and ubiquitous in swine environment, and may be associated with many diseases (99,100). A survey published in 1976 estimated that 2.4% of liveborn pigs die of streptococcosis during the preweaning period (101). Of these, 57.6% may have streptococci isolated from the lung. Alpha-hemolytic streptococci, most notably S. suis, have been considered primary causes of septicemia,

arthritis, meningitis, and, occasionally, pneumonia in young pigs (12,102). S. suis infection of the upper respiratory tract apparently causes no lesions, but it does enable the organism to invade the lower respiratory tract or cause septicemia in times of stress (103,104).

Alpha-hemolytic streptococci have been isolated from outbreaks or sporadic cases of suppurative pneumonia in nursing pigs two to three weeks of age (18), and may be associated with pericarditis, endocarditis, arthritis, enteritis, or meningitis (17,24,105,106). One author isolated an alpha-hemolytic streptococcus from a lung lesion in a market hog, then inoculated the culture intranasally into several gnotobiotic pigs three to five days of age (12). No lesions were observed, but the organism did establish itself on mucosae of the tonsils and the nasal cavity.

Diagnosis of streptococcal disease is based on isolation of these organisms, but streptococci are also common in normal animals. A microbiologic study of grossly normal lungs from market hogs revealed that alpha-hemolytic streptococci were the only organisms isolated in significant numbers (48). Serogrouping of the alpha-hemolytic streptococci has just begun to be utilized on the diagnostic level (101, 102,104,105,107); however, the significance of these organisms as respiratory tract pathogens has not yet been determined.

Pasteurella multocida

Pasteurella multocida is a common component of the oropharyngeal and nasal flora of swine (44,45,51,92). Stress from disease, malnutrition

or poor environmental conditions enable the organism to infect the respiratory tract. After establishment in the upper respiratory tracts of pigs, P. multocida may initiate or potentiate atrophic rhinitis caused by other organisms (58,93,108,109). Recent reports suggest that strains of P. multocida most often associated with atrophic rhinitis are toxin producers (109,110,111).

When P. multocida invades the lower respiratory tracts of swine, it is invariably secondary to viral, mycoplasmal, or bacterial pneumonia (58,112). In separate abattoir studies, 35% and 40.7% of market hogs with pneumonia had P. multocida (12,48). Clinical signs of coughing, fever, nasal discharge, and dyspnea may occur in forty to ninety percent of infected pigs, and five to ten percent may die (113). Purulent bronchopneumonia will initially develop, then will become fibrinous or fibrous in nature; both acute and chronic lesions may occur in the same animal (92,113). Serofibrinous pleuritis, pericarditis, and peritonitis may also be present. In gnotobiotic pigs three to five days old inoculated intranasally with P. multocida, a transient bronchopneumonia (duration of three to six days) developed (12). Some so-called "dubious" isolates may cause severe pneumonia and septicemia within three to ten days after inoculation into young pigs (110).

While passaging samples through mice may increase the chances of recovering toxin-producing strains of P. multocida (109), isolation of the organism from diseased tissue is generally not difficult. The diagnostician must keep in mind that the organism could mask or overwhelm the primary cause of the disease.

Salmonella choleraesuis

Salmonella choleraesuis is an occasional isolate from outbreaks of pneumonia in growing swine (114). Clinical signs may include coughing and cyanosis, although clinical disease may be inapparent (115). The short incubation period (24 to 48 hours) may cause for a confusing diagnostic history (115). Morbidity of 10% may occur, and mortality may be quite high (115). Signs of septicemia (epithelial petechiation), lung abscessation and consolidation, and fibrinopurulent pleurisy may be seen at post mortem (51). The organism may be hard to detect, while at other times the diagnostician may fail to relate the isolation of S. choleraesuis to the presence of pneumonia.

Corynebacterium pyogenes and Actinobacillus suis

Sporadic outbreaks of lung abscesses and suppurative pneumonia have occurred in young swine due to Corynebacterium pyogenes and Actinobacillus suis. Disease is generally associated with the presence of other microorganisms. Septicemia and arthritis due to these organisms may also be present (114,116,117,118).

Viruses

Swine influenza virus

Swine influenza virus spreads quickly through a swine herd, with clinical signs similar to human "flu" (51). Anorexia, fever, dyspnea,

and coughing occur after a one to three day incubation period, and may last from two to six days (119). All exposed swine may be affected (120). Most pigs recover rapidly as long as there are no secondary complications. Fetal lung hypoplasia has occurred after experimental in utero infection of porcine fetuses (121).

Necropsy of pigs infected with swine influenza virus reveals well demarcated, depressed, discolored areas throughout the cardiac and apical lobes of the lung (122). There may be serosanguinous pleural exudate and excessive bronchial and tracheal catarrah (36). Chronic lesions consist of grey foci of consolidation in the lung. Histologically, lung edema and bronchopneumonia are present; disruption of bronchial epithelium with a neutrophilic exudate, and perivascular and peribronchiolar mononuclear cell accumulations, are especially noted (36).

Diagnosis of swine influenza virus infection is made tentatively from microscopic lesions, and isolation of the virus may confirm the diagnosis. Ante-mortem diagnosis may be achieved by serologic testing of sera from convalescent animals (119).

Porcine cytomegalovirus

Porcine cytomegalovirus is the cause of inclusion body rhinitis in pigs less than twelve weeks of age (123,124). Pigs infected in utero may transmit the infection to littermates, or pigs may become infected through a carrier sow after birth (92,125). Affected littermates become anorexic and sneeze often by five to ten days of age (126). Anemia and enteritis have been reported to occur in pigs of this age with inclusion

body rhinitis (126). Young pigs can excrete virus for three to eight weeks, thus spreading it to other nursery pigs after weaning (125). Frequent sneezing, conjunctivitis, and infrequent coughing are seen in affected pigs two to eight weeks of age (124). Pigs older than nine weeks of age seem to acquire resistance to inclusion body rhinitis, but may be latent carriers of porcine cytomegalovirus (127).

Generalized porcine cytomegalovirus infection in the baby pig may be lethal (97,125,126). Hemorrhage may be present in alveolar walls and interlobular septae of the lungs, with a scattered macrophage exudate (97). Intranuclear inclusion bodies may be seen in tubular epithelium of the kidneys associated with an interstitial infiltrate of mononuclear cells (97,126). Inclusion body rhinitis is invariably present in these pigs.

Diagnosis of inclusion body rhinitis is confirmed by examination of turbinates from affected pigs. A catarrhal rhinitis may be present (36). Microscopic examination reveals large, basophilic, intranuclear inclusion bodies within the tubuloalveolar gland and duct epithelial cells of the nasal mucosa; there is usually heavy infiltration of the lamina propria with mononuclear cells (123). It has been postulated that natural resistance mechanisms to bacteria may be compromised in the young pig with inclusion body rhinitis (124,125).

Adenovirus

Adenovirus serotypes 1, 2, 3, and 4 have been shown to cause enteritis in swine (128,129) and have been considered a cause of pneumonia in young pigs. Signs of respiratory disease are often

subclinical, but it is suspected that the adenovirus may predispose young pigs to other respiratory pathogens (130,131). Pigs less than two weeks of age inoculated with adenovirus by a respiratory route developed focal interstitial pneumonia within 13 to 21 days (128,132, 133,134,135). If also exposed to the virus orally, alimentary tract disease developed in some, but not all pigs (128,134).

Diagnosis of adenoviral pneumonia is usually based on histologic examination. Thickened alveolar septae due to proliferation of septal cells are the most common lesions (132,133,134). Intranuclear inclusions typical of adenovirus infection have been reported in septal cells at the center of these proliferative areas (133,134). Prominent lymphoreticular cell aggregates have been reported at all levels of the bronchial tree in young pigs infected with adenovirus (136).

Pseudorabies virus

Pseudorabies virus causes abortions, stillbirths, and neonatal deaths in swine. Affected sows may show signs of respiratory distress, but few lesions are recognized. Baby pigs with pseudorabies usually exhibit central nervous system disorders and die from generalized infection, but signs of respiratory disease may be prominent with some strains of the virus. Mortality of up to 100% of baby pigs is not unusual (112,137).

When young pigs were inoculated with pseudorabies virus via the respiratory tract, sneezing and coughing were not unusual (138). Necrotizing pneumonia was present when pigs were necropsied from one

to six days post inoculation (139,140,141). Intranuclear inclusion bodies were detected in bronchiolar epithelial cells (139,140) and, on one occasion, intranuclear inclusion bodies were noted in a few nasal mucosal gland cells (138).

Definitive diagnosis of pseudorabies is based on isolation of the virus or by direct fluorescent antibody examination of affected tissues (the tonsil is the tissue most likely to be positive). The virus neutralization test may be used to detect pseudorabies virus antibody in convalescent swine (142).

Parasites

Verminous pneumonia may occur in young pigs infected with Metastrongylus spp. or Ascaris lumbricoides var. suis. Although different stages of these parasites initiate the clinical signs observed, both predispose the pig to severe viral, mycoplasmal, or bacterial pneumonia.

Three species of Metastrongylus are recognized as porcine lungworms: M. apri (the most common), M. pudendotectus and M. salmi (143,144). The pig is exposed to infective larvae either by ingesting earthworms (usually Eisenia foetida) or soil carrying recently liberated larvae (144,145). The larvae travel via lymph vessels to the lung and clinical signs occur within ten days after ingestion (144). The dry, hacking cough is usually very mild, but heavy infestations in the young pig may cause dyspnea and, eventually, stunted growth (146). Conditions necessary for development of such severe parasite loads would be unusual

with modern confinement housing. In many instances secondary infections may lead to pulmonary consolidation and death (146). Lesions in the lung are generally limited to the diaphragmatic lobes and the air passages (147); vesicular emphysema also may be present (148). Infiltration with polymorphonuclear cells occurs along the migratory paths, and nodules of histiocytes may surround molted larval sheaths (144). The adult is found embedded in the epithelium of the trachea, bronchi, or bronchioles (146), usually with associated lymphoid hyperplasia (149).

Ascaris suum larvae may cause severe damage to the lungs during their migratory phase. About five days after ascarid eggs are ingested, larvae pass through the liver and begin migration through the lung (122); within eight to ten days after ingestion of the eggs, the pigs usually begin coughing. Lesions are hemorrhagic, and a considerable eosinophilic inflammatory reaction may be expected where the larvae have broken through alveolar walls or bronchiolar epithelium (150). The adult ascarid is found in the lumen of the small intestine (147).

PART I. CHRONOLOGICAL STUDIES OF RESPIRATORY DISEASE IN BABY PIGS: EXAMINATION OF NASAL SECRETIONS FOR, AND PREVALENCE OF ANTIBODIES IN SERA TO, CERTAIN MICROORGANISMS

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CHRONOLOGICAL STUDIES OF RESPIRATORY DISEASE IN BABY PIGS: EXAMINATION OF NASAL SECRETIONS FOR, AND PREVALENCE OF ANTIBODIES TO, CERTAIN MICROORGANISMS

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SUMMARY

The prevalence of infections with Bordetella bronchiseptica, Pasteurella multocida, Haemophilus parasuis, and Mycoplasma hyorhinis in the nasal cavities of 187 young pigs in six conventional Iowa herds were studied. The producers at each of these farms had indicated that substantial coughing and sneezing were a continuing problem in pigs one to seven weeks of age. Nasal secretions and sera were collected at one to two week intervals from pigs one-half to seven and one-half weeks of age.

The most prevalent isolates from the nasal secretions of pigs less than five weeks of age were H. parasuis and M. hyorhinis. Isolation of H. parasuis decreased as pigs became older, while that of M. hyorhinis remained relatively constant. Isolation of B. bronchiseptica and P. multocida tended to increase as pigs became older. In almost all instances where pigs were culture-positive for B. bronchiseptica or P. multocida, either H. parasuis, M. hyorhinis, or both, had been isolated at prior sample intervals. Haemophilus pleuropneumoniae was isolated from nasal secretions of five pigs in one herd.

Complement-fixing antibodies to Mycoplasma hyopneumoniae, M. hyorhinis, H. pleuropneumoniae, and H. parasuis were present in swine from each of the six herds. Antibodies to M. hyopneumoniae were present in a few samples from each herd, and in many one- to two-week old pigs in two herds. The incidence of CF antibodies to M. hyorhinis varied from herd to herd, but they were uncommon in pigs two to three

weeks of age and tended to increase in incidence as pigs became older. CF antibodies to H. pleuropneumoniae were found in 78.3% to 100% of the pigs at all sample intervals. Similarly, antibodies to H. parasuis were prevalent at all sample intervals, except for one herd where most pigs were CF-negative at one-half week of age but became positive by five weeks of age.

INTRODUCTION

Respiratory disease in swine is widely recognized as an important cause of economic loss in modern intensified production systems. Specific infectious agents which are common in swine reared under such conditions include Bordetella bronchiseptica, Pasteurella multocida, Haemophilus parasuis/suis, and Mycoplasma hyorhinis (1,2,3). Although all four of these agents are common in the nasal flora of pigs, only B. bronchiseptica and P. multocida are known important causes of disease in the respiratory tract; the roles of H. parasuis and M. hyorhinis in respiratory disease are not yet understood.

Even though the herd prevalence of various respiratory disease agents has been determined, little is known about the sequence of occurrence of these organisms in the respiratory tracts of baby pigs. In the present study, we have determined the prevalence of infections with B. bronchiseptica, P. multocida, H. parasuis, and M. hyorhinis in the nasal cavities of young pigs in six Iowa herds at various intervals from one-half to seven and one-half weeks after birth. Swine in each of the herds were kept in complete confinement and had ongoing respiratory disease, including sneezing and coughing in baby pigs. In addition to documenting the sequence of infection with these four agents, the prevalence of complement-fixing antibodies to Mycoplasma hyopneumoniae, M. hyorhinis, Haemophilus pleuropneumoniae, and H. parasuis was also determined at various intervals.

MATERIALS AND METHODS

Source Of Pigs

Six conventional herds (A through F) with persistent respiratory disease in young pigs were studied. On initial visit, the management was evaluated and the respiratory disease status of the herd was determined (see Table 1). The producers at each of these farms had indicated that substantial coughing and sneezing were a continuing problem in pigs one to seven weeks of age. In each case, our clinical evaluation indicated that young pigs in the farrowing units were coughing and sneezing. Groups of 8 to 32 neonatal pigs (beginning age of ½ to 2 weeks) were selected and identified with ear tags for on-the-farm sequential sampling.

Herd A

The initial visit to this central Iowa herd was in early 1981. Farrowing was continuous, with crates cleaned with high pressure water between litters. Conditions in the farrowing house were generally fair (crates clean and dry, slight irritating odor, few drafts) although the humidity was high. Ventilation was poor (dusty, irritating atmosphere) in the nursery. Pigs were kept in the nursery until the finishing floor or other farm facilities were available, generally at 8 to 12 weeks of age. An atrophic rhinitis bacterin (*B. bronchiseptica* and *P. multocida*) was in use on the farm.

Clinical signs of respiratory disease observed during the initial visit (see Table 1) persisted throughout the winter. Three small groups of pigs (numbering 8, 8, and 10) farrowed in late December, mid-January, and late January, respectively, were sampled.

Herd B

This central Iowa herd was visited initially in the winter of 1981. At this time, sows were paired two weeks post-farrowing and moved together with their litters into lactation pens for three to four weeks. The pigs were then weaned when they were six to seven weeks of age and placed in a double-deck nursery. Conditions in the farrowing house were fair (crates clean and dry, slight irritating odor, no drafts). A group of 18 pigs farrowed the first week in January, exhibiting sneezing and coughing (Table 1), was studied.

The management scheme was altered for this herd one month after commencement of the initial study. Pigs were weaned at three weeks of age and moved into a triple-deck nursery. After six to seven weeks, the pigs were moved into feeder-pig pens (partial slats) and conditions became rather crowded. The overflow of larger pigs were moved to dirt lots or barns, where they were finished. Clinical signs were less severe than previously recorded. A group of 20 pigs farrowed in late February was studied. An atrophic rhinitis bacterin (B. bronchiseptica, P. multocida, and Pseudomonas sp.) was in use.

Herd C

In early summer, 1981, signs of respiratory disease were severe in this west central Iowa herd (see Table 1). Five to ten percent of the nursery pigs were gaunt and in poor condition. Transmissible gastroenteritis (TGE) was reported to have been a frequent problem in the nursery. An atrophic rhinitis bacterin (B. bronchiseptica, P. multocida, and Pseudomonas sp.) and a TGE vaccine were in use on this farm. The facilities were kept in good condition (clean, dry, and free of drafts). One group of 28 pigs was sampled beginning when they were one week of age.

Herd D

Many pigs in the nursery of this central Iowa herd had clinical signs of respiratory disease (see Table 1), but as the pigs were moved to growing pens the signs diminished and pigs appeared to be healthy. The producer complained of a growth plateau in pigs two to three weeks of age. Up to two percent of finishing hogs had some snout deviation. Conditions in both farrowing and nursery failities were good (clean, dry, and with little irritating odor). It was noted that for a condensed stack nursery, this one was well maintained. A B. bronchiseptica intranasal vaccine was used in baby pigs in this herd. A group of 31 pigs farrowed in late winter, 1982, was examined.

