INACTIVATION OF HAEMOPHILUS PLEUROPNEUMONIAE BY PIG EMBRYO TRACHEAL EXPLANTS

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Pulmonary infections caused by Haemophilus pleuropneumoniae (HP) have become of great economic significance in intensive swine operations. The rapid course and high mortality characteristic of the acute form of the disease suggest that this organism, unlike most bacteria, can act as a primary agent and invade the uncompromised lung. In other bacterial pneumonias of the pig, such as Pasteurellosis, the mucociliary apparatus (comprised of the ciliated tracheobronchial epithelium and mucus) has been shown to have a remarkable capacity for inactivating the organism. This inactivation is due to the activity of a bacteriostatic glycoprotein found in the mucus (Pijoan and Ochoa, 1978, 1980; Iglesias and Pijoan, 1982). This has led to speculation that in healthy animals, the particle size of airborne bacteria has a direct bearing on the establishment of infection. Only droplets of small diameters have the ability to penetrate into the alveolus, bypassing the mucociliary clearing system.

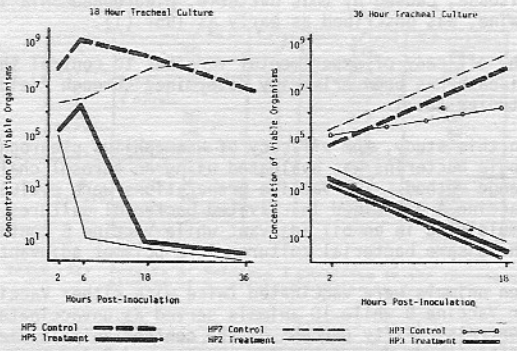
In HP infections, impact on the alveolar wall probably leads to infection, because the organism produces toxins capable of killing the alveolar macrophage (Bendixen et. al. 1981). The alveolar macrophage is the main defense mechanism at this level of the respiratory system. It is not known whether impact in the tracheobronchial system leads to infection, or if the microbiocidal substances of the mucus can control the replication of the organisms. This information has important practical applications on environmental control of pneumonia.

MATERIALS AND METHODS

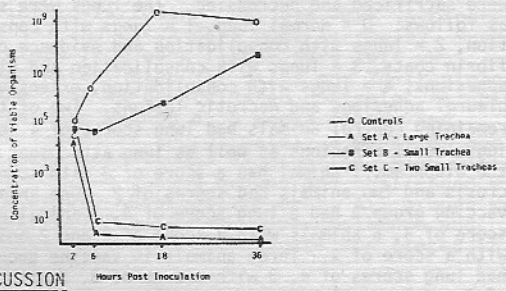
Tracheas were obtained at a slaughterhouse from ± 90 day-old pig embryos and cultured as described by Pijoan and Ochoa (1978). The Eagle's MEM culture medium was modified by the addition of 100 µg/ml NAD to promote the growth of HP. Experiment 1: Twenty-one tubes containing one tracheal ring each, were infected with a single serotype of HP. Serotypes 2, 3 and 5 were tested. At the same time, tubes containing media without tracheas were inoculated as controls. Bacterial inoculation was performed at 18 and 36 hrs. after initiation of tracheal culture. At 2, 6, 18 and 36 hours postinoculation (PI), the supernatants of four tubes were pooled, and viable counts performed by the pour-plate method. The controls were counted at the same times. Inoculated and non-inoculated tracheas were collected and formalin-fixed, and histopathological studies performed. Experiment 2: This was similar to Experiment 1, but tracheas were obtained from large (± 95 days) and small (± 73 days) embryos, to see if there was an age difference in the inactivation of bacteria. Three sets of tubes were infected with HP-2. Set A had large embryo tracheas; set B had small embryo tracheas and set C had two small-embryo tracheas per tube. Viable counts and histopathology were made as detailed above, but only after 18 hrs. of tracheal culture.

RESULTS

Experiment 1: Fig. 1 shows the viable bacterial numbers in the presence of tracheas (treated) or in the same medium without tracheas (control). It can be seen that a sharp decline of viable numbers occurred in the treated bacteria, especially after 5 hrs. PI. The same results were obtained if bacterial infection was performed after cultivation of tracheas for 30 hrs. The histopathology did not show important differences between infected and non-infected tracheas which all had some degree of epithelial desquamation and erosion. These changes were most pronounced in the epithelial crypts.



Experiment 2: The results are shown on Fig. 2. Large tracheas (set A) had a more pronounced effect than small (set B). However, when 2 small tracheas were put in the same tube (set C), the effect was similar to the large.



DISCUSSION

A pronounced inactivating effect on the bacteria could be demonstrated. The effect was noticeable after 5 hrs., but not 2 hrs. PI. This suggests that bacterial replication is necessary, which points to a replication-dependent bactericidal activity. This is in agreement with the findings in similar work with P. multocida (Iglesias and Pijoan, 1982). All ages of embryos tested had this bacteriostatic capability, but a minimum amount of tissue is necessary to inactivate the organisms in the amount of culture media used (1.5 ml).

Similar to other respiratory infections of the pig, the tracheobronchial system seems able to cope with HP infections in the uncompromised animal. Therefore, colonization of the lung can occur only if: a) a previous infection (probably viral) or some immunosuppressive event prevents the secretion of the bacteriostatic substance. b) if the airborne microorganisms are contained in small droplet sizes, therefore being able to bypass the system and colonizing the alveolus.

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