Pathogenesis of Pleuropneumonia in Swine Caused by Haemophilus Pleuropneumoniae: Pathologic, Immunofluorescent, and Bacteriologic Studies

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Porcine pleuropneumonia caused by Haemophilus pleuropneumoniae (HP) has been described in Argentina, Brazil, Bulgaria, Denmark, East Germany, England, France, Japan, Mexico, Sweden, Switzerland Taiwan ROC and the United States, and the incidence has shown a very marked increase in recent years. Although extensive work on the bacteriology, serology, and pathology of HP has been described in recent reports, much remains to be explained of the pathogenesis of HP. This paper reports the progressive development of HP and the distribution of the organisms in the tissues and organs of the exposed pigs.

In experiment 1, sixteen eight-week-old crossbred pigs were used. Twelve pigs were inoculated intranasally with 5 ml of an 18 hr broth culture and the remaining 2 pigs received 5 ml of broth without HP organisms. As controls, Haemophilus pleuropneumoniae serotype 5 strain No. TSC-127 isolated from the pneumonia lungs of naturally infected pigs was used. HP pleuropneumoniae was grown in 5% rabbit blood-chocolate agar and the suspected HP colonies were inoculated into chocolate heart infusion broth with 5% rabbit blood. The HP inoculum was an 18-hour culture. The inoculum contained approximately 10^9 CFU/ml.

The animals were observed several times daily. Venous blood was collected from each pig one day prior to being tested at 1, 3, 7, 14 days inoculation (PI) for the bacteriologic evaluation. Two pigs from the inoculated treatment were killed at 5, 1, 3, and 14 days PI for complete bacteriologic examination. Necropsies were performed on all pigs that died or were euthanized. Representative samples were submitted for bacteriologic, immunofluorescent, and bacteriologic examinations. Excised tissues were frozen by immersion in deep freezer, cut at 0.6 mm thickness, dried and fixed in acetone prior to staining with direct immunofluorescent methods. For the conjugate, a specific antiserum to the TSC-127 strain of HP was produced in a specific-pathogen-free pig. The immunofluorescent serum was fractionated by diethylaminoethyl Sephadex A-50 and conjugated with fluorescein isothiocyanate following standard procedures. The isolation technique of HP described by Hsu was employed.

In experiment 2, four feeder pigs which had died of acute HP obtained from an outbreak farm were submitted for pathologic, immunofluorescent and bacteriologic studies using the procedures mentioned above.

In experiment 1, most of the infected pigs developed respiratory distress with severe abdominal retraction, pyrexia, vomiting and hyperthermia, and became reluctant to move starting from 4 to 6 hrs PI. The white blood cell count (WBC) before inoculation was 14,700 to 17,600 /μl. Six hours PI, the WBC had increased to 20,500 to 33,600 /μl, but the values decreased at 24 hrs PI. In addition, there was increase in the percentage of immature neutrophils in the pig with the most leukocytosis. However, the WBC increased again at 3 days PI. The gross lesions were limited in the thoracic cavity and consisted of pneumonia and pleuritis. Of 14 pigs infected with HP organisms, 12 (86%) developed various degrees of hemorhagic-fibrinous pleuropneumonia. The remaining one (pig no. 7) was normal. The pathologic lesions were divided into four phases based on clinicopathologic, acute, subacute, and chronic. Pigs killed at 6 hrs PI or dying at 8-10 hrs PI showed the lesions of sutured pleuropneumonia, where the lungs were sutured with a swollen, firm, dark red in color, with very prominent edema in the interlobular septa. Histologically, there were severe hemorrhage and exudation in the alveoli, fibrinous thrombi, interlobular edema and pleura without much acute neutrophils. Pigs killed or dying at 20-24 hrs PI showed the lesions of acute pleuropneumonia, where several 'limbo' hemorrhagic circumscribed lesions were scattered in the lungs. A thick layer of fibrinoid exudate covered over and loosely adhered to the adjacent pulmonary areas of the lungs. Histologically, the above reactions were observed in the affected lungs; additionally, the large numbers of macrophages and lymphocytes occluded the alveoli, and bronchioles and fibrin thrombi with portal connective tissue were seen in the blood vessels and lymphatics. The subacute form was observed at 7 day PI. The pulmonary lesions were firmer, sharply demarcated and red-brown in color. The cut surfaces showed bands of white fibrous tissue with necrotic center. The pleura was usually adhered to the adjacent pulmonary areas. The chronic form was observed at 14 and 15 days PI. The lesions of pulmonary areas resembled those mentioned in the subacute form.

Specific immunofluorescence was most consistently detected in the affected lungs, pulmonary and hepatic lymph nodes, pleural exudate, and bone marrow, but also frequently in tonsil, blood of heart chambers and spleen. Bacterial isolations were performed from various tissues of pigs sacrificed at different times after infection. The distributions of HP antigens corresponded completely with those by immunofluorescence.

In experiment 2, the symptoms and pathologic lesions of these four pigs were compatible with those in the pigs intranasally inoculated with HP in experiment 1. The distribution of HP organisms in the tissues or organs of pigs with naturally occurring HP was very similar to the patterns found in the experimentally inoculated pigs.

Conclusion
Pigs inoculated intranasally with higher dose of HP organisms showed typical clinical symptoms and lesions of HP. Four phases of clinical response of HP were observed: peracute, acute subacute and chronic. In the peracute phase the pigs died within 6-10 hrs PI, whereas in the acute, subacute and chronic forms, the pigs usually died or necropsied in 1-3, 7 and over 14 days, respectively. The clinical signs and pathologic lesions in each phase were described.

Both immunofluorescent and bacteriologic study demonstrated that lungs and thoracic and nasal exudate consistently had the massive localization of HP organism. This distribution of HP organisms in the tissues or organs of pigs intranasally inoculated with HP was very similar to the patterns found in the field case.