Herd E

This north central Iowa herd was examined in late spring, 1982. The clinical signs of respiratory disease in baby pigs in this herd were not severe (see Table 1), but by weaning age many pigs were coughing. There was little to no unthriftiness associated with these signs, although the producer complained of "chronic weanlings" or pigs that would not grow well. Pigs leaving the nursery at ten to twelve weeks of age appeared healthy. The facilities were generally clean and dry. Straw bedding was used at farrowing, then removed. Odors were not bad except on still, hot days, when ventilation in the nursery was inadequate. A group of 32 pigs farrowed in late May were sampled.

Herd F

There were many farrowing facilities on this central Iowa farm. Pigs in this study were housed in a converted dairy barn, which could house over ninety litters. There were hutch boxes in the "crates", with straw on the floors. The sows were small, and many had histories of mastitis-metritis-agalactia (MMA). Attempts were made to keep the facilities clean, and humidity and odors were no problem (natural ventilation was very good in this barn). The clinical signs observed during the summer of 1982 are presented in Table 1. A group of 32 pigs farrowed mid-June were selected for study.

Table 1. Herd description and presenting clinical signs of respiratory disease in young pigs

Herd	Number of sows in herd	Description of facilities	Pig age at weaning	Weeks in nursery	Signs of pig respiratory disease
A	150 to 200	Continuous farrowing with crates on wood or expanded metal slats. Nursery on concrete slats.	3 to 5 weeks	3 to 7 weeks	2 to 3 weeks old: sneezing and tearing in 3-4% 3 to 4 weeks old: sneezing or coughing in 1-2% 6 to 10 weeks old: productive cough and nasal discharge in 1-2%
B	270	All-in all-out farrowing with crates on concrete slats. Nursery multi-deck; concrete slats below, rubber-coated wire mesh above.	3 to 6 weeks	5 to 7 weeks	2 weeks old: sneezing or coughing in 2-3% 4 to 5 weeks old: sneezing in 20% productive cough in 5-10% 10 to 12 weeks old: pigs appeared healthy
C	400 to 430	All-in all-out farrowing with raised crates on rubber-coated wire mesh. Nursery of raised pens on slats of expanded metal.	3 weeks	5 to 7 weeks	2 weeks old: sneezing or coughing in 20% 3 weeks old: almost all sporadically coughing 6 to 7 weeks old: pigs appeared healthy

D	375	All-in all-out farrowing with crates on concrete slats. Nursery double-deck with pens on wire mesh.	2 to 2½ weeks	3 weeks	1 week old: sneezing in 1-2% coughing sporadic 2 to 3 weeks old: sneezing in 10-15%, productive cough in 20-30% 6 to 7 weeks old: little sneezing or coughing
E	275	Seasonal all-in all-out farrowing with raised crates on rubber-coated wire mesh. Nursery double-deck with concrete slats below, wire mesh above.	4 weeks	8 weeks	1 week old: sneezing or coughing in 1% 3 to 4 weeks old: sneezing or coughing in 20-50% 10 weeks old: little sneezing or coughing
F	800	Continuous farrowing in individual solid-bottom pens. Nursery of raised pens on wire mesh.	4 weeks	6 to 7 weeks	2 to 3 weeks old: sneezing in 10-15%, coughing in 2-3% 6 to 7 weeks old: little sneezing or coughing

Collection of Samples

Nasal secretions and sera were collected from the selected group of pigs in each herd at 1 to 2 week intervals, beginning when the pigs were ½ to 2 weeks of age. Nasal secretions were collected using calcium alginate-tipped wire swabs[a] which were then placed in Hanks' balanced salt solution[b] with 10% fetal calf serum (HBSS WITH FCS) for transport. During transport the samples were stored at 4°C. Blood was collected by the pre-vena-cava technique, allowed to clot, centrifuged, and sera then drawn off and frozen at -20°C. Sera were collected from sows during the first visit to the farm and stored in the same manner.

Microbiologic Studies

Nasal secretions on swabs were streaked on 5% horse blood agar and MacConkey agar[c] with 1% dextrose, then the swabs were swirled in mycoplasma broth (BHI) (4,5) with 3,000 IU penicillin[d] per ml and thallium acetate[e] at 1:3000 dilution. Blood agar plates were streaked

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[a]Calgiswab Type 1. Inolex Division, American Can Company, Glenwood, Illinois.

[b]Hanks' balanced salt solution. GIBCO Laboratories, Grand Island, New York.

[c]MacConkey agar. Difco Laboratories, Detroit, Michigan.

[d]Penicillin G potassium for injection, USP. Eli Lilly and Company, Indianapolis, Indiana.

[e]Thallium(ous) acetate. Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, New Jersey.

with a Staphylococcus epidermidis nurse culture to support Haemophilus spp. growth. Samples from herds D, E, and F were also inoculated on 5% horse blood agar with 2 μg clindamycin/ml[f] to facilitate isolation of P. multocida. Plates were incubated at 37°C, then examined after 24 and 48 hours incubation.

Colonies suspected to be P. multocida and B. bronchiseptica were inoculated to tryptose phosphate broth. Typical P. multocida produced large, white, mucoid colonies on blood agar and did not grow on MacConkey agar; produced acid from dextrose but not from lactose; were citrate- and urease-negative; and were non-motile. B. bronchiseptica was recognized as small blue-tan colonies on MacConkey agar, or pearl-like colonies with a small zone of complete hemolysis on blood agar. Isolates confirmed as B. bronchiseptica alkalinized dextrose, lactose, and litmus milk, and were citrate- and urease-positive.

Small semi-translucent colonies satelliting along the growth of S. epidermidis on blood agar were suspected to be Haemophilus spp. All suspected haemophili were examined using Gram's stain, and re-inoculated to blood agar. Biochemical identification was done according to a modification of procedures described by Biberstein et al. (6). Haemophili were inoculated to enriched broth medium (M96) (7). After 24 hours, the broth was supplemented with 100 μg nicotinamide adenine dinucleotide/ml and subsequently inoculated to carbohydrate broth[g] (6)

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[f]Clindamycin hydrochloride USP. The Upjohn Company, Kalamazoo, Michigan.

[g]Phenol red broth base. Difco Laboratories, Detroit, Michigan.

and to PPLO agar[h] (8). Colonies were harvested from the agar with 0.1 M phosphate-buffered saline and washed twice. A small loop of cells was transferred to both 2% urea (9) and δ-aminolevulinic acid (ALA) substrate[i] (10). After 24 hours, bromthymol blue indicator was used to detect urease, and Kovacs reagent was used to detect porphobilinogen (→ porphyrin) production. Acid reaction in phenol red broth base supplemented with dextrose, sucrose, lactose, mannite, or xylose was considered indicative of carbohydrate fermentation. Haemophili were considered to be H. parasuis if they fermented dextrose and sucrose; caused no change in lactose, mannite, or xylose; were urease-negative; and converted ALA to porphyrin (10). Identification of H. pleuropneumoniae was based on fermentation of dextrose, sucrose, mannite, and xylose; failure to ferment lactose; production of urease; and conversion of ALA to porphyrin (11).

Inoculated BHI broth was incubated at 36°C, and after 72 hours, 0.5 ml was passaged blindly to a fresh tube of BHI. Second passage cultures were examined for growth daily over the next two weeks. Mycoplasma growth was recognized as faint turbidity with or without a pink color change indicating reduction of tetrazolium. Cultures with suspected growth were inoculated on BHI agar and incubated at 37°C in

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[h] PPLO agar. Difco Laboratories, Detroit, Michigan.

[i] δ-aminolevulinic acid hydrocloride. Sigma Chemical Company, St. Louis, Missouri.

a humid incubator. When colonies suspected to be mycoplasma were seen, the direct epi-immunofluorescent antibody technique (12) was used for identification using specific antisera for M. hyorhinis, M. hyosynoviae, M. arginini, and Acholeplasma laidlawii. If colonies failed to fluoresce with M. hyorhinis- or M. hyosynoviae-conjugated antisera, single colony isolates were evaluated for ability to utilize arginine or dextrose (13) and ability to grow in serum-free medium (NSR) (14). Isolates suspected to be Acholeplasma spp. were evaluated for lack of sensitivity to 5% sodium polyanethol sufonate (SPS)[j] (15). Other mycoplasma were identified by growth inhibition (GI) tests (16) using the appropriate most likely antisera (17).

Serologic Studies

The complement fixation (CF) test was done according to Slavik and Switzer (18) using antigens prepared from M. hyopneumoniae, M. hyorhinis, H. pleuropneumoniae, and H. parasuis. M. hyopneumoniae strain 11 and M. hyorhinis strain 7 antigens were provided by T. F. Young (Veterinary Medical Research Institute, Ames, Iowa). H. pleuropneumoniae serotypes 1 through 5 were merthiolate-inactivated antigens prepared and used as a pool as done by Schultz et al. (19). H. parasuis antigens were prepared from cultures isolated from pigs on each farm, for testing of sera from that farm. The cultures were grown in M96 overnight, supplemented with NAD (100 ug/ml), then 0.7 to 0.8 ml of each culture

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[j] Grobax. Roche Diagnostics, Nutley, New Jersey.

was inoculated on four PPLO agar plates.[h] After 6 hours incubation at 37°C, colonies were harvested with 5 ml veronal buffered diluent (VBD) (20) and centrifuged. After washing the pellet twice, the cells were resuspended in VBD to a turbidity visually corresponding to McFarland nephelometer barium sulfate standard tube no. 3 (21) and heat inactivated at 50°C for thirty minutes. H. parasuis antigens were stored at 4°C. These antigens did not cross-react with H. pleuropneumoniae on subsequent CF testing.

Convalescent antisera to M. hyopneumoniae strain 11, M. hyorhinis strain 7, and H. pleuropneumoniae serotypes 1 through 5 were used as positive controls when performing the CF test with homologous antigens.[k] Hyperimmune sera to H. parasuis isolated from Herds A, B, and C were used as positive controls for the respective farms. The hyperimmune antisera was produced by inoculating 2-week-old weaned pigs intrathoracically with 2 ml of a 6 hr culture in M96 medium. Homologous H. parasuis vaccines were then prepared by incubating 0.5 ml of 0.9% formalin with 2 ml of a 6 hr culture in M96 overnight at 4°C. The vaccines were administered intramuscularly to respective pigs 26 days after infection. Pigs were bled out 10 days later, and sera were harvested and stored at -20°C. For herds D, E, and F, sow or older pig sera selected from each farm were used as H. parasuis-positive controls; these control sera had CF titers comparable to those obtained with the hyperimmune antisera prepared from H. parasuis strains isolated from herds A, B, C.

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[k] T. F. Young. Veterinary Medical Research Institute, Ames, Iowa.

Sera with reactions at dilutions of 1:4 or higher were considered positive for CF antibodies to the antigen used. Results obtained with sera which were anticomplementary have been removed from the data.

RESULTS

Herd A

Twenty-six pigs from Herd A were examined in three groups of 8, 8, and 10 pigs each, beginning when they were two to three weeks of age. Nasal secretions and blood samples were collected at one to five week intervals. Most pigs did not show overt signs of disease while nursing, although nasal discharge with productive cough was not uncommon after weaning. A tabulation of B. bronchiseptica, P. multocida, H. parasuis, and M. hyorhinis isolated from nasal secretions of pigs sampled at one to five week intervals is presented in Figure 1. In addition, M. hyosynoviae was isolated from nasal secretions of one pig at four weeks of age and another at six weeks of age.

Percentages of pigs with CF antibodies to H. parasuis and M. hyorhinis are also presented in Figure 1. Sera from all pigs were negative for CF antibodies to M. hyopneumoniae except for one pig which had a titer of 1:4 when it was six weeks of age. None of the sows (see Table 2) had CF antibodies to M. hyopneumoniae. Antibodies to H. pleuropneumoniae were detected in most of the pigs (92.9% to 100%) at all sample intervals.

Herd B

In herd B, two groups consisting of 18 and 20 pigs each were examined beginning at one to two weeks of age. Nasal and blood samples

were collected at one to six week intervals. The incidence of organisms isolated from the nasal cavities of these pigs is presented in Figure 2. Nasal secretions of one pig were culture-positive for M. hyosynoviae when it was six to seven weeks of age.

Figure 2 also presents the percentage of pigs with CF antibodies to H. parasuis and M. hyorhinis at one to five week intervals. CF antibodies to M. hyopneumoniae were not detected in any pigs during the nursery period, but when the pigs were seventeen weeks of age, three of ten sampled had titers (1:8, 1:16, 1:64) to the organism. Antibodies to H. pleuropneumoniae were detected in a majority of pigs (78.3% to 100%) throughout the study. Antibody titers in sera collected from sows at one to two weeks postpartum are presented in Table 2.

Herd C

A group of 28 pigs were examined beginning when they were one week of age. Samples were collected at one to two week intervals until the pigs were seven weeks old. The pigs appeared healthy throughout the study. Isolates from the nasal secretions are presented in Figure 3. H. pleuropneumoniae was isolated from nasal secretions of pigs at two, three, and four weeks of age (2, 1, and 2 respectively). Initially, the colonies produced no hemolysis on 5% horse blood agar, but after several passages through M96 and on blood agar, hemolysis was comparable to other strains of the organism. The cultures were identified as serotype 5 by plate agglutination and indirect fluorescent antibody tests, although apparent cross-reactions to serotype 3 antiserum occurred

with the agglutination test.[l]

Percentages of pigs with CF antibodies to H. parasuis and M. hyorhinis are presented in Figure 3. CF antibodies to M. hyopneumoniae were detected in 6 pigs at one week of age, and 1 pig at two weeks of age. Similarly, CF antibodies to the organism were detected in the dams of these pigs (see Table 2). CF antibodies to the organism were not detected in pigs from three to five weeks of age. At seven weeks of age, 2 of 16 pigs sampled had positive titers (1:4 and 1:32). CF antibodies to H. pleuropneumoniae were detected in all of the pigs at all sample intervals.

Herd D

A group of 31 pigs were selected for study; they were sampled at one, two, four, and six weeks of age. Percentages of pigs with B. bronchiseptica, P. multocida, H. parasuis, and M. hyorhinis isolated from the nasal seretions are presented in Figure 4. In addition, M. salivarium was isolated from 1 pig at two weeks of age and M. buccale from 1 pig at six weeks of age.[m]

Percentages of pigs with antibodies to H. parasuis and M. hyorhinis are presented in Figure 4. CF antibodies to M. hyopneumoniae were present in 2 pigs (titer 1:4) at one week of age, and in 2 different pigs (titer 1:4) at two weeks of age. Antibodies were not detected in sera

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[l] V. J. Rapp. Veterinary Medical Research Institute, Ames, Iowa.

[m] B. J. Zimmermann. Veterinary Medical Research Institute, Ames, Iowa.

from the sows (see Table 2) or any other pigs sampled. Antibodies to H. pleuropneumoniae were present in most of the pigs (95.7% to 100%) at all sample times.

Herd E

One group of 32 pigs was examined at two week intervals, beginning when they were one week of age. Isolates from the nasal secretions are presented in Figure 5. M. hyosynoviae was isolated from one pig at five weeks of age. The percentages of pigs with antibodies to H. parasuis and M. hyorhinis are given also in Figure 5. In addition, 67.7% of the one-week-old pigs had titers (ranging 1:4 to 1:128) to M. hyopneumoniae; only one of the dams had a positive titer (1:4) (See Table 2). CF antibodies were not detected in pigs tested at three weeks of age (1:4) or 7 weeks of age; one of 27 five-week-old pigs had a 1:4 titer to the organism. Antibodies to H. pleuropneumoniae were also present; CF antibodies were present in 100% of pigs at one week of age, 79% at three weeks of age, 88.5% at five weeks of age, and 54% at seven weeks of age.

Herd F

Thirty-two pigs from Herd F were examined beginning when they were less than a week old. The percentages of pigs culture-positive for B. bronchiseptica, P. multocida, H. parasuis, and M. hyorhinis are given in Figure 6. M. buccale was isolated from the nasal secretions from one pig each at 3, 5 and 7½ weeks of age (3%, 4% and 4.5% of pigs sampled, respectively). M. hyosynoviae was isolated from pigs at 5 and 7½ weeks

of age (16% and 9% of pigs sampled, respectively). M. salivarium also was isolated from pigs at 5 and 7½ weeks of age (12% and 9% of pigs sampled, respectively). One mycoplasma isolated from the nasal secretions of a 7½ week-old pig was identical biochemically and by growth inhibition test to Mycoplasma sp. H4-4B F^m. Acholeplasma were isolated from pigs ½ week, 1 week, and 3 weeks of age (9.4%, 9.4% and 16%, respectively).

