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

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Porcine pleuropneumonia caused by Haemophilus pleuropneumoniae(Hp) has been described in Argentina, Australia, Belgium, Canada, 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 weeks old crossbred pigs were used. Fourteen pigs were inoculated intranasally with 5 ml of an 18 hrs 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 pneumonic lungs of naturally infected pigs was used. H. pleuropneumoniae was grown in 5% rabbit blood-chocolate agar and the suspected Hp colonies were inoculated into chocolate heart brain infusion broth with 5% rabbit blood. The Hp inoculum was an 18-hour growth culture. The inoculum contained approximately 10^9 FCU/ml.

The animals were observed several times daily. Venous blood was collected from each pig one day prior to being tested and at ¼, 1, 3, 7 and 14 days inoculation(PI) for the hematological evaluation. Two pigs from the inoculated treatment were killed at ¼, 1, 3, 7 and 14 days PI for complete pathological examination. Necropsies were performed on all pigs that died or were euthanatized. Representative samples were submitted for histopathological, immunofluorescent and bacteriological examinations. Excised tissues were frozen by immersion in deep freezer, cut at 6 um 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 hyperimmune serum was fractioned by diethylaminoethyl sephadex A-50 and conjugated with fluorescence 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 pathological, immunofluorescent and bacteriologic studies using the procedures mentioned above.

In experiment 1, most of the infected pigs developed respiratory distress with severe abdominal respiration, pyrexia, vomiting and hyperpnea, 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 /mm^3. Six hours PI, the WBC had increased up to 20,500 - 33,600 /mm^3, 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 leucocytosis. However, the WBC increased again at 3 days PI.

The gross lesions were limited in the thoracic cavity and consisted of pneumonia and pleurisy. Of 14 pigs infected with Hp organisms, 13 (92.8%) developed various degrees of hemorrhagic-fibrinous pleuropneumonia. The remaining one (pig no 7) was normal. The pathological lesions were divided into four phases based on clinical courses: peracute, acute, subacute and chronic. Pigs killed at 6 hrs PI or dying at 8-10 hrs PI, showed the lesions of peracute pleuropneumonia, where the lungs were extensively 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 with fibrinous exudate mixed with some neutrophils. Pigs killed or dying 20-24 hrs PI showed the lesions of acute pleuropneumonia, where several firm, hemorrhagic circumscribed lesions were scattered in the lungs. A thick layer of fibrinous exudate covered over and loosely adhered to the adjacent pneumonic areas of the lungs. Histologically, the above reactions were observed in the affected lungs; additionally, great numbers of macrophages and lymphocytes occluded the alveoli, and bronchioles and fibrinous thrombi with inflammatory cells were seen in the blood vessels and lymphatics. The subacute form was observed at 7 day PI. The pneumonic areas were firm, sharply demarcated and reddish-grey in colour. The cut surfaces showed bands of white fibrous tissue with necrotic center. The pleura usually adhered to the adjacent pneumonic areas. The chronic forms were observed at 14 and 16 days PI. The lesions of pneumonic areas resembled those mentioned in the subacute one.

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 sacrified at different times after infection. The districations of Hp antigens corresponded completely with those by immunofluorescence.

In experiment 2, the symptoms and pathological 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 developed 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 pathological lesions in each phase were described.

Both immunofluorescent technigue 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.

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