Experimental reproduction of acute pneumonic pasteurellosis in rabbits

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Summary. A histomorphometric and physiopathological study was made of the lung parenchyma of Belgian White SPF rabbits infected experimentally with Pasteurella multocida type A.

Symptoms observed were characteristic of the acute respiratory syndrome. Mean serum cortisol concentration and rectal temperature increased in all experimental groups.

Histopathological changes included alveolitis and leukocytic bronchitis. Changes in alveolar and bronchial cytoarchitecture were attributed to the degeneration and necrosis of constituent epithelial cells.

Key words: P. multocida, Lung, Structure, Pathophysiology, Ultrastructure, Rabbit

Materials and methods

Animals

30 Belgian White SPF rabbits, of about 1 kg body weight were used. 25 animals were inoculated by intratracheal application 0.5 x 3 x 10^8 bacterial/ml (P. multocida A.). The remaining 5 animals served as control. Animals were killed by exanguination under ether anaesthesia at 12, 24, 48, 72, 120 h.p.i.

Clinical Examination

The following clinical features were recorded: rectal temperature; respiratory rate; presence and nature of the cough; ocular and nasal discharges; auscultation; and behaviour.

Serum cortisol levels

Prior to sacrifice, blood samples from control and experimental animals were taken for serum cortisol determinations using a radioimmunoassay technique (Slocombe et al., 1984).

Light microscopic findings

2 cm² sections from all lobes of the lungs were collected and fixed with 10% formaldehyde and 5% glutaraldehyde in phosphate buffer.

The samples were stained with H-E, PAS and Toluidine blue.

Morphometrical examination

The morphometric findings were realised with a Semiautomatic Image Analyser (Olivetti M-24), loaded with «General area» Software. The parameters studied can be seen in Table 1.

Statistical comparison of the morphometric parameters was made using Student’s t-test.
Pneumonic pasteurellosis in rabbits

**Ultrastructural examination**

Small sections of all the lobes were fixed with 5% glutaraldehyde in phosphate buffer, and embedded in Epon-Araldite. Sections of 0.4 μm were cut from the blocks obtained and counterstained with lead citrate and uranyl acetate.

**Results**

**Clinical Observations**

At 12 and 24 hours p.i., rectal temperature in experimental animals had increased (Table 2) and dyspnoea, tachycardia and serous nasal discharge were observed.

At 48 hours p.i., a mucopurulent nasal discharge with intense dyspnoea, increased bronchial rales, and vesicular murmurs were present.

By 72 and 120 hours p.i., clinical signs were more severe, with intense bronchial rales and vesicular murmurs, especially in cranial parts of the lung. Progressive emaciation was evident in the final stages.

**Serum cortisol levels**

Mean serum cortisol concentration increased in all experimental groups, reaching maximum levels of 27.2 ng/ml at 24 h.p.i. (Table 3).

**Table 1. Mean (X±SE) of temperature at various times after inoculation.**

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL RABBITS</th>
<th>EXPERIMENTAL RABBITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h.p.i.</td>
<td>42±0.2</td>
<td>26.4±1.3</td>
</tr>
<tr>
<td>24 h.p.i.</td>
<td>42±0.3</td>
<td>37.2±1.8</td>
</tr>
<tr>
<td>48 h.p.i.</td>
<td>40.5±0.5</td>
<td>13.2±2.3</td>
</tr>
<tr>
<td>72 h.p.i.</td>
<td>39.5±0.2</td>
<td>12.1±3.2</td>
</tr>
<tr>
<td>120 h.p.i.</td>
<td>39.0±0.2</td>
<td>9.7±0.5</td>
</tr>
</tbody>
</table>

**Table 2. Mean (X±SE) serum cortisol levels at various times after inoculation.**

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL RABBITS</th>
<th>EXPERIMENTAL RABBITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h.p.i.</td>
<td>2.32±0.03</td>
<td>24 h.p.i.</td>
</tr>
<tr>
<td>48 h.p.i.</td>
<td>9.1±0.5</td>
<td>72 h.p.i.</td>
</tr>
<tr>
<td>120 h.p.i.</td>
<td>9.7±0.5</td>
<td></td>
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</tbody>
</table>

Table 3. Morphometrical findings.