Percentages of pigs with CF antibodies to H. parasuis and M. hyorhinis are presented in Figure 6. CF antibodies to M. hyopneumoniae were present when the pigs were initially sampled, then waned until none had titers (56.7% at the age of ½ week, 28.1% at 1 week, 3.23% at 3 weeks, and 0% at 5 and 7 weeks). One sow sampled had a positive titer (1:16) (See Table 2). Almost all pigs (96.8% to 100%) had CF antibodies to H. pleuropneumoniae at all sample intervals.

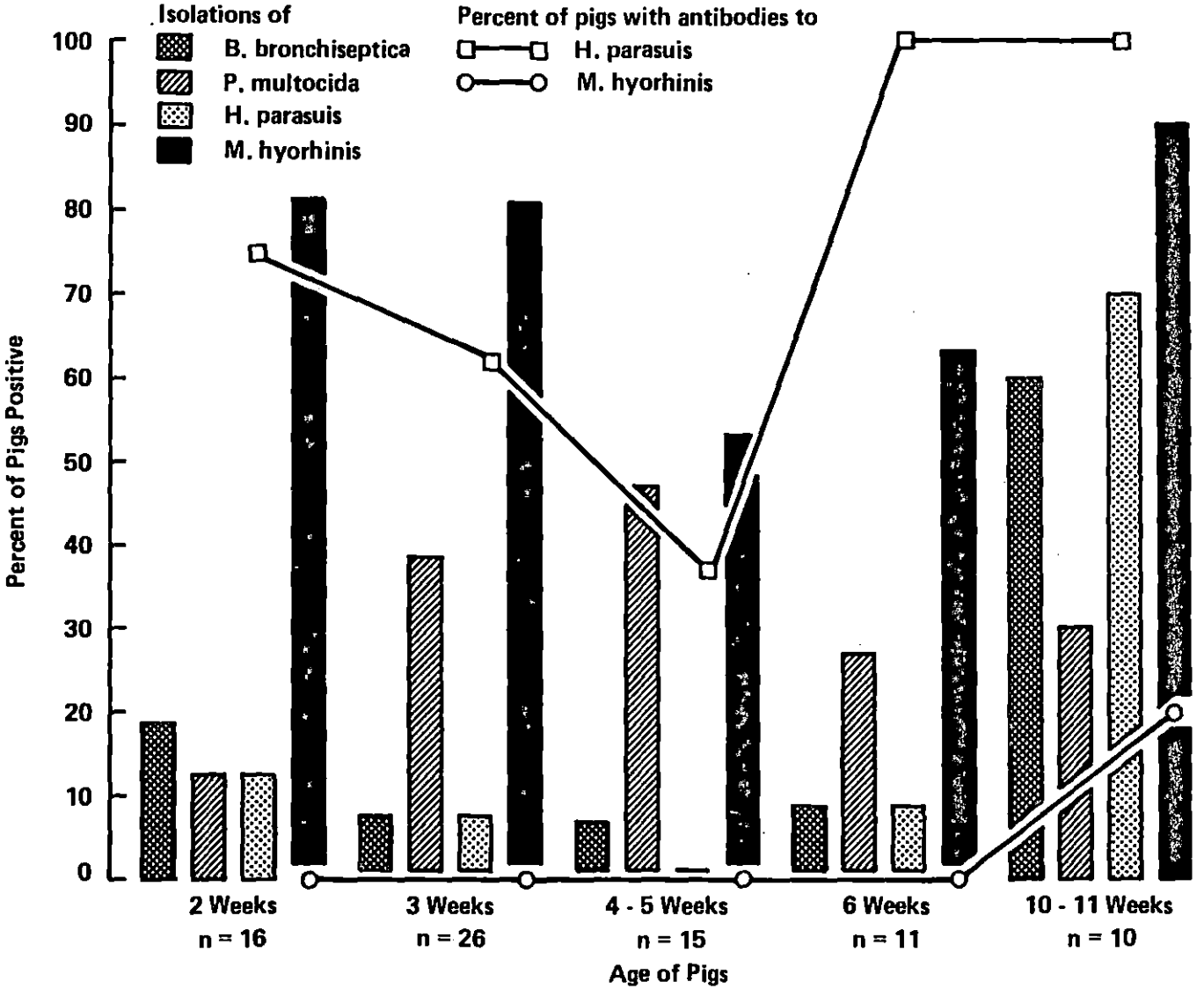

Figure 1. Microbiologic and serologic findings - Herd A

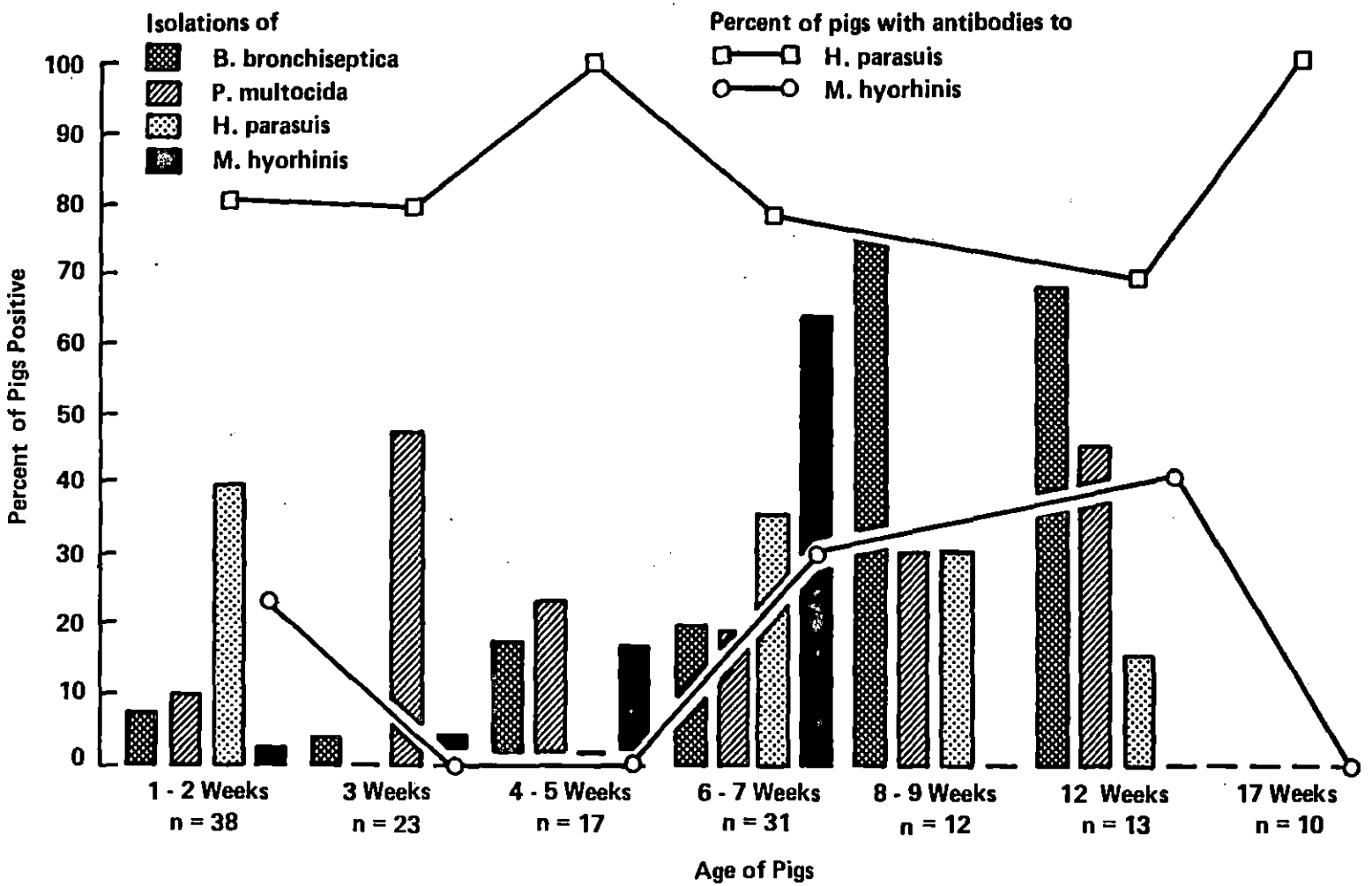

Figure 2. Microbiologic and serologic findings - Herd B

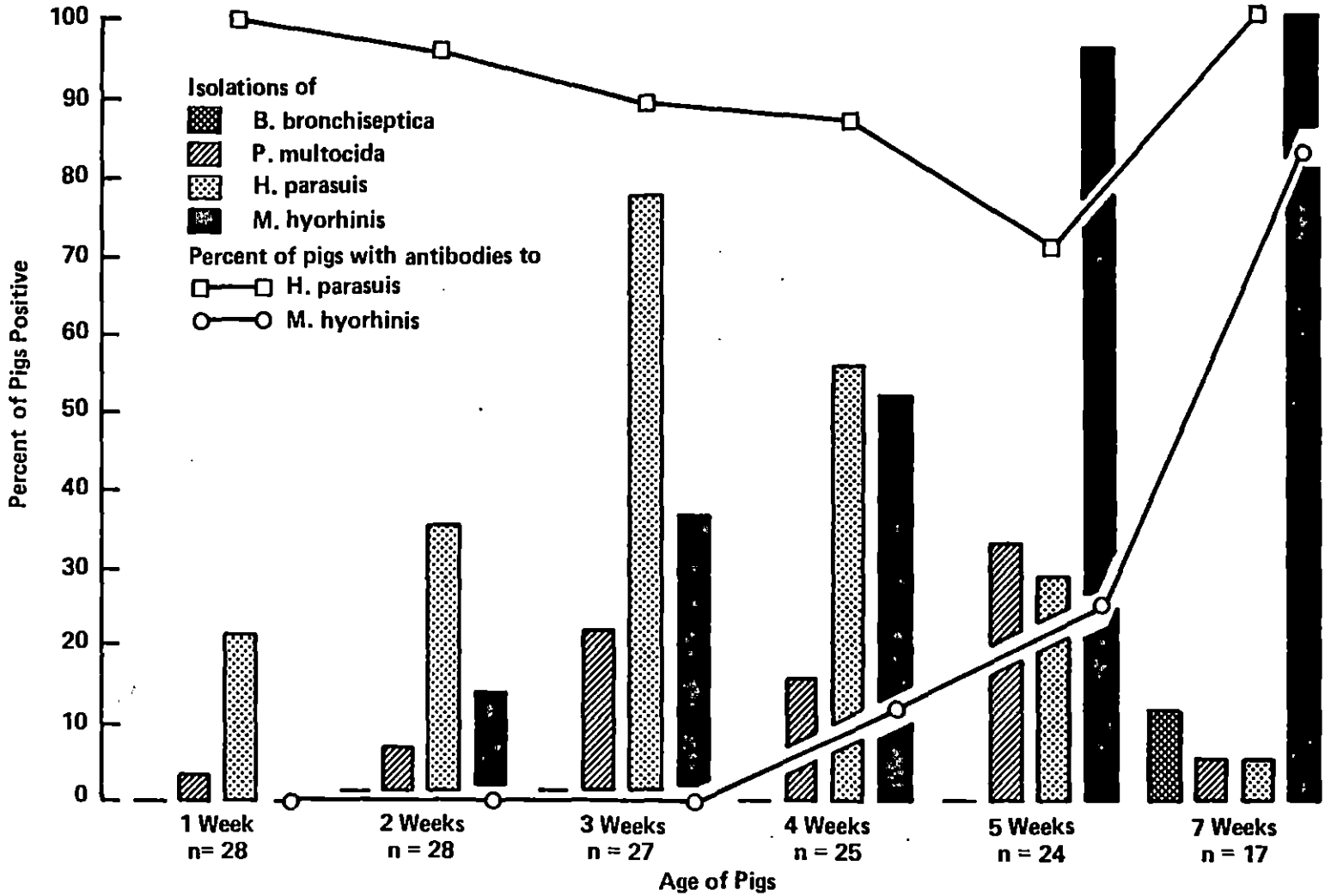

Figure 3. Microbiologic and serologic findings - Herd C

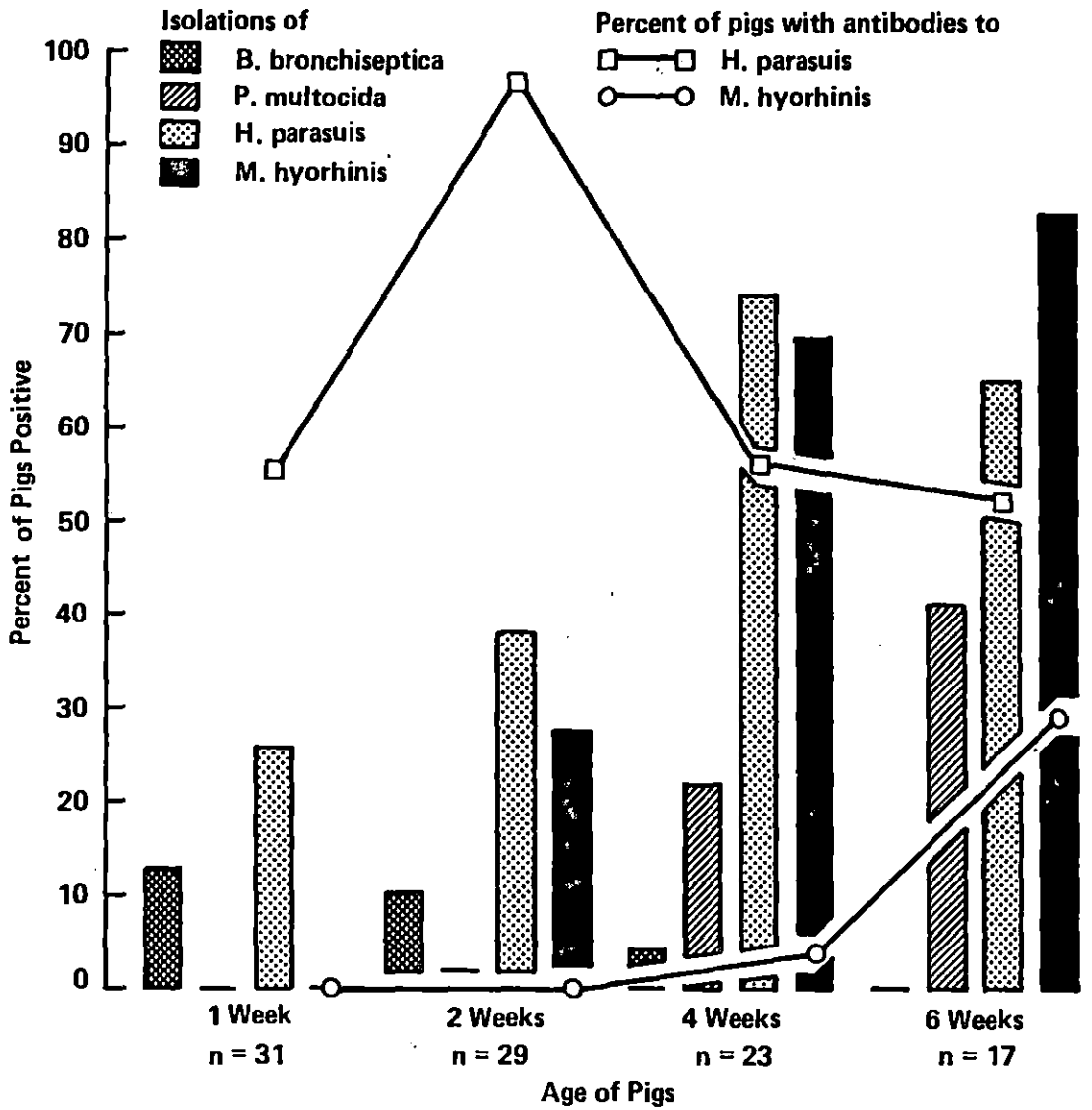

Figure 4. Microbiologic and serologic findings - Herd D

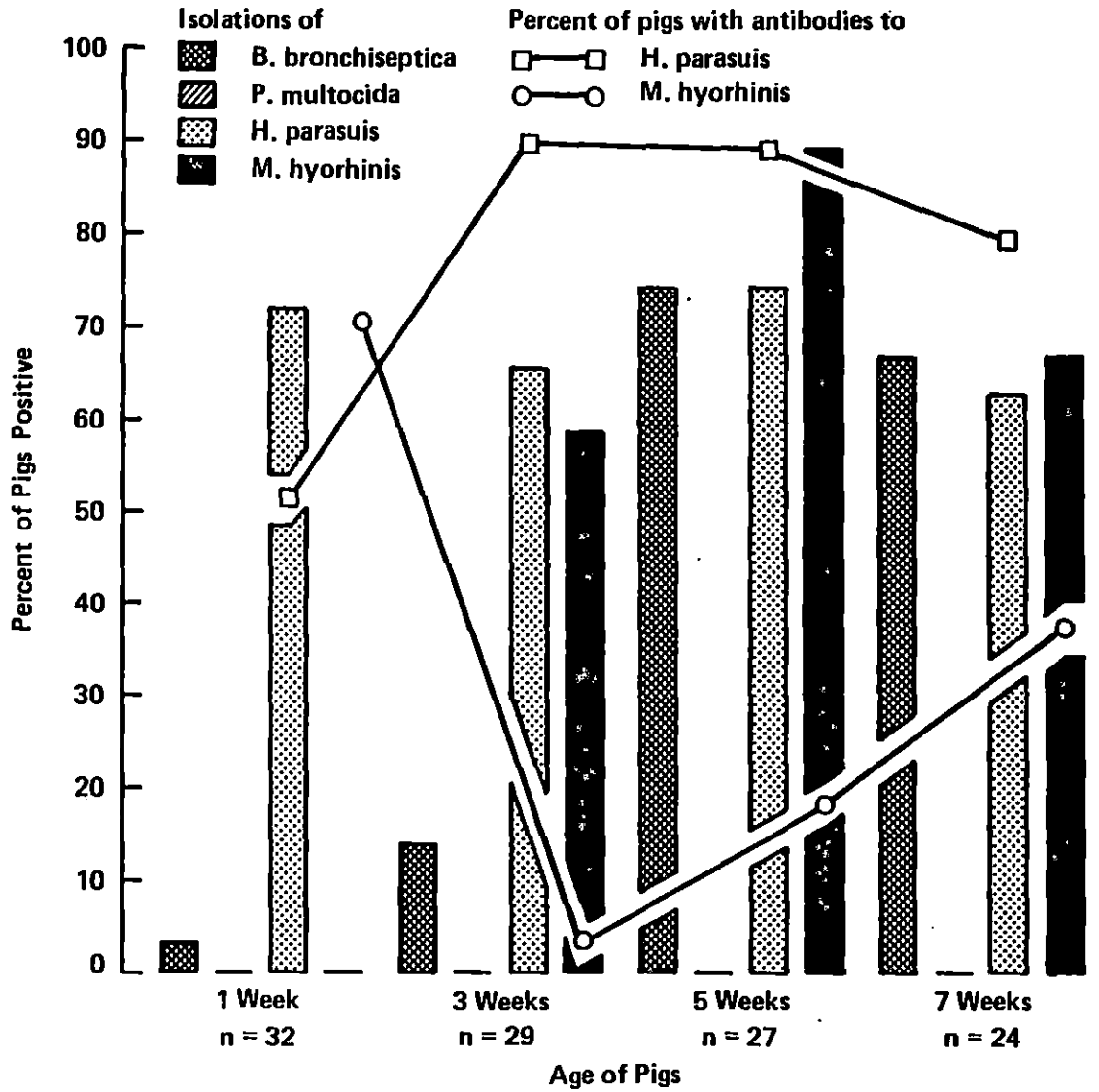

Figure 5. Microbiologic and serologic findings - Herd E

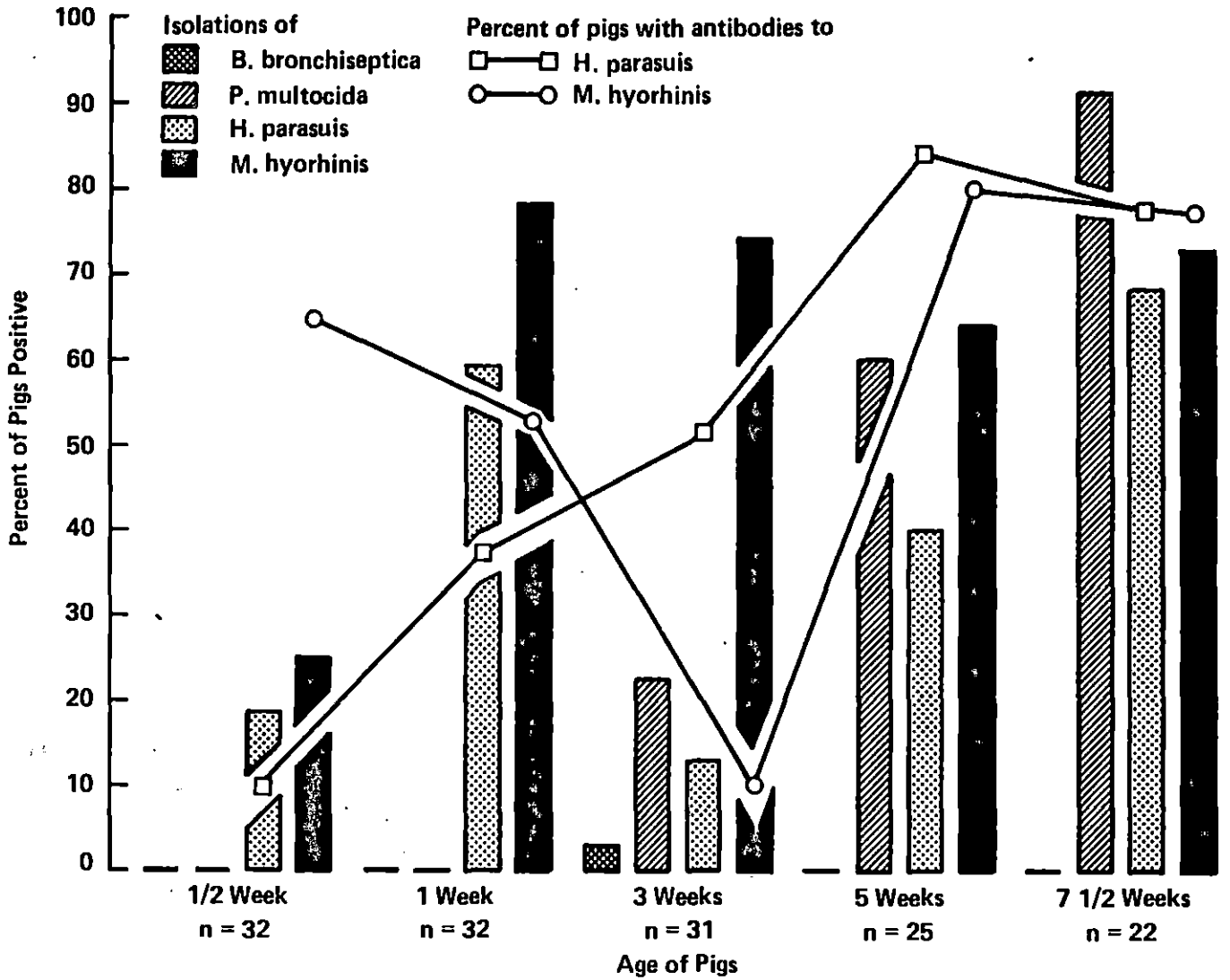

Figure 6. Microbiologic and serologic findings – Herd F

Table 2. Complement fixing antibody titers in sows

Organism	Herd											
	A(8)[a]		B(11)		C(6)		D(6)		E(5)		F(5)	
	No. pos.	Titers	No. pos.	Titers[b]	No. pos.	Titers	No. pos.	Titers	No. pos.	Titers	No. pos.	Titers
Mycoplasma hyopneumoniae	0	NA	1	1:4(1)	4	1:4(1) 1:8(1) 1:16(1) 1:64(1)	0	NA	1	1:4(1)	1	1:16(1)
Mycoplasma hyorhinis	0	NA	2	1:8(1) 1:16(1)	1	1:32(1)	0	NA	3	1:4(2) 1:8(1)	0	NA
Haemophilus pleuropneumoniae	8	1:16(1) 1:64(2) 1:128(5)	11	1:128(11)	6	1:128(6)	6	1:16(3) 1:32(2) 1:64(1)	4	1:4(1) 1:16(2) 1:32(1)	5	1:64(4) 1:128(1)
Haemophilus parasuis	8	1:32(3) 1:64(4) 1:128(1)	11	1:16(1) 1:32(5) 1:64(4) 1:128(1)	6	1:128(6)	5	1:32(3) 1:64(1) 1:128(1)	4	1:4(1) 1:16(2) 1:64(1)	0	NA

[a]Parenthetical numbers indicate number of sows sampled or number with a given titer.

[b]Titers of sows that were positive.

DISCUSSION

During this study, 715 samples of nasal secretions were collected from 187 pigs in six Iowa swine herds. The samples were collected from pigs ½ week to 7½ weeks of age with signs of sneezing or coughing. The organisms isolated most often were H. parasuis and M. hyorhinis. Frequency of isolation of these organisms did not correspond to the severity of clinical signs. Pigs necropsied from the groups in this study frequently had lesions of rhinitis and pneumonia (22). Both H. parasuis and M. hyorhinis were recovered frequently from the nasal secretions, tracheal secretions, and lungs of pigs examined at necropsy (22). H. parasuis and M. hyorhinis have been shown previously to be common in nasal secretions of young pigs. Harris et al. (2) isolated H. parasuis from 70% of the herds in their study, and M. hyorhinis from 42%; pigs six to eight weeks of age in 102 herds were examined. Bertschinger and Nicod (3) surveyed five hundred six-to-ten-week-old pigs in 50 herds and isolated H. parasuis from almost all of the pigs from each herd, and M. hyorhinis from at least one pig in 32% of the herds.

In the present study, 0% to 19% of pigs less than three weeks of age had B. bronchiseptica in their nasal secretions and 0% to 13% had P. multocida. While there was considerable variation between herds and from one sample interval to the next within a herd, frequency of both organisms tended to increase as pigs became older. In herds A and B the percentages of pigs with both B. bronchiseptica and

P. multocida were highest when they were sampled after eight weeks of age. In herds C, D, and F there were few isolations of B. bronchiseptica at any sample interval while P. multocida was isolated from 37% of the five-week-old pigs in herd C, 41% of the six-week-old pigs in herd D, and 60% of the five-week-old pigs in herd F. In herd E, P. multocida was not recovered at any time; however, the percentage of pigs with B. bronchiseptica increased to 74% by the time pigs were five weeks of age. Thus, each of the six herds included in our study had B. bronchiseptica and five had P. multocida. B. bronchiseptica was isolated from 25% and 8% of the herds examined respectively by Harris et al. (2) and Bertschinger and Nicod (3), while P. multocida was isolated from 9% and 20%, respectively. There is a possibility that sulfa drugs were used during the times of these studies (1969 and 1970, respectively), thus decreasing the percentage of herds from which the organisms were isolated. Because there are now restrictions on the use of sulfa drugs, pork producers are more wary of using them in rations at all stages of production.

Atrophic rhinitis (AR) vaccines were used in four of the herds in the present study. In herds A, B, and C parenteral vaccines containing B. bronchiseptica and P. multocida were given to the pigs at two to three weeks of age, and in herd D an intranasal B. bronchiseptica vaccine was given to the pigs when they were two to three days of age. Herd E had used an intranasal vaccine previously, but discontinued its use after AR scores at slaughter did not decrease. Herd F had no AR prevention program. In retrospect, B. bronchiseptica and P. multocida

were isolated frequently regardless of whether a vaccine was used. Development of infection with B. bronchiseptica and P. multocida in herds A and B may have been delayed by bacterin usage, thus minimizing the impact of AR. B. bronchiseptica was isolated from only a few pigs in herd C, which was using a parenteral vaccine. The absence of the organism in herd F illustrates that the organism may be absent even when there are no vaccines used. Only rare isolations of B. bronchiseptica were made from the nasal secretions of pigs in herd D, where intranasal vaccine was in use. Forty-eight percent of the pigs in herd D were infected with P. multocida, and necropsy of selected pigs from this group revealed that AR was common (22). Herd E had the highest incidence of B. bronchiseptica; they were not using any vaccine, although an intranasal vaccine had been used several months previously. AR was common in pigs necropsied from this herd (22).

