

CHANGES IN THE LUNGS OF GOATS WITH ACUTE PNEUMONIA FOLLOWING EXPERIMENTAL CHALLENGE WITH *PASTEURELLA HAEMOLYTICA* AND *PASTEURELLA MULTOCIDA*

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SUMMARY

Forty goats, approximately 7 months old, were divided into 4 groups of 10 animals each. Those in group 1 were challenged intratracheally with 4 mL inoculum containing 1.0×10^8 cfu/mL of *Pasteurella haemolytica* A2 isolated from pneumonic lungs of a goat. Goats in group 2 were similarly challenged with *P. haemolytica* A2 isolated from the nasal cavity of a healthy goat and goats in groups 3 and 4 were challenged with *P. multocida* types A and D isolated from pneumonic lungs respectively. Two goats from group 1, four from group 3 and five from group 4 died within 8 h of challenge. Gross changes included pulmonary oedema, petechiations and patches of acute pneumonia while histological changes included sequestration of neutrophilic leucocytes in capillaries, thrombosis and endothelial degeneration. These changes were typical of those caused by endotoxin on the endothelium of pulmonary blood vessels. *P. haemolytica* was not isolated from blood whereas *P. multocida* was readily observed in and isolated from blood. It is concluded that acute lesions in both *P. haemolytica* and *P. multocida* infections of goats involved endotoxin and developed in similar manner. *P. haemolytica* infection, however, resulted in toxæmia rather than the septicaemia as in *P. multocida* infections.

Keywords: Lung pathology, acute pneumonia, *Pasteurella haemolytica*, *Pasteurella multocida*, goats

INTRODUCTION

Pasteurella haemolytica has been recognised as an important pathogen in small ruminants leading to either pneumonic or systemic pasteurellosis (Gilmour, 1993). *P. haemolytica* type A has often been associated with pneumonic lesions in lambs while *P. multocida* types A and D have always been associated with systemic diseases in ruminants (Schiefer *et al.*, 1978; Links *et al.*, 1993). However, *P. multocida* types A and D have been shown to be able to produce acute and chronic pneumonic lesions in goats either experimentally (Zamri-Saad *et al.*, 1996) or naturally (Loganathan and Chandrasekaran, 1992). This paper compares the changes in the lungs of goats, which developed acute pneumonia following intratracheal challenge with either *P. haemolytica* type A, *P. multocida* type A or *P. multocida* type D.

MATERIALS AND METHODS

Animals

Forty clinically healthy goats about 7 months old were divided into four groups of 10 animals each. Nasal swabs were taken immediately after selection and every three days for at least two weeks to ensure that the goats were free of *P. haemolytica* and *P. multocida*. Each group was kept in a separate room and fed cut grass and supplemented feed at the rate of 0.5 kg/goat/day. The challenge trial was started when

all goats were free of either *P. haemolytica* or *P. multocida* from their nasal cavity for at least two consecutive weeks.

Inoculum

Two isolates of *P. haemolytica* A2 were used. The first was from pneumonic lungs of a goat and the second from the nasal mucosa of a healthy goat. Both *P. multocida* types A and D were isolated from pneumonic lungs of goats. The isolates were cultured on blood agar and incubated at 37°C overnight. Thirty same-sized colonies were selected and inoculated into 50 mL brain-heart infusion broth (BHI) and incubated at 37°C for 18 h. The colony-forming units (cfu) were estimated using the standard total plate count method before they were diluted with sterile phosphate buffered saline (PBS) to give an end concentration of approximately 1.0×10^8 cfu/mL.

Experimental design

All goats of group 1 were challenged intratracheally with 4 mL inoculum containing *P. haemolytica* A2 isolated from pneumonic lungs while those of group 2 were challenged similarly with *P. haemolytica* A2 isolated from healthy nasal mucosa. Goats of groups 3 and 4 were challenged with *P. multocida* types A and D respectively. The goats were observed hourly for clinical signs of acute respiratory infection, particularly high body temperature ($>40^\circ\text{C}$), nasal discharge, dyspnoea, recumbency and death. Goats that died

within 24 hours were considered to have died of acute infection and were examined immediately for gross lung lesions before the entire right apical lobes were collected for bacterial, histopathological and transmission electron microscopic examinations. Lung and heart blood swab samples were also taken for bacteriological isolation of *P. haemolytica* and *P. multocida*.

RESULTS

Clinical observations

Two (20%) goats from group 1, four (40%) from group 3 and five (50%) from group 4 died within 8 hours post-challenge. They were found either on sternal or lateral recumbency with body temperatures exceeding 41°C, showed labored mouth breathing with small amounts of serous nasal discharge, moderate hypersalivation and congested mucous membranes. None of the goats of group 2 died, but remained healthy throughout the study period while the remaining goats that survived showed slight to moderate nasal discharge without increased body temperature between 4 to 10 days post-challenge.