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL 12 h.p.i.</th>
<th>24 h.p.i.</th>
<th>48 h.p.i.</th>
<th>72 h.p.i.</th>
<th>120 h.p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar area</td>
<td>692±458</td>
<td>3730±2560</td>
<td>3322±2560</td>
<td>1806±1363</td>
<td>1439±507</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Bronchial area</td>
<td>8225±2587</td>
<td>7792±2587</td>
<td>8203±2901</td>
<td>9234±2901</td>
<td>7871±1807</td>
</tr>
<tr>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Pneumocyte II area</td>
<td>46±9</td>
<td>88±19</td>
<td>82±19</td>
<td>92±17</td>
<td>147±14</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Number of measurements= 100.

**Pneumonic (Pn) and Haemorrhagic (H) areas**

*P. multocida* infection in rabbits

![Fig. 1. Pneumonic (Pn) and Haemorrhagic (H) areas of pulmonary lobes in *P. multocida* infection in the rabbit (AI-Right apical lobe, CRI-Right cranial lobe, CAI-Right caudal lobe, AD-Left apical lobe, CRD-Left cranial lobe, M-Medium lobe, CAD-Left caudal lobe).](chart)
**Pneumonic pasteurellosis in rabbits**

Gross Pathology

The Gross Pathology findings are presented in Fig. 1. Animals killed at 12 and 24 hours postinoculation

Histopathology

The bronchioles, alveolar ducts and alveoli of animals sacrificed 12 h.p.i. with *Pasteurella multocida type A* contained a mixed inflammatory cell exudate with many erythrocytes, degenerating neutrophils and bacteria. Signs of cell degeneration were evident in alveolar, bronchial and bronchiolar epithelium, due to the accumulation of bacteria which occasionally gave rise to necrosis.

Interalveolar spaces were widened. The pulmonary vessels were congested. Dilatation of lymphatic capillaries containing proteinaceous material, edema, infiltration by neutrophils, although some eosinophilic granulocytes were observed.

The pulmonary lesions of animals sacrificed 24 h.p.i. were similar to those observed at 12 h.p.i., except that they were more numerous and more extensive (Fig. 2).

Ultrastructural findings

No noteworthy alterations were found in pneumocytes I.

The laminar bodies of pneumocytes II were observed with variable morphology and perinuclear distribution. They were bordered by thick membrane and contained osmiophilic structures.

The cytoplasmic vacuolar system of alveolar macrophages was well developed.

Alveolar, bronchial and bronchiolar structures

![Image](2)

![Image](3)

![Image](4)

**Fig. 2.** Lung (24 h. p.i.). Bacterial action on alveolar epithelium (arrow). Degenerated neutrophils and necrotic debris. H-E. x 280

**Fig. 3.** Lung (24 h. p.i.). Lumen of alveoli containing aggregates of *Pasteurella multocida A* (arrows). x 18,000

**Fig. 4.** Lung (24 h. p.i.). Eosinophil granulocytes in interalveolar septum. x 11,500
Pneumonic pasteurellosis in rabbits

showed intense edema, accompanied by inflammatory exudation consisting of a small number of erythrocytes, neutrophils, eosinophils and aggregates of bacteria (Fig. 3).

Intense edema and polymorphonuclear granulocytes were observed in the interalveolar septa (Fig. 4).

Animals killed at 48 hours post inoculation

Histopathology

Alveolitis and fibrino-leukocyte bronchitis could be observed. Necrosis of alveolar, bronchial and bronchiolar epithelia, lumina full of degenerated neutrophils (Fig. 5), and aggregates of bacteria and cell debris were detected. Edema and marginated inflammatory cells were detected in the lumen of interalveolar arterioles. The tunica media of some arterioles contained fibrinoid necrosis (Fig. 6).

Ultrastructural findings

Pneumocytes I were similar in appearance to those of controls. The endoplasmic reticulum cisternae of pneumocytes II were dilated, multivesicular structures of varying morphology were observed. The laminar bodies had increased in number and were of variable shape (Fig. 7).

Pneumocytes contained abundant lysosomal material and isolated waste products. Alterations were particularly evident in the capillary endothelium of the blood-air barrier, giving rise to intense cell and plasma exudation into interalveolar septa.
Pneumonic pasteurellosis in rabbits

Animals killed at 72 and 120 hours postinoculations

Histopathology

The process observed in animals killed at 72-120 h.p.i. may be defined as alveolitis and suppurative-bronchitis, extending to the interalveolar septa, which contained degenerated inflammatory masses, haemorrhages (Fig. 8) and homogeneous proteinaceous material (Fig. 9).

The lumen of bronchi contained many neutrophils (Fig. 10). The alveolar walls were greatly thickened and contained mononuclear and neutrophil cells within the interstitial tissue.