Complement-fixing (CF) antibodies to M. hyopneumoniae, M. hyorhinis, H. pleuropneumoniae, and H. parasuis were detected in sera from pigs in each of the six herds. CF antibodies to M. hyopneumoniae were present in 66 of the 701 sera tested. Pigs two weeks of age or less in herds C (6 pigs), D (4 pigs), E (21 pigs), and F (17 pigs) accounted for 88% of the positives. Previous reports indicate that actively acquired antibody to M. hyopneumoniae may develop in pigs by ten weeks of age (23) or as early as five weeks post-contact (18). Four of the pigs with CF antibodies to M. hyopneumoniae in the present study were five to seven weeks of age (one in herd A, two in herd C, and one in herd E), and three were seventeen weeks of age (all from herd B). The presence or

absence of CF antibodies in sows had no bearing on their detection in neonatal nursing pigs. Four sows in herd C had CF antibodies to M. hyopneumoniae, yet only six pigs had CF antibodies prior to weaning. Two of these pigs were from sero-positive sows and four were from sero-negative sows. Herds E and F had only one sow with CF antibodies to the organism, yet 68% and 57% of the pigs, respectively, had CF antibodies during the first week of life. Determination of the presence of CF antibodies to M. hyopneumoniae in sera from baby pigs may give a better indication of herd status than sera from adult swine.

CF antibodies to M. hyorhinis were present in 29% to 82% of sera from five- to seven-week-old pigs in herds B, C, D, E, and F. In herds B, E, and F the prevalence of CF antibodies in pigs sampled at one or two weeks of age was 22%, 71%, and 66%, respectively. When the pigs were three weeks of age, the prevalence of CF antibodies had declined to 0%, 3%, and 10%, respectively. Prevalence of these antibodies increased over the five- and seven-week-old sample intervals. Ross et al. (24) indicated that CF antibodies to M. hyorhinis may be detected two weeks post-infection. Thus, the earlier antibodies were likely maternal in origin, and the later antibodies may have been actively stimulated by infection with the organism. M. hyorhinis was isolated in herds D, E, and F from 70%, 89%, and 64% of the pigs, respectively, when the incidence of CF antibodies to the organism was increasing. CF antibodies were not present in herd A until pigs were sampled at ten to twelve weeks of age, although M. hyorhinis had been isolated from 53% to 81% of the pigs at each previous sample interval. In herds C

and D, development of CF antibodies to M. hyorhinis seemed to follow colonization of the nasal cavity by the organism. Kirchhoff et al. (25) have reported that a high percentage of pigs in herds with atrophic rhinitis were sero-positive for M. hyorhinis while Goiš et al. (26) have associated antibodies with the presence of M. hyorhinis in the lungs of market hogs. Pigs necropsied from the group in herd C had mild rhinitis and only two of seven had pneumonia, but M. hyorhinis was consistently isolated from all areas of the respiratory tracts sampled in pigs three to six weeks of age (22). Rhinitis and pneumonia were present in almost all of the pigs necropsied from herd D; M. hyorhinis was isolated from all animals (22).

H. pleuropneumoniae was isolated rarely during the present study, but 78.3% to 100% of the pigs in five of the six herds (A, B, C, D, and F) had CF antibodies to the organism at all sample intervals. Schultz et al. (19) have reported that on the average, 47% of samples from any herd sero-positive for H. pleuropneumoniae will have CF antibodies to the organism. In herd E, the incidence of CF antibodies decreased from 100% in pigs sampled at one week of age to 54% of those sampled at seven weeks of age. The organism may not have been as active in herd E, because one of the sows was not CF antibody-positive; the herd should be considered vulnerable to an acute outbreak of pleuropneumonia. The consistently high percentage of pigs with CF antibodies to H. pleuropneumoniae in herds A, B, C, D, and F may indicate stimulation of active immunity, especially in pigs which were positive at eight to seventeen weeks of age (herds A and B). The organism may be present in

the upper respiratory tract, as was found in herd C. The organism is not commonly reported in nasal secretions; possibly antibodies in the secretions may alter H. pleuropneumoniae in such a way as to make primary isolates difficult to differentiate from H. parasuis. Nicolet[n] reported a few isolates of H. pleuropneumoniae of nasal origin that were serotype 3. Our isolates were identified as serotype 5 by fluorescent antibody technique; when tested by agglutination procedure, the isolates were identified as belonging to serotype 5 but they cross reacted with serotype 3.

Most pigs sampled in the present study were sero-positive for H. parasuis by five to seven weeks of age. Also, the organism was isolated frequently from the nasal secretions of pigs, except in herd A where a large percentage of pigs were culture-negative until they were ten to eleven weeks of age. Percentage of pigs in herd A with CF antibodies to H. parasuis declined to 36% by four to five weeks of age, but increased to 100% of the pigs at six weeks of age. At that interval H. parasuis was isolated infrequently from the nasal secretions. None of the dams of pigs sampled in herd F had CF antibodies to H. parasuis, and only 10% of the pigs were sero-positive at one-half week of age. By five weeks of age 84% of these pigs had developed CF antibodies with no evidence of Glässer's disease or overt disease other than sneezing and coughing. Nielsen (27) and Riising (28) concluded

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[n]J. Nicolet. Institute for Veterinary Bacteriology, University at Berne, Berne, Switzerland, Personal communication, 1983.

that pigs without CF antibodies to H. parasuis were susceptible to Glässer's disease. Nielsen also concluded that most animals in chronically infected herds should be sero-positive for the organism, and that Glässer's disease would be uncommon.