Gross pathology

The entire musculature and all other internal organs, particularly the lungs, liver, spleen and kidneys of the 11 goats that died acutely were severely congested. The thoracic cavities were filled with excessive amounts of straw-coloured fluid, ranging between 500 to 800 mL. The posterior two-thirds of the tracheas were filled with the same type of fluid while the lungs were heavy and glistening. There were numerous patches of depressed dark red discolouration ranging from 1.0 to 3.5 cm diameter on the lung surface, particularly on the anterior lobes of both left and right lungs. Numerous petechiations were observed throughout the lung surface.

Histopathology

Marked thickening of the interstitium with prominent and congested capillaries made the interstitium appeared red. Oedema fluid was found in both interstitium and most alveolar spaces with few red blood cells in the alveolar spaces. An on-going acute inflammatory reaction was evident from the presence of margined neutrophilic leucocytes in the congested blood vessels of the lungs and some neutrophils in the alveolar spaces. Some pneumocytes were swollen with cytoplasmic vacuolation while numerous bacterial cells were found in the alveolar spaces among the neutrophils (Fig. 1). There were hardly any bacterial

cells within the cell cytoplasm of either neutrophils or pneumocytes.

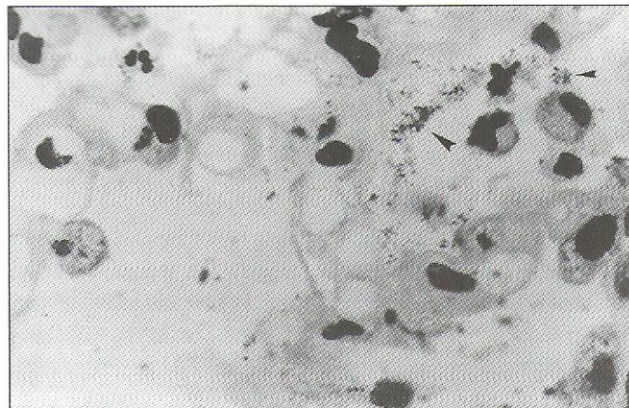


Fig. 1. Lung section of a goat that died acutely following challenge with *P. haemolytica* A2 showing swollen pneumocytes with cytoplasmic vacuolation and numerous bacterial cells (arrowheads) in the alveolar space among the neutrophils. Giemsa x1,000

Ultrastructural observations

Ultrastructurally, there was evidence of disrupted surfactant covering the alveolar wall following challenge by both *Pasteurella* spp. while some bacteria were observed to attach themselves to the wall of the alveoli. The entire alveolar spaces were filled with protein-rich oedema fluid and some alveoli contained red blood cells. All capillaries in the interstitium were severely congested and filled with red blood cells, while the major blood vessels of the lungs had numerous thrombi (Fig. 2). The endothelial membranes of lung capillaries showed degenerative changes. Bacterial cells of *P. multocida* were readily observed in the lumens of pulmonary blood vessels of goats challenged with either *P. multocida* type A or D (Fig. 3).

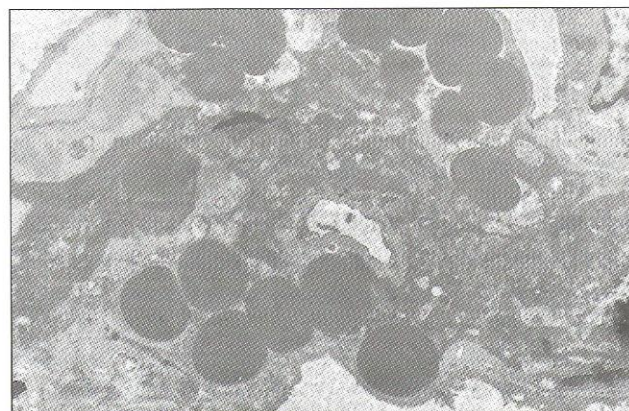


Fig. 2. Lung capillary of goat that died acutely following *P. haemolytica* A2 infection. The capillary is congested with thrombus. TEM x2,700



Fig. 3. Lung capillary of a goat that died acutely following infection with *P. multocida* type D. The organisms are readily observed in the lumen of the capillary (arrowheads). TEM x2,750

Bacteria isolation

P. haemolytica A2 and *P. multocida* A and D were re-isolated from all lung samples. However, only *P. multocida* types A and D were readily isolated from the heart swab samples. Neither of the two goats challenged with *P. haemolytica* A2 had the organism in the heart blood swab samples.

DISCUSSION

Inoculation of goats with either *P. haemolytica* or *P. multocida* isolated from lungs of goats with typical pneumonic pasteurellosis resulted in some acute deaths within 8 hours post-challenge. Similar acute infections have been reported in sheep infected with *P. haemolytica* biotype T as well as in goats infected with *P. multocida* types A, B and D (Gilmour *et al.*, 1991; Loganathan and Chandrasekaran, 1992; Zamri-Saad *et al.*, 1996). In the present case, *P. multocida* produced more acute deaths than *P. haemolytica* A2, a finding in agreement with a similar study that compared infections by *P. multocida* type B and *P. haemolytica* A2 (Loganathan and Chandrasekaran, 1992).

Acute infections by both *P. haemolytica* and *P. multocida* has been associated with their ability to produce endotoxin (