Evidence was also found of alveolar fibrosis extending towards interalveolar septa (Fig. 11). In visceral pleurae, pleurisy was accompanied by intense cell infiltration.

Ultrastructural findings

Structures similar to multivesicular bodies in cellular extension of pneumocytes I were evident. Pseudopods containing small vacuoles were evident in cell membrane of pneumocytes II. The cytoplasm contained numerous free ribosomes, cisternae of the endoplasmic reticulum were dilated, and mitochondria were swollen with dense matrix. Laminar bodies contained osmiophilic structures and extensions of external membrane which circumscribed portions of cytoplasm (Fig. 12).

Neutrophil and mononuclear cells, with signs of phagocytosis, were detected in the interalveolar space (Figs. 13, 14).
Macrophages with waste products of cell digestion were observed in alveolar lumina.
The alveolar epithelium showed cell degeneration, with intense descamation into the lumen (Fig. 15).

Morphometrical and statistical findings

The results are shown in Table 3.

Discussion

The histopathological changes observed in lung of rabbits experimentally infected with Pasteurella multocida A were similar to those in naturally occurring disease; however the experimental lesion was more severe, presumably because of number of organisms (Flatt and Dungworth, 1971a,b).
The response may be classified as a fibrino-exudative pneumonia developing fundamentally in dorsal and declive areas of pulmonary lobes, and accompanied by cellular bronchitis and pleurisy (Flatt and Dungworth, 1971a,b; Panciera and Corstret, 1984). The location of lesions in these areas may be due to these being the areas of greatest ventilation in the lung (Martel and Michel, 1985; Parodi and Labarre, 1985).
The Gram-negative organism releases peptides that are similar to complement and which may attract neutrophil without intervention of the complement system of macrophages (Wark et al., 1968).

Bacterial endotoxin released by P. multocida exerts a
chemotactic effect, favouring the migration of neutrophils to alveolar and bronchial lumina, demonstrating the importance of these cells in the pathogenesis of acute pneumonic pasteurellosis in the rabbit. Neutrophils were required because their selective phagocytosis of bacteria led to cell disgranulation and the release of endogenous pyrogens with the subsequent increase in temperature (Slocombe et al., 1984, 1985).

The initial response of *P. multocida*, was probably related to immunosuppression by corticosteroids triggered off by stress. Increased concentrations of glucocorticoids inhibit the immune response of animals by diminishing antibody production, diminishing lymphocyte blastogenesis, altering granulocyte and monocyte concentrations and functions and by inhibiting phagocytosis (Parrillo and Fauci, 1979; Roth and Kaeberle, 1982; Roth and Kaeberle, 1982; Suarez-Güemes et al., 1986).

The toxic effects of bacterial endotoxin give rise to degeneration and disgranulation of neutrophils and subsequently the chemostatic action of mononuclear cells and phagocytes (Wark et al., 1968; Markham and Wilkie, 1980). As the pneumonia progressed and resolution occurred, the macrophages and mononuclear cells become the predominant cell types (Gilha et al., 1974).

From 72 hours p.i., onwards, mononuclear phagocytes were detected, with bacterial phagocytosis and abundant cellular debris. Some highly-degenerated macrophages were observed, which were attributed to the presence of toxins lethal to alveolar macrophages (Markham and Wilkie, 1980).

The pulmonary lesion in *P. multocida* infection in the rabbit was consistent with the changes in morphometrical parameters.

Endotoxin may be the factor responsible for pulmonary edema and cellular exudate formation, since its administration causes an initial pulmonary edema due to pulmonary hypertension and a second phase related to leukocyte invasion of the lung, leading to increased vascular permeability (Helfin and Bringhim, 1981; Issekutz et al., 1983; Morrison and Uleritch, 1987; Redondo et al., 1987).

Pulmonary vessel occlusion by hyperemia and fibrin deposits gave rise to hypoxia and consequently necrosis of alveolar and bronchiolar epithelium (Morrison and Uleritch, 1987).

Lesions to the alveolar epithelium were not uniform particularly in type II alveolar epithelial cells. This is thought to be related to the reactive hypertrophy of these cells (Gilmour et al., 1982; Slocombe et al., 1984).

Pneumocyte type II cytoplasm contained remarkably well-developed endoplasmic reticulum, which is felt to be related to the production of phospholipids for cell surfactant. The presence of mitochondria in the vicinity of these cisternae would provide the energy necessary for synthesis (Pastor et al., 1985).

**References**


Pneumonic pasteurellosis in rabbits


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