Goiš et al. (29) have speculated that H. parasuis may cause a mild rhinitis in young pigs that predisposes to secondary infection by P. multocida. Combining all sample intervals for the pigs in the present study, large percentages of animals in herds A, B, C, D and F had nasal infections with H. parasuis and P. multocida. Except for one pig in herd D, each of these pigs were infected also with M. hyorhinis. Atrophic rhinitis was detected in just two herds, D and F (22). Herds A, B, and C were using a parenteral AR vaccine that contained P. multocida. Although mild rhinitis was present in pigs necropsied from herds A, B, and C (22), it is possible that pigs in these herds had sufficient immunity to P. multocida to prevent development of severe turbinate atrophy.

During the present study, there were 292 isolates of H. parasuis. These isolates were identified as H. parasuis by their ability to synthesize porphyrin from δ-amino levulinic acid (i.e., did not require the X, or hemin, factor for growth); requirement of V factor (nicotinamide adenine dinucleotide); ability to ferment glucose and sucrose; and failure to ferment lactose, xylose, and mannitol. This is identical to H. suis, except for the absence of the requirement for X factor. The overwhelming majority of strains previously reported as H. suis have also not required the X factor for growth (i.e., have been capable

of forming hemin in the absence of pre-formed iron porphyrins) (30, 31,32). Therefore, the name H. parasuis has been used in this paper under the assumption that many previously reported isolations of H. suis were actually H. parasuis.

In overview, the sequence of isolations of the organisms from the nasal cavities of baby pigs was H. parasuis, M. hyorhinis, and then B. bronchiseptica and/or P. multocida (these latter two depended on the herd in question). H. parasuis was the first organism isolated from 50.3% of the pigs, whereas M. hyorhinis was the first from 26.6%. M. hyorhinis was isolated subsequent to H. parasuis from 33.2% of the pigs sampled, while H. parasuis was subsequent to M. hyorhinis from only 8%. H. parasuis and M. hyorhinis both were present in 16.4% of the pigs at their first culture-positive sample. H. parasuis was most prevalent in pigs sampled by two weeks of age (51.4% were culture-positive) and decreased to 39% of the five- to eight-week-old pigs. M. hyorhinis was prevalent in pigs three to four weeks of age (51.9% were culture-positive) and remained prevalent in pigs through five to eight weeks of age. B. bronchiseptica and P. multocida were isolated more commonly as pigs got older (44.4% of the pigs with these organisms were first culture-positive after five to eight weeks of age). In 92.6% of the cases where pigs were culture-positive for B. bronchiseptica or P. multocida, either H. parasuis, M. hyorhinis, or both, had been isolated at prior sample intervals. The likelihood of H. parasuis and M. hyorhinis enhancing rhinitis or other respiratory diseases in the young pig needs to be considered further.

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PART II. CHRONOLOGICAL STUDIES OF RESPIRATORY DISEASE IN BABY PIGS: NECROPSY, HISTOLOGIC, AND MICROBIOLOGIC EXAMINATION OF RESPIRATORY TRACTS FROM FIFTY-FIVE COUGHING PIGS

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CHRONOLOGICAL STUDIES OF RESPIRATORY DISEASE IN BABY PIGS: NECROPSY, HISTOLOGIC, AND MICROBIOLOGIC EXAMINATION OF RESPIRATORY TRACTS FROM FIFTY-FIVE COUGHING PIGS

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SUMMARY

Fifty-five coughing pigs one to eight weeks of age from six conventional Iowa herds were necropsied. Forty-three had either gross or microscopic evidence of pneumonia, or microbiologic evidence of lung infection. Mild interstitial pneumonia with or without foci of suppurative bronchopneumonia was the most common microscopic finding. Adenovirus-like inclusions were located within nuclei of bronchiolar epithelial cells of pigs in one herd. Clubbing or atrophy of the ventral nasal turbinates was present in 41 pigs. Microscopic evidence of rhinitis was present in 39, 4 with inclusion body rhinitis.

The most common isolates from lung were Mycoplasma hyorhinis and Haemophilus parasuis. Of 31 pigs culture-positive for M. hyorhinis, 24 had pneumonia. Of 20 pigs culture-positive for H. parasuis, 18 had pneumonia. Three of 5 pigs culture-positive for Bordetella bronchiseptica had pneumonia. Other isolates, all from pneumonic lung, were Streptococcus suis, from 4 pigs, and Mycoplasma hyosynoviae, from 1 pig. The cause of mycoplasmal pneumonia, Mycoplasma hyopneumoniae, was not detected in any of the pigs. M. hyorhinis and H. parasuis were also the most common isolates from the nasal cavity secretions; 37 and 34 pigs, respectively. B. bronchiseptica and Pasteurella multocida were isolated from 12 and 15 pigs, respectively.

Chi-square analysis indicated that isolation of B. bronchiseptica, P. multocida, H. parasuis, or M. hyorhinis was independent of the presence or absence of rhinitis or pneumonia.

INTRODUCTION

Respiratory diseases are often given little consideration in young pigs because of recurring problems with stillbirths, weak pigs, overlaying, and diarrhea. However, baby pigs are known to be susceptible to various agents involved in respiratory diseases, and it is assumed that these diseases often begin during the suckling and nursery phases of swine production. The overt signs and economic loss due to respiratory diseases are more noticable in the growing and finishing phases of production.

Because it is likely that first exposure to respiratory pathogens occurs early in the pig's life, disease prophylaxis could be most efficacious and most cost effective at this time. Recently, considerable interest has been shown in antibiotic treatment of baby pigs for control of mycoplasmal pneumonia (1,2,3), one of the most important respiratory diseases in growing and finishing swine. Prophylaxis or treatment for any disease will be best selected if the etiologic agent(s) involved are known. Because there has been little documentation of the agents involved in baby pig pneumonia, we decided to investigate the problem in 6 herds with histories of respiratory disease in baby pigs. Fifty-five pigs 1 to 8 weeks of age, which had been observed coughing, were necropsied and their respiratory tracts were examined for lesions of rhinitis and pneumonia, and for bacteria and mycoplasmas known to be involved in respiratory disease. Our results indicate that the lesions occurring in baby pig pneumonia were predominantly interstitial- and

suppurative broncho-pneumonia and that the predominant microbes isolated were H. parasuis and M. hyorhinis. The cause of mycoplasmal pneumonia in swine, M. hyopneumoniae, was not detected in any of the pigs.

MATERIALS AND METHODS

Source of Pigs

Six conventional herds (see Table 1) with persistent respiratory disease in young pigs were examined. The producers indicated that sneezing and coughing were a continuing problem, affecting pigs one to seven weeks of age. Groups of neonatal pigs (age one to two weeks) were selected and identified with ear tags for on the farm study. Sequential microbiological examination of nasal secretions for selected respiratory pathogens and of sera for antibodies to these agents have been presented in a separate report (4). In the work presented here, pigs observed coughing were selected from the groups at one to eight weeks of age, transported to Iowa State University, and evaluated by necropsy, histologic and microbiologic examination.

Necropsy Technique

All pigs were necropsied within twelve hours after arrival at ISU. Euthanasia was by electrocution and exsanguination by incision of the axillary arterial and venous complex. Necropsied were done as aseptically as possible and tissues and secretions for microbiological examination were refrigerated immediately. Samples taken for microbiological examination consisted of tissues from right and left cardiac lobes of the lung and secretions from the right and left bronchi, trachea, tonsil, nasopharynx, and nasal cavity, as well as heart blood. Secretions were collected with sterile calcium alginate swabs and placed in 0.75 ml

Table 1. Herd description and presenting clinical signs of respiratory disease in young pigs

Herd	Number of sows in herd	Description of facilities	Pig age at weaning	Weeks in nursery	Signs of pig respiratory disease
A	150 to 200	Continuous farrowing with crates on wood or expanded metal slats. Nursery on concrete slats.	3 to 5 weeks	3 to 7 weeks	2 to 3 weeks old: sneezing and tearing in 3-4% 3 to 4 weeks old: sneezing or coughing in 1-2% 6 to 10 weeks old: productive cough and nasal discharge in 1-2%
B	270	All-in all-out farrowing with crates on concrete slats. Nursery multi-deck; concrete slats below, rubber-coated wire mesh above.	3 to 6 weeks	5 to 7 weeks	2 weeks old: sneezing or coughing in 2-3% 4 to 5 weeks old: sneezing in 20% productive cough in 5-10% 10 to 12 weeks old: pigs appeared healthy
C	400 to 430	All-in all-out farrowing with raised crates on rubber-coated wire mesh. Nursery of raised pens on slats of expanded metal.	3 weeks	5 to 7 weeks	2 weeks old: sneezing or coughing in 20% 3 weeks old: almost all sporadically coughing 6 to 7 weeks old: pigs appeared healthy

D	375	All-in all-out farrowing with crates on concrete slats. Nursery double-deck with pens on wire mesh.	2 to 2½ weeks	3 weeks	1 week old: sneezing in 1-2% coughing sporadic 2 to 3 weeks old: sneezing in 10-15%, productive cough in 20-30% 6 to 7 weeks old: little sneezing or coughing
E	275	Seasonal all-in all-out farrowing with raised crates on rubber-coated wire mesh. Nursery double-deck with concrete slats below, wire mesh above.	4 weeks	8 weeks	1 week old: sneezing or coughing in 1% 3 to 4 weeks old: sneezing or coughing in 20-50% 10 weeks old: little sneezing or coughing
F	800	Continuous farrowing in individual solid-bottom pens. Nursery of raised pens on wire mesh.	4 weeks	6 to 7 weeks	2 to 3 weeks old: sneezing in 10-15%, coughing in 2-3% 6 to 7 weeks old: little sneezing or coughing

Hanks' balanced salt solution[a] with 10% fetal calf serum (HBSS with FCS) prior to refrigeration. Gross lesions were recorded and sketched (where applicable). The snout was examined following removal of the lower jaw, splitting the head longitudinally, and transecting the snout slightly rostral to the second premolar teeth.

Histologic Technique

Tissue samples for microscopic examination included sections of each lobe of the lung, trachea (caudal), and ventral nasal turbinate. Samples were fixed in neutral buffered 10% formalin, sectioned at 6 μ, and stained with hematoxylin and eosin (sectioning and staining were done by R. O. Ross, Veterinary Medical Research Institute, Ames, Iowa).

Microbiological Studies

Inoculation of media

Approximately 1 gram samples of right and left cardiac lobes of the lung were ground and suspended in HBSS with FCS. After large particles had settled, the supernate was inoculated as follows: 0.05 ml aliquots were streaked on 2 blood agar plates (one for incubation in air and one for anaerobic incubation[b]) and MacConkey agar[c] with 1%

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[a]Hanks' balanced salt solution. GIBCO Laboratories, Grand Island, New York.

[b]Gas Pak, Hydrogen & Carbon Dioxide. BBL, Cockeysville, Maryland.

[c]MacConkey agar. Difco Laboratories, Detroit, Michigan.

dextrose, 0.2 ml was inoculated into Friis mycoplasma broth (5,6), and 0.1 ml was transferred to HBSS with FCS. The Friis broth was subsequently passaged (0.2 ml per 1.8 ml Friis) through 10^{-7}. The HBSS with FCS was also passaged through 10^{-7} (0.1 ml inoculum per 0.9 ml HBSS with FCS); a 0.05 aliquot of each dilution was streaked on a blood agar plate.

The mycoplasma broth used for samples from herds A, B, and C was prepared according to Friis with 12.5% swine serum (5) and 12.5% horse serum (6). Because most samples from herds A, B, and C were 'overgrown' by M. hyorhinis, some alterations were made in the Friis broth. The special broth (Formula B) used for herds D, E, and F was also prepared according to Friis; acid extract of yeast (shown to retard growth of M. hyorhinis) was substituted for the water extract of yeast (6) and modified Hanks' balanced salt solution was increased to 50%.[d] Primary passages in Friis broth B also contained 0.3 mg cycloserine[e]/ml and hyperimmune antiserum against M. hyorhinis prepared in rabbits. Friis broth B without the cycloserine and M. hyorhinis antiserum was used for subcultures.

Secretions collected on swabs from the right bronchus, left bronchus, trachea, tonsil, nasopharynx, and nasal cavity were streaked on 5% horse blood agar and MacConkey agar with 1% dextrose, then the swabs were swirled in mycoplasma broth (BHI) (7,8) with 2000 IU

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[d] N. F. Friis, State Veterinary Serum Laboratory, Copenhagen, Denmark. Personal communication, 1981.

[e] D-cyclosine. Sigma Chemical Company, St. Louis, Missouri.

penicillin[f]/ml and thallium acetate[g] at 1:4000 dilution. Blood agar plates were streaked once with a Staphylococcus epidermidis nurse culture to support Haemophilus spp. growth. Samples of tonsil, nasopharyngeal, and nasal secretions from pigs from herds D, E, and F were also inoculated on five percent horse blood agar with 2 ug clindamycin[h]/ml to facilitate isolation of P. multocida.

The transport medium containing secretions collected with swabs was inoculated into Friis broth (about 0.2 ml per 1.8 ml of Friis broth). Due to problems of contamination of cultures from herds A and B, antibiotics were added to the medium used for herd C. Additional problems with M. hyorhinis overgrowth led to changing the Friis formula used for herds D, E, and F.

Friis broth A without any additives was used for herds A and B. The Friis broth inoculated with secretion samples were passaged through 10^{-2}. From herd B, the right bronchial dilutions were carried out through 10^{-5}. Friis broth A was used for secretions collected from herd C, and 0.13 mg/ml each of meticillin[i] and bacitracin[j] were added to tubes inoculated with tonsil, nasopharynx, and nasal cavity samples.

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[f]Penicillin G potassium for injection, USP. Eli Lilly and Company, Indianapolis, Indiana.

[g]Thallium(ous) acetate. Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, New Jersey.

[h]Clindamycin hydrochloride USP. The Upjohn Company, Kalamazoo, Michigan.

[i]Staphcillin. Bristol Laboratories, Syracuse, New York.

[j]Bacitracin micronisee sterile, USP. Roussell UCLAF, c/o Dipl. Ing. Z. Szabo, Vienna, Austria.

Samples from the left bronchus, trachea, tonsil, nasopharynx, and nasal cavity were passaged through 10^{-3} in Friis broth A, except samples from right bronchus, which were passaged through 10^{-5}.

From herds D, E, and F, tonsil, nasopharyngeal, and nasal inocula were passaged through 10^{-3} in Friis broth B with additional meticillin (0.13 mg/ml), bacitracin (0.13 mg/ml) and thallium acetate (to 1:4000 dilution); passage was continued through 10^{-7} in Friis broth B without the additional antibiotics. Right bronchus, left bronchus, and tracheal secretions were passaged through Friis broth B similar to the lung supernates from these herds.

Isolation and identification of microbes

Aerobic plates were incubated at 37°C, then examined for growth after 24 to 48 hours. Identification of the common respiratory pathogenic bacteria was done as described previously (4).

Blood agar plates inoculated for anaerobic growth were incubated in 85% hydrogen and 15% carbon dioxide[b] at 36°C and examined after 48 hours. Colonies that grew were reinoculated to blood agar to determine their tolerance to air and examined using Gram's stain.

Cultures in BHI broth were incubated at 36°C and, after 72 hours, 0.5 ml was passaged blindly to a fresh tube of BHI. Second passage cultures were examined for evidence of growth daily over the next two weeks. Cultures with suspected mycoplasma growth were inoculated on BHI agar and placed at 37°C in a humid incubator. Identification of colonies was by epi-immunofluorescence (9) or disc growth inhibition (10), as described previously (4).

Primary passages of Friis broth were incubated on roller drums[k] at 36°C, and were examined for color change (acid) or turbidity daily for six weeks. The Friis broth cultures from herds E and F were removed from the roller drums after four weeks and placed in stationary racks at 36°C for the remaining two weeks of primary incubation. Subcultivation of suspected growth was carried out for four passages, each incubated in stationary racks at 36°C. The last passage of the highest dilution of each inoculum with growth was plated on regular Friis agar and incubated in a candle jar at 36°C. After 3 to 6 days incubation, plates were inspected for growth; mycoplasma colonies were identified by epi-immunofluorescence with the plates being refrigerated for 24 hours then methanol-fixed for 40 minutes prior to staining with conjugate (11). To reduce background color, the agar was counterstained with chelated azo-dye for 3 minutes. The chelated azo-dye was prepared according to Potgieter and Ross (12) as modified by Amanfu (11). The azo-dye was prepared by dissolving 15.6 mg of Eriochrome Black T[l] in 20 ml N, N dimethyl formide. The chelating agent was prepared by mixing 50 ml N, N dimethyl formide, 10 ml distilled water, 10 ml 0.1 M aluminum chloride, and 10 ml acetic acid, adjusting the pH of the mixture to 5.2 with 1 N sodium hydroxide solution, then adding distilled water to a total volume of 100 ml. The chelating agent was added slowly to the azo-dye; the chelated azo-dye was then stored at 4°C.

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[k] Dual Tissue Culture Rotator. Lab-Line Instruments, Inc., Melrose Park, Illinois.

[l] Eriochrome Black T. Hartmann Leddon Company, Philadelphia, Pennsylvania.

Direct fluorescent antibody testing (DFAT) for M. hyopneumoniae

At necropsy, small (about 1 cm square) sections of right and left cardiac lobes of the lung were frozen at -70°C in embedding medium for frozen tissue specimens.[m] Each section contained cross-sections through various bronchi and bronchioles. At a later, convenient date, sections (4 μ) were cut with a cryostat, placed on glass slides, fixed in chilled absolute methanol for 10 min, and stored at -20°C. The procedure for the DFAT was done according to Amanfu et al. (13). Conjugated antisera to M. hyopneumoniae and M. hyorhinis, and positive control lung sections were provided by B. J. Zimmermann (Veterinary Medical Research Institute, Ames, Iowa).

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[m]Tissue-Tek II O.C.T. Compound. Lab-Tek Products, Naperville, Illinois.

RESULTS

Necropsy and microbiological findings in pigs from herds A through F are presented in Table 2. A total of 55 pigs were examined; 30 had gross lesions in their lungs while microscopic lesions of mild interstitial pneumonia and/or suppurative bronchopneumonia were found in 27. Peribronchiolar or perivascular cuffing by lymphohistiocytic cells was present to some extent in 22 pigs. Typical gross and microscopic lesions are depicted in Figures 1 through 8. Gross examination of the nasal turbinates from these 55 pigs revealed clubbing or atrophy of the ventral turbinates in 41. Microscopic examination supported the diagnosis of rhinitis in 39 of the pigs. Inclusion body rhinitis was diagnosed in 4 pigs. Large basophilic, intranuclear bodies within the tubuloalveolar glands of the nasal mucosa were observed in these animals. In addition there was a heavy mononuclear cell infiltration of the lamina propria around the glands which were three to four times larger than normal.

The most common isolates from lung were H. parasuis (from 20 pigs) and M. hyorhinis (from 31 pigs). B. bronchiseptica was isolated from 5 of the lungs and Streptococcus suis was isolated from 4. Mycoplasma hyosynoviae was isolated from the lung of one pig. The most common isolates from secretions collected at the abrostral portion of the nasal cavity were H. parasuis and M. hyorhinis (from 34 pigs and 37 pigs, respectively). B. bronchiseptica was isolated from the nasal cavities of 12 pigs and P. multocida was isolated from 15 pigs. Two pigs had Mycoplasma salivarium and 2 had Mycoplasma buccale in their nasal

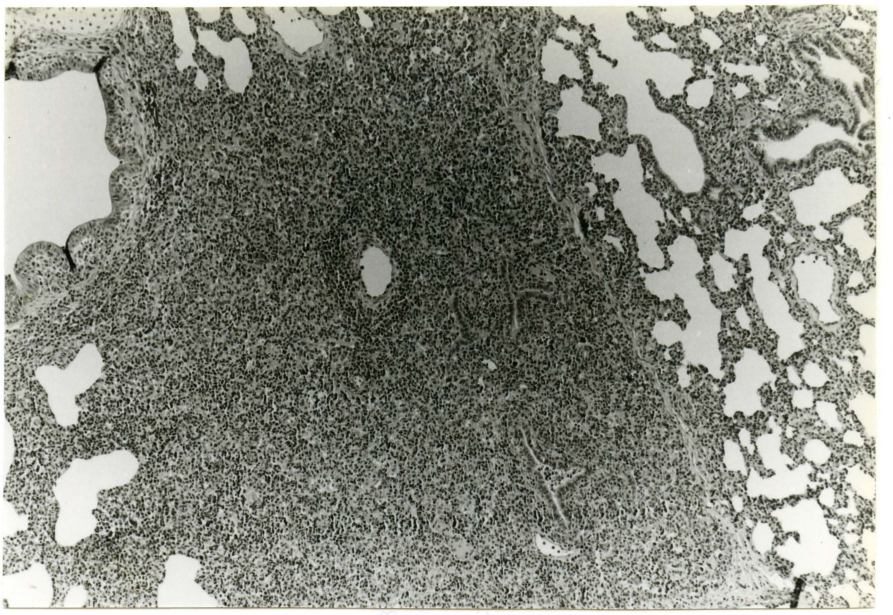

Figure 1. Acute to chronic interstitial pneumonia in a four- to five-week-old pig (no. 011) from herd B. Note perivascular lymphohistiocytic cuff. H & E - 262X

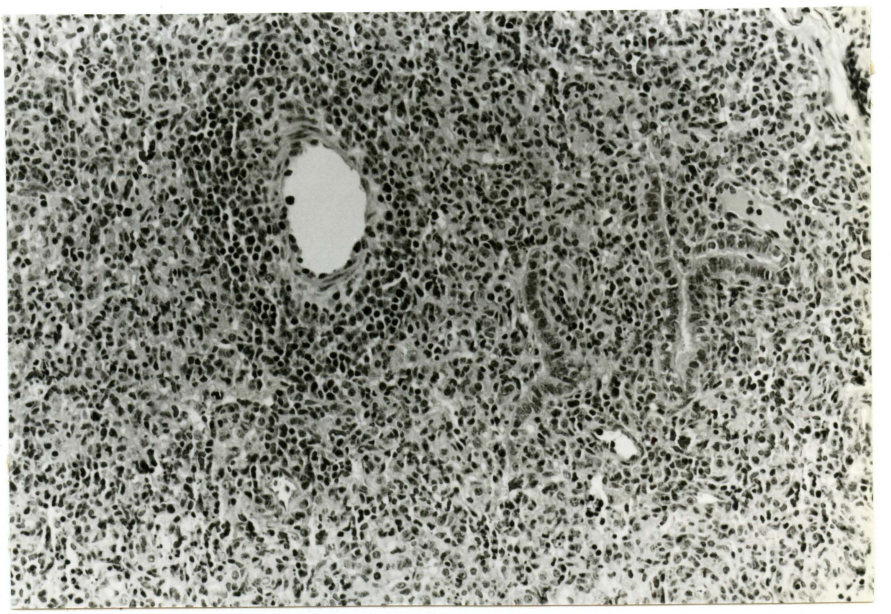

Figure 2. Perivascular lymphohistiocytic cuff in four- to five-week-old pig (no. 011) from herd B with interstitial pneumonia. H & E - 667X

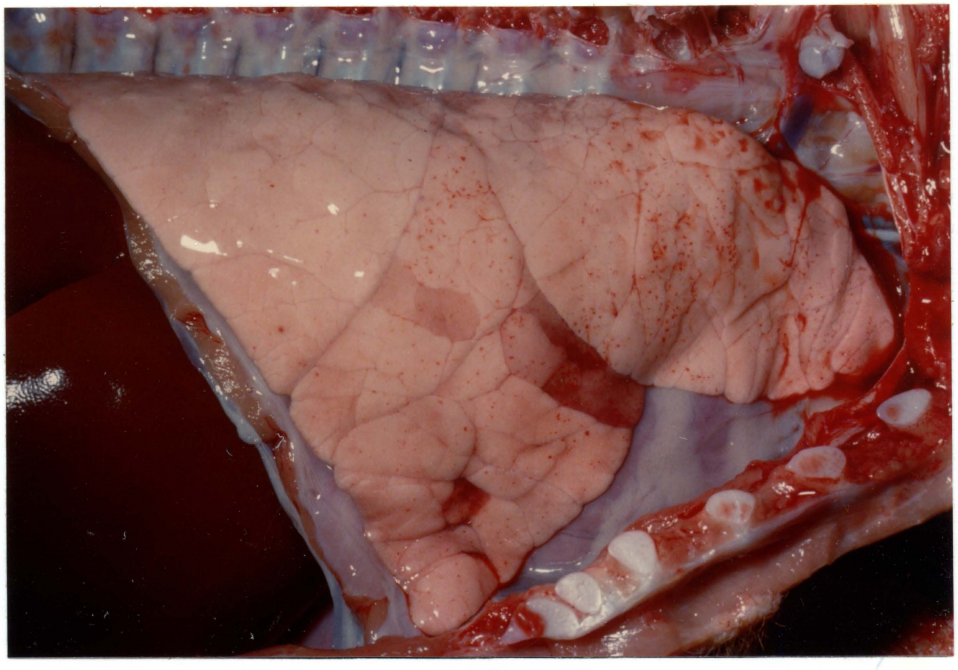

Figure 3. Gross appearance of lung in four-week-old pig (no. 032) from herd C. M. hyorhinis was isolated from the right cardiac lobe

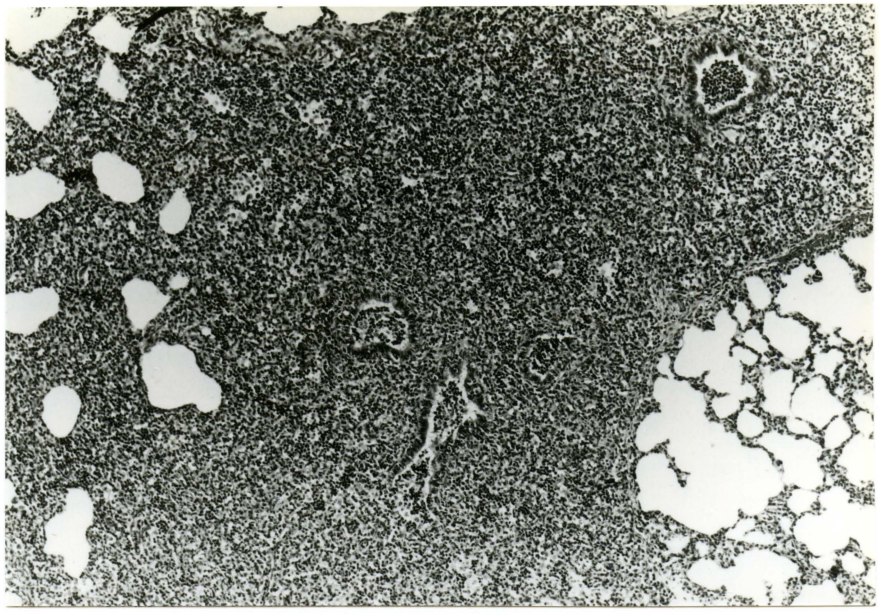

Figure 4. Acute bronchopneumonia in a four-week-old pig (no. 032) from herd C. H & E - 262X

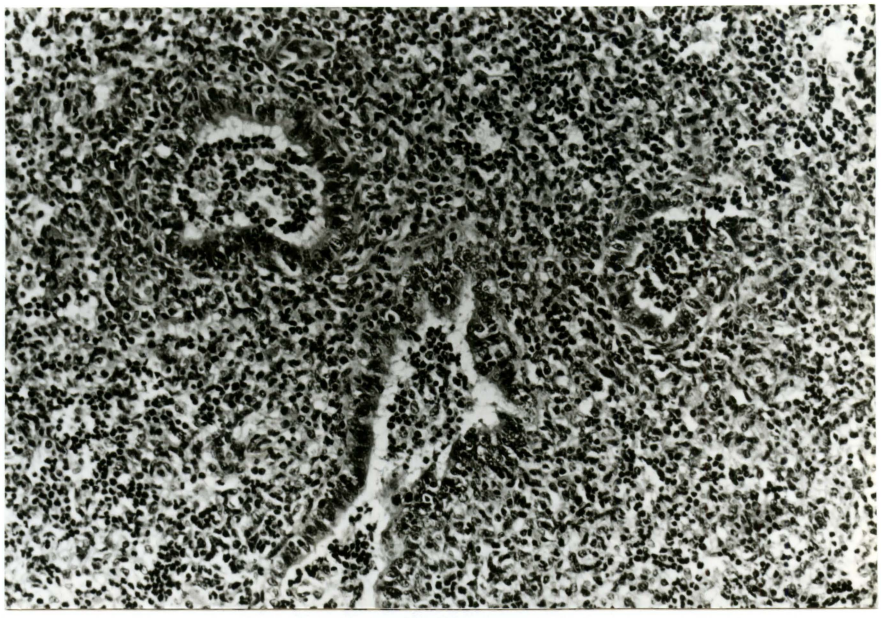

Figure 5. Acute bronchopneumonia in a four-week-old pig (no. 032) from herd C. H & E - 667X

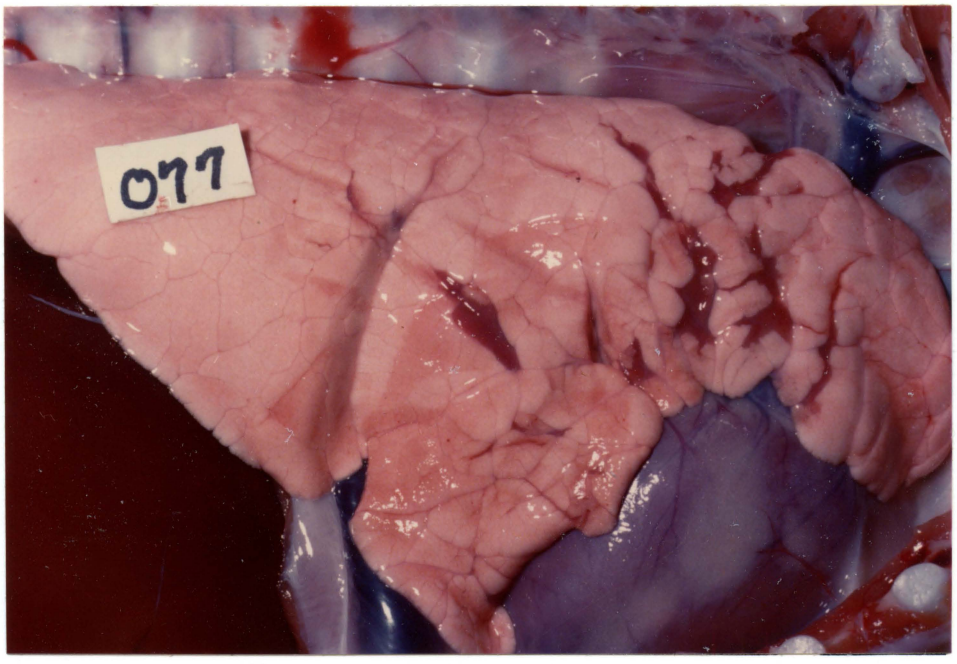

Figure 6. Gross appearance of lung in eight-week-old pig (no. 077) from herd F. H. parasuis and M. hyorhinis were isolated from the right and left cardiac lobes

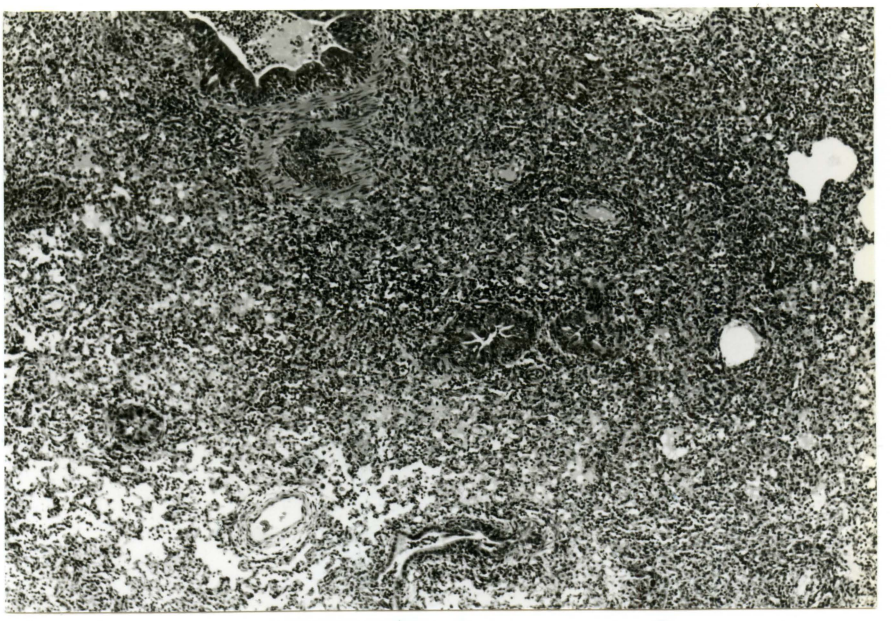

Figure 7. Subacute to chronic bronchopneumonia in an eight-week-old pig (no. 077) from herd F. H & E - 262X

Figure 8. Subacute to chronic bronchopneumonia in an eight-week-old pig (no.077) from herd F. H & E - 667X

secretions. Table 3 presents the common microorganisms isolated from pigs with or without lesions. The presence or absence of lesions could not be correlated by chi-square analysis to the isolation of B. bronchiseptica, P. multocida, H. parasuis, or M. hyorhinis from that tissue.

Samples of lung, bronchi, trachea, nasopharynx, and nasal cavity from each of the 55 pigs were all culture-negative for M. hyopneumoniae in Friis media. Examination of frozen sections of lung from the pigs by direct immunofluorescence did not demonstrate the presence of M. hyopneumoniae in any lungs.

Herd A

Three-week-old pigs had no gross lesions, but microscopic examination of lung tissue from the pigs revealed small foci of suppurative and interstitial pneumonia. H. parasuis and M. hyorhinis were isolated from the lung samples. Extensive consolidation was present in the lung of a five-week-old pig. B. bronchiseptica and M. hyorhinis were isolated.

Herd B

Pigs necropsied at 1 to 2 and 3 weeks of age had no lesions of pneumonia, nor were any microbes isolated from their lungs. Suppurative pneumonia was present in pig no. 011 (4 to 5 weeks of age) and H. parasuis was isolated. Pig no. 017 (6 to 7 weeks of age) also had suppurative pneumonia; B. bronchiseptica was isolated.

Herd C

Pigs necropsied from this herd had few lesions, and the only isolate from lung was M. hyorhinis. The health of pigs from this group in herd C appeared to improve as the study progressed.

Herd D

Pneumonia was found in pigs beginning when they were 1 to 2 weeks of age. Eight of twelve pigs less than 6 weeks of age had extensive gross and microscopic lesions of suppurative interstitial and bronchopneumonia. From the lung samples of the 15 pigs necropsied, H. parasuis was isolated from 9, M. hyorhinis from 8, and S. suis from 2. Lesions in lungs of pigs 2 to 3 weeks of age had edema fluid with the pneumonia. In lung sections from 2-week-old pigs (nos. 038 and 039), there were dense basophilic intranuclear structures resembling adenoviral inclusions. The inclusions, seen in pig 038, were located within nuclei of bronchiolar epithelial cells (Figure 9). The nuclear membranes were distinctly basophilic, containing a homogenous, slightly purple mass within a halo (Figure 10). These intranuclear inclusions prompted further investigation into a possible viral etiology of pneumonia in herd D. Sera from 11 pigs six weeks of age and 5 sows were tested for antibodies to swine influenza virus (SIV)[n] and pseudorabies virus (PRV)[o]. Hemagglutination inhibiting antibodies to SIV were present in 2 pigs at titers of 1:10

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[n]G. A. Erickson. National Veterinary Services Laboratory, Ames, Iowa.

[o]H. T. Hill. Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa.

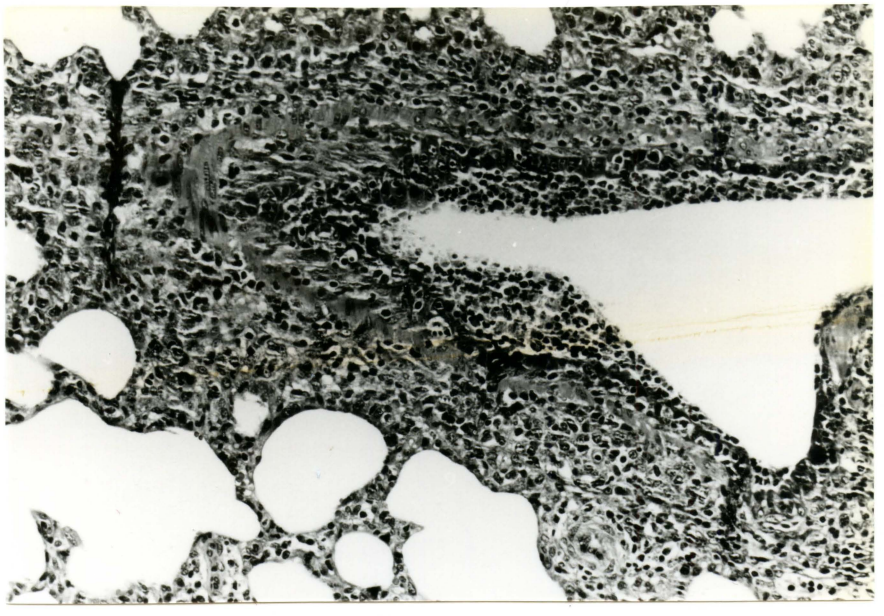

Figure 9. Bronchiolitis associated with adenovirus-like inclusions in two-week-old pig from herd D. H & E - 667X

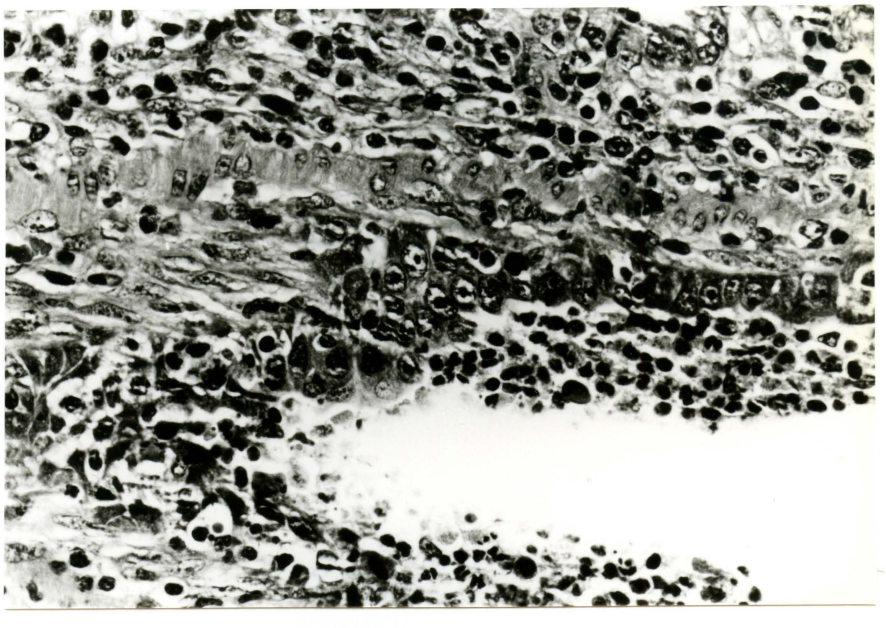

Figure 10. Adenovirus-like intranuclear inclusions in bronchiolar epithelium of two-week-old pig from herd D. H & E - 1674X

and 1:20 , and 1 sow at 1:40; this sow was the dam of the pig with the 1:10 titer. Serum neutralizing antibody titers of 1:4 to PRV were present in 10 of 11 pigs sampled. The sows had titers of 1:8, 1:16, 1:16, 1:64, and 1:245. Adenovirus serology examinations were not available.

Herd E

Pneumonia was not recognized in pigs from this herd until they were 6 and 8 weeks of age, when suppurative broncho- and interstitial-pneumonias were recorded. Microbes isolated from the lung samples of 12 pigs included H. parasuis from 6, M. hyorhinis from 6, B. bronchi-septica from 3, S. suis from 2, and M. hyosynoviae from 1.

Herd F

Foci of chronic suppurative bronchopneumonia were noted in pigs necropsied at 5 through 8 weeks of age from this herd. H. parasuis was isolated from the lung samples of 3 pigs, and M. hyorhinis was isolated from 9 pigs.

Table 2. Summary of necropsy and microbiologic findings in 55 pigs

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
A	3 wks	007 017	Lung	None	Minute foci acute suppurative & interstitial pneumonia	H. parasuis H. hyorhinis
			Bronchi & trachea	None	Slight bronchitis with purulent exudate; mild tracheitis	H. parasuis M. hyorhinis
			Turbinate	Slight clubbing of ventral scrolls	Mild rhinitis	P. multocida M. hyorhinis
	5 wks	019	Lung	Consolidation >50% right cardiac, <25% right apical & left cardiac & 100% intermediate lobes	Acute to chronic suppurative broncho-pneumonia with focal necrosis	B. bronchiseptica M. hyorhinis
			Bronchi & trachea	Mucopurulent exudate in bronchi & trachea	Chronic tracheitis with squamous metaplasia	B. bronchiseptica H. parasuis M. hyorhinis
			Turbinate	Severe turbinate atrophy	Osteoclastic hyperplasia	B. bronchiseptica
B	1-2 wks	008 009	Lung	None	None	None
		020	Bronchi & trachea	None	None	H. parasuis

		Turbinate	Slight clubbing of ventral scrolls	Extensive lymphohistiocytic cell infiltration of nasal mucosa	B. bronchiseptica H. parasuis
3 wks	010 022	Lung	None	None	None
		Bronchi & trachea	None	None	H. parasuis
		Turbinate	Ventral scrolls slightly blanched & clubbed	Very mild rhinitis with some squamous metaplasia	H. parasuis
4-5 wks	011	Lung	Patchy consolidation (<25%) right & left cardiac & apical lobes	Very small foci subacute suppurative bronchopneumonia	H. parasuis
		Bronchi & trachea	None	Mild tracheitis	H. parasuis M. hyorhinis
		Turbinate	Ventral scrolls slightly clubbed, blanched, with mild catarrh	Inclusion body rhinitis with extensive lymphohistiocytic cell infiltration of nasal mucosa	M. hyorhinis

Table 2 (continued)

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
B	6-7 wks	018	Lung	Irregular consolidation (<25%) right & left cardiac, & intermediate lobes	Very small foci subacute suppurative bronchopneumonia	B. bronchiseptica
			Bronchi & trachea	None	Mild tracheitis	B. bronchiseptica
			Turbinate	Catarrhal exudate	Severe rhinitis with microabscesses & extensive lymphohistiocytic cells	B. bronchiseptica
C	1 wk	025 026	Lung	None	None	None
			Bronchi & trachea	None	None	P. multocida
			Turbinate	Slightly blanched mucosa	Mild focal suppurative rhinitis	None
	2 wks	029	Lung	None	None	None
			Bronchi & trachea	None	None	H. parasuis
			Turbinate	Slight clubbing of right ventral scroll; mild catarrhal exudate	Very mild rhinitis	None

3 wks	031	Lung	Small lesions, dorsal aspect right cardiac lobe	None	*M. hyorhinis*
		Bronchi & trachea	None	None	*H. parasuis* *M. hyorhinis*
		Turbinate	Catarrhal exudate	Mild rhinitis	*H. parasuis* *M. hyorhinis*
4 wks	032	Lung	Consolidation <25% right cardiac lobe	Very small foci acute purulent bronchopneumonia	*M. hyorhinis*
		Bronchi & trachea	Catarrhal exudate in bronchi & trachea	None	*P. multocida* *H. parasuis* *M. hyorhinis*
		Turbinate	Mucopurulent exudate	Very mild rhinitis	*H. parasuis* *M. hyorhinis*
5 wks	033	Lung	None	None	*M. hyorhinis*
		Bronchi & trachea	None	None	*M. hyorhinis*
		Turbinate	Mucopurulent exudate	Focal suppurative & necrotic rhinitis	*M. hyorhinis*

Table 2 (continued)

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
C	6 wks	030	Lung	Consolidation <50% right, <25% left, cardiac lobes	Small foci chronic interstitial pneumonia with bronchiolar cuffing	M. hyorhinis
			Bronchi & trachea	None	Very mild tracheitis	M. hyorhinis
			Turbinate	Mild catarrhal exudate	None	M. hyorhinis
D	1 wk	036	Lung	Consolidation <25% right cardiac lobe	None	None
			Bronchi & trachea	None	None	H. parasuis
			Turbinate	None	Very mild focal rhinitis	H. parasuis M. hyorhinis
	2 wks	038 039	Lung	50-75% right & left cardiac & apical lobes, & <25% left diaphragmatic lobe edematous	Extensive chronic suppurative broncho- & interstitial pneumonia with necrotic bronchiolitis; slight bronchiolar cuffing by eosinophils	H. parasuis S. suis
			Bronchi & trachea	None	Adenovirus-like intranuclear inclusions in few bronchial epithelial cells; mild chronic tracheitis	H. parasuis M. hyorhinis

		Turbinate	Mucopurulent exudate	Mild rhinitis	B. bronchiseptica H. parasuis M. hyorhinis
3 wks	043 044 045	Lung	Intermediate consolidation & edema 25-50% all lobes	Moderate chronic suppurative broncho-pneumonia with multifocal interstitial pneumonia; bronchiolar cuffing	H. parasuis M. hyorhinis
		Bronchi & trachea	Bronchial catarrh	Bronchitis; mild to chronic tracheitis	H. parasuis M. hyorhinis
		Turbinate	Mucopurulent exudate; slight clubbing of ventral scrolls	Mild rhinitis	B. bronchiseptica P. multocida H. parasuis M. hyorhinis
4 wks	046	Lung	Consolidation 100% right cardiac, <50% right apical, <25% left cardiac, apical & intermediate lobes	Extensive chronic suppurative broncho- & interstitial pneumonia with vascular & bronchiolar cuffing	H. parasuis M. hyorhinis
		Bronchi & trachea	None	Very mild tracheitis	H. parasuis M. hyorhinis

Table 2 (continued)

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
D	4 wks		Turbinate	Mucopurulent exudate	Mild rhinitis	None
	5½ wks	047 048 049	Lung	Discolored, firm lesions 25-100% cardiac & apical lobes	Patchy foci resolving chronic interstitial pneumonia with some suppurative broncho-pneumonia; extensive bronchiolar & vascular lymph cell cuffs	H. parasuis M. hyorhinis
			Bronchi & trachea	Bronchial muco-purulent exudate	Mild bronchitis; mild tracheitis	H. parasuis M. hyorhinis
			Turbinate	Slight clubbing of ventral scrolls; mucopurulent exudate	Mild rhinitis	H. parasuis M. hyorhinis M. salivarium
	6 wks	035 050	Lung	Consolidation 25-100% right cardiac & apical lobes	Foci chronic suppurative broncho- & interstitial pneumonia with bronchiolar lymphohistiocytic cuffing	M. hyorhinis S. suis
			Bronchi & trachea	Bronchial muco-purulent exudate	Mild tracheitis	H. parasuis M. hyorhinis
			Turbinate	Slight clubbing of ventral scrolls	Chronic rhinitis	H. parasuis M. hyorhinis M. salivarium

	7½ wks	051 052 053	Lung	None	Small foci chronic interstitial pneumonia with some suppurative broncho-pneumonia; few small foci granulomatous pneumonia; vascular lymph cell cuffs	H. parasuis M. hyorhinis
			Bronchi & trachea	None	Bronchial lympho-histiocytic cuffing	B. bronchiseptica H. parasuis M. hyorhinis
			Turbinate	Clubbing of ventral scrolls	None	B. bronchiseptica H. parasuis M. hyorhinis
E	2 wks	056 057 058	Lung	None	None	None
			Bronchi & trachea	None	None	H. parasuis
			Turbinate	Slight clubbing of ventral scrolls	None	H. parasuis
	4 wks	059 061	Lung	Consolidation <25% left cardiac lobe	Multifocal bronchiolar mixed cell exudate	B. bronchiseptica H. parasuis M. hyorhinis

Table 2 (continued)

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
E	4 wks		Bronchi & trachea	None	Mild tracheitis	B. bronchiseptica H. parasuis M. hyorhinis
			Turbinate	Slight clubbing of ventral scrolls	None	B. bronchiseptica H. parasuis M. hyorhinis
	5 wks	060	Lung	Irregular consolidation <25% left cardiac lobe	None	H. parasuis
			Bronchi & trachea	None	Mild tracheitis	H. parasuis M. hyorhinis
			Turbinate	Slight clubbing of ventral scrolls	Mild rhinitis	H. parasuis
	6 wks	064 065 066	Lung	Consolidation 50% right cardiac & apical, <25% left cardiac & apical lobes	Foci of mild chronic suppurative bronchopneumonia with bronchiolar lymphohistiocytic cuffing	B. bronchiseptica H. parasuis M. hyorhinis S. suis
			Bronchi & trachea	Bronchial & tracheal catarrh	Mild bronchitis with neutrophilic exudate; mild tracheitis with microabscesses in mucosa	B. bronchiseptica H. parasuis M. hyorhinis

			Turbinate	Ventral turbinate atrophy with blanched mucosa & mucopurulent exudate	Moderate chronic rhinitis with some squamous metaplasia	B. bronchiseptica H. parasuis M. hyorhinis
	8 wks	070 071 072	Lung	Slight mottling of cardiac & apical lobes	Few small foci resolving chronic interstitial pneumonia, sometimes suppurative	B. bronchiseptica H. parasuis M. hyorhinis M. hyosynoviae S. suis
			Bronchi & trachea	Bronchial & tracheal catarrh	Moderate tracheitis	B. bronchiseptica H. parasuis M. hyorhinis
			Turbinate	Loss of turbinate scroll definition; mucopurulent exudate	Moderate chronic rhinitis with some squamous metaplasia	B. bronchiseptica P. multocida H. parasuis M. hyorhinis
F	3½ wks	062 063	Lung	Consolidation <25% right cardiac lobe	None	M. hyorhinis
			Bronchi & trachea	None	Very mild tracheitis	H. parasuis M. hyorhinis

Table 2 (continued)

Herd	Age	Pig no.	Tissue	Gross lesions	Microscopic lesions	Microbiology
F	3½ wks		Turbinate	Turbinate mucosa blanched with muco-purulent exudate	Mild rhinitis; inclusion body rhinitis	H. parasuis M. hyorhinis
	5 wks	067 068 069	Lung	Edema & consolidation 50% right cardiac, & <25% right apical & diaphragmatic, left cardiac & apical, & intermediate, lobes	Foci of resolving chronic suppurative broncho-pneumonia with focal edema	H. parasuis M. hyorhinis
			Bronchi & trachea	Bronchial catarrh	Chronic bronchitis with some necrosis of bronchial epithelium	H. parasuis M. hyorhinis
			Turbinate	Clubbed ventral scrolls; nasal catarrh	None	H. parasuis M. hyorhinis
	7 wks	073 074 075	Lung	Consolidation <25% all lobes	Foci resolving mild chronic suppurative broncho-pneumonia with some fibrosis & bronchiolar hyperplasia; scattered accumulations of lymphocytes, especially peribronchiolar	H. parasuis M. hyorhinis

		Bronchi & trachea	Bronchial & tracheal catarrh	Very mild tracheitis	H. parasuis M. hyorhinis
		Turbinate	Turbinate atrophy with blanched mucosa & slight mucopurulent exudate	Mild rhinitis with focal osteoclastic activity; inclusion body rhinitis	P. multocida H. parasuis M. hyorhinis M. buccale
8 wks	076 077 078	Lung	Consolidation <25% cardiac & apical lobes	Foci chronic suppurative bronchopneumonia with areas of granulomatous & giant cell reaction; bronchiolar & vascular lymphohistiocytic cuffing	H. parasuis M. hyorhinis
		Bronchi & trachea	None	Mild tracheitis	H. parasuis M. hyorhinis
		Turbinate	Severe clubbing of ventral scrolls	Mild rhinitis	P. multocida H. parasuis M. hyorhinis

Table 3. Prevalence of B. bronchiseptica, P. multocida, H. parasuis, and M. hyorhinis in the respiratory tracts of 55 pigs

Tissue	No. of pigs[a]	Organism			
		B. bronchiseptica	P. multocida	H. parasuis	M. hyrohinis
Turbinate		21.8%[b]	27.3%	61.8%	67.3%
rhinitis	40	9[c]	12	24	29
no rhinitis	15	3	3	10	8
Trachea		14.5%	1.8%	70.1%	61.8%
tracheitis	34	7	1	27	25
no tracheitis	21	1	0	12	9
Lung		9.1%	0%	36.4%	56.4%
pneumonia	34	3	0	18	24
no pneumonia	21	2	0	2	7

[a]Number of pigs with or without lesions.

[b]Percentage of pigs with organism isolated from that tissue.

[c]Number of pigs with organism.

DISCUSSION

Respiratory diseases in the baby pig have been of minor concern to the pork producer. Pneumonia usually accounts for only 1.2% to 1.7% of baby pig mortatlity prior to weaning, while over 50% of pre-weaning mortality is attributed to stillbirths, trauma, and starvation (14,15). Recently, however, there has been an increasing awareness that sneezing and coughing is common among young pigs in many herds and may be the initial phase of well-known, economically important respiratory diseases of growing and finishing swine. If early signs of respiratory disease can be recognized, there is potential for treating the disease before it becomes chronic. It is the chronic disease that contributes to economic post-weaning loss, with up to 5% decrease in feed efficiency and decrease in daily gain to market (16,17). Prior signs of respiratory disease have not always been noted in pigs that had pneumonia at slaughter, but pen mates of coughing pigs are more than twice as likely to have pneumonia as those pigs from pens with no clinical signs (17). It is possible that porcine pneumonia is at least in part initiated during the suckling and nursing period, and that clinical signs of disease may be recognized during this time.

In our study, emphasis was placed on pigs less than eight weeks of age that were coughing at the time of necropsy. The 55 pigs were from a group selected from litters with at least one coughing pig. There were 43 pigs with either gross or microscopic evidence of pneumonia, or microbiologic evidence of lung infection. The predominant isolates

from the lung were M. hyorhinis (from 31 pigs) and H. parasuis (from 20 pigs). Interstitial pneumonia (or the presence of histiocytes within the interstitium) with foci of suppurative pneumonia was the most common microscopic finding.

M. hyorhinis is often isolated from the lungs of market hogs with pneumonia (18,19) and is extremely common in the nasal cavities of young pigs (4,20,21). It has been implicated as a secondary invader in pneumonia caused by M. hyopneumoniae; however, its ability to produce a naturally occurring pneumonia by itself has only been speculated. Gnotobiotic pigs inoculated with various strains of M. hyorhinis developed catarrhal bronchopneumonia within two to three weeks (22). The principal microscopic changes in these experimentally infected pigs were perivascular, peribronchiolar, and interstitial lymphohistiocytic infiltrates in the lungs. In our study, 24 of the pigs with M. hyorhinis also had pneumonia. Peribronchial and peribronchiolar, and sometimes perivascular, cuffing with lymphohistiocytic cells were present to some extent in 20 of the pigs examined. Most of these lesions were quite mild, but a few were pronounced. Lesions of this type are often associated with mycoplasmal pneumonia, but M. hyopneumoniae is generally recognized to cause a much more extensive infiltration of the bronchial mucosa with lymphohistiocytic cells (23). The prevalence of M. hyorhinis in respiratory tracts of young pigs and its persistence in many chronic pneumonias associated with M. hyopneumoniae strongly suggests that M. hyorhinis plays a larger role in porcine pneumonia than previously suspected. It is possible that different strains of M. hyorhinis may be virulent enough to

complicate M. hyopneumoniae disease.

M. hyopneumoniae disease in the field is virtually always complicated by bacteria and other mycoplasmas. In healthy pigs, or in the absence of bacteria or other mycoplasmas, induction of M. hyopneumoniae disease is variable and large inocula must often be used (23). Young pigs of any age have eventually been shown to be susceptible to infection by the organism (24). Contact exposures have been less successful, though Piffer (25) identified the organism in twelve-week-old pigs that had been exposed to heavily inoculated pigs from three weeks to five or six weeks of age. Microscopic lesions were present and were fluorescent antibody positive for mycoplasmal pneumonia. From field studies Holmgren (26) has identified macroscopic pneumonias in pigs two to five weeks of age that were fluorescent antibody positive for M. hyopneumoniae. Some other agents may actually predispose the pig to M. hyopneumoniae and initiate the chronic disease for which the organism is so well-known. It is possible that M. hyorhinis compromises the lung to an extent that M. hyopneumoniae may colonize more readily.

It must be stressed that isolation of M. hyorhinis from lung, and failure to isolate M. hyopneumoniae, does not prove that M. hyorhinis caused pneumonia. M. hyorhinis grows fairly well in vitro while M. hyopneumoniae is quite fastidious. It has been postulated that 10^3 to 10^5 M. hyopneumoniae in lung may be necessary for isolation of the organism (27). Pneumonic lung may contain 10^7 M. hyorhinis and this organism often overgrows M. hyopneumoniae (28). Attempts were made throughout our study to inhibit growth of M. hyorhinis

and allow M. hyopneumoniae to grow by using selective Friis media which inhibit the former organism. Seemingly M. hyorhinis was not markedly suppressed because we still readily isolated the organism in high titer from all areas of the respiratory tracts, from many pigs. It is possible that M. hyopneumoniae was present in the lesions but that our diagnostic methods were not sensitive enough to allow identification concurrent with the overabundance of M. hyorhinis. Fluorescent antibody testing was no more sensitive, because all of the pigs in our study were FA-negative for M. hyorhinis as well as M. hyopneumoniae.

H. parasuis was present in the respiratory tracts of 85.5% of the pigs necropsied in our study. The prevalence of this organism in nasal secretions has been reported previously (4). As indicated in Table 3, the organism is not unusual in the lower respiratory tract of the young pig. H. parasuis has not been mentioned in many reports on pneumonias, but this may be due to the failure of isolation because of improper selective media or failure to recognize the organism as significant. However, Little (29) did isolate H. parasuis from 1.7% of pneumonic lungs from market hogs examined specifically for haemophili. It is possible that when the young pig is stressed the organism may invade the lower respiratory tract.

H. parasuis has been isolated occasionally from interstitial pneumonia and bronchiolitis in the young pigs, but in most cases septicemia or Glässer's disease has been concurrent (30). No signs of Glässer's disease were present in any of the herds examined during

our study, but H. parasuis was isolated from the lungs of 20 of the 55 pigs necropsied. Eighteen of these pigs had pneumonia, eleven with an interstitial component and nine with concurrent M. hyorhinis infection. H. parasuis may be the first organism to colonize the pig's respiratory tract in large numbers, eventually being replaced by other organisms (especially M. hyorhinis) as the pig matures (4). Čois et al. (31) have suggested that H. parasuis may cause a mild rhinitis in young pigs that predispose to secondary infection by P. multocida. There is also a possibility that the nasal cavity harbors the organism in high enough numbers that it may invade the lower respiratory tract. In the present study, 16 of the 20 pigs with H. parasuis infection in their lungs also had the organism in their nasal secretions.

B. bronchiseptica and P. multocida are two organisms which may become part of the respiratory tract flora as pigs get older (4). B. bronchiseptica was isolated from the lungs of only five pigs in the present study, three with pneumonia. The organism has been incriminated as a cause of severe pneumonia in baby pigs by several authors (32,33), but in our study this was not the case. The herds with a high incidence of B. bronchiseptica in their nasal and tracheal secretions (especially herd E) did not have any higher incidence of B. bronchiseptica infection in the lung than the herds with a low incidence of B. bronchiseptica. It may be that pulmonary bordetellosis is a sporadic disease and occurs only in debilitated or exceptionally susceptible swine. Possibly different strains of B. bronchiseptica vary in their pneumotropism.

P. multocida is considered secondary to other pneumonia-causing microbes in the lung, although in chronic disease it may be the only agent recovered. In our study, P. multocida was present in the upper respiratory tract but not in the lung. The organism may invade in the latter stages of disease or in the severely compromised lung. P. multocida was a common isolate from pneumonic lungs of market hogs (18,19).

S. suis was isolated from lungs of pigs necropsied from herd D, associated with adenovirus-like lesions, and from herd E, associated with several other microbes. S. suis is becoming more recognized as a possible cause of pneumonia in the baby pig (34,35), though pneumonia has not been produced experimentally. S. suis is an alpha-hemolytic streptococcus. Baby pig pneumonia and septicemia of alpha-hemolytic-streptococcus origin were reported by Brown in 1969 (36). The organism he described was probably S. suis. Koehne et al. (34) has described a suppurative bronchopneumonia associating S. suis with B. bronchiseptica and P. multocida. Sanford and Tilker (35) identified S. suis in association with bronchopneumonia in pigs from nursing- to market age. It is possible that S. suis only occurs in lung compromised by viral or mycoplasmal infection, but L'Ecuyer et al. (18) reported that the only significant isolates from normal lungs were alpha-hemolytic streptococci. Various Lancefield's groups have been proposed for S. suis (37,38, and it is likely that strains vary markedly in virulence and pathogenicity.

Initially, swine influenza virus was suspected as the primary etiology of the acute bronchiolitis and pneumonia in pigs from herd D. Closer examination revealed adenovirus-like inclusions within bronchiolar epithelial cells. Attempts to identify virions by electron microscopy in multiple sections with inclusions were inconclusive.[p] Onset of coughing in this herd had been at one to two weeks of age. Lung edema with extensive suppurative broncho- and interstitial pneumonia were noted in the pigs that we necropsied, and H. parasuis and S. suis were isolated. The necrosis of many bronchi and bronchioles made it difficult to identify more than a few inclusions. Pigs with experimental adenoviral pneumonia had lymphohistiocytic cell aggregates around bronchi, bronchioles, and blood vessels, which extended into alveolar septae (39). It has been reported that the inclusions were most often located within these alveolar cell aggregates (40,41). We found no inclusions in these areas, possibly due to the secondary bacterial infections in these lungs. Andrews[q] suspects that adenovirus infection may begin in the airway epithelium and therefore inclusions in the bronchial or bronchiolar epithelium may be observed only early in the disease.

Results obtained in this study suggest that H. parasuis

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[p]J. A. Fagerland. Electron Microscopy Laboratory, Iowa State University, College of Veterinary Medicine, Ames, Iowa.

[q]J. J. Andrews. Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa. Personal communication, 1983.

and M. hyorhinis must be suspected as important factors in the coughing young pig. These two organisms are consistently present in the nasal cavity of the young pig (4). At the very least, H. parasuis and M. hyorhinis may invade the trachea, bronchi, and lungs of pigs when other agents, such as swine influenza virus or adenovirus, cause primary damage, or possibly at times when the baby pig is stressed. There is strong reason to believe that H. parasuis and M. hyorhinis are contributing to disease in the young pig and are complicating farrowing and nursery production. Respiratory disease due to these organisms may make the baby pig more susceptible to other diseases as well as to other causes of pneumonia (i.e., M. hyopneumoniae or H. pleuropneumoniae).

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CONCLUSION

Management of respiratory disease in swine requires an understanding of the organisms involved and their sequential appearance in the respiratory tract. In a report prepared by Little (92) in 1975, a possible sequence of upper respiratory tract infection by various respiratory tract pathogens was suggested. Within the first two weeks of life the baby pig may become infected with Haemophilus spp., Bordetella bronchiseptica, and Mycoplasma hyorhinis; at six weeks of age Mycoplasma hyopneumoniae may infect the upper respiratory tract; and by eight weeks of age Pasteurella multocida may be present. The data were compiled from nineteen problem and clinically normal herds, and the age groups studied in each herd varied from six weeks to fourteen months.

In the present studies, we examined almost two-hundred pigs from six Iowa herds. Owners of these herds complained of coughing in baby pigs that seemed to be associated with retarded growth. A possible sequence of infection may be proposed from results we obtained by microbiologic examination of the nasal secretions in pigs ½ to 7 weeks of age for certain respiratory tract pathogens. Haemophilus parasuis was most prevalent in pigs sampled by 2 weeks of age, and decreased in incidence as pigs became older; M. hyorhinis was prevalent in pigs 3 to 4 weeks of age, and remained so; and B. bronchiseptica and P. multocida were isolated more commonly from pigs five to eight weeks of age. In almost all cases where pigs were culture positive for B. bronchiseptica

or P. multocida, either H. parasuis, M. hyorhinis, or both had been isolated at prior sample intervals. Fifty-five pigs ranging from 1 to 7 weeks of age, were necropsied and M. hyopneumoniae could not be detected from any level of the respiratory tracts.

M. hyopneumoniae is believed to be the primary agent leading to increased susceptibility of pigs to other pneumonia-causing organisms. The incubation period for M. hyopneumoniae is 2 to 3 weeks (32,41), with clinical evidence of the disease possible in pigs 5 to 8 weeks of age (34,38). Acute clinical signs of disease include a persistent non-productive cough (40), while chronic disease is evidenced by reduced growth rate, decreased feed conversion, and general debility (30,31). Microscopic lesions of M. hyopneumoniae pneumonia include extensive peribronchiolar and perivascular lymphohistiocytic cell accumulations (31).

Coughing pigs were selected from the groups studied, and, as stated previously, M. hyopneumoniae was not detected in their respiratory tracts. The most common lung isolates from these 55 pigs were M. hyorhinis and H. parasuis. Interstitial pneumonia with or without foci of suppurative bronchopneumonia was the most common lung lesion. Of the 31 pigs from which M. hyorhinis was isolated, 20 had peribronchial, peribronchiolar, and, in some cases, perivascular lymphohistiocytic cell accumulations. This cuffing was not as extensive as that involved with M. hyopneumoniae disease; however, the observations suggest that M. hyorhinis plays a larger role in porcine pneumonias than previously suspected. It is possible that certain strains of M. hyorhinis may help

initiate or aggravate M. hyopneumoniae lung infection.

H. parasuis was isolated from the lungs of 20 of the pigs necropsied, and 16 of these also had the organism in their nasal secretions. The high incidence of this organism in nasal secretions, and its prevalence in the lungs of coughing pigs leads us to suspect that H. parasuis may be the first organism to compromise the respiratory tract. Goiš et al. (93) speculated that the organism may cause a mild rhinitis in young pigs, thus predisposing them to secondary infection. Therefore, it is feasible that H. parasuis infection in the lung could predispose the lung to infection by other microbes. Our studies indicate that in both cases M. hyorhinis may subsequently infect the nares and the lung.

Originally, our studies were undertaken in anticipation of a treatment to prevent M. hyopneumoniae disease. We felt that for such a program to be successful, the chronological prevalence and progression of bacteria and mycoplasmas in the respiratory tracts of baby pigs should be determined. It is very important to consider respiratory disease during the pre-weaning stage of production because this is often when exposure first occurs, where early signs may first be noted, and where disease prophylaxis is most economically feasible. Our studies of sneezing and coughing baby pigs indicate that M. hyorhinis and H. parasuis are the first organisms to infect the respiratory tract. It is possible that these organisms could be causing the clinical signs of respiratory disease in these pigs, but by chi-square analyses,

isolation of M. hyorhinis or H. parasuis could not be correlated with rhinitis, tracheitis, or pneumonia. However, M. hyorhinis and H. parasuis may make the baby pig more susceptible to other causes of pneumonia, thus complicating hog production schemes. The likelihood that M. hyorhinis and H. parasuis are a major factor in the pigs' susceptibility to M. hyopneumoniae infection needs to be studied further.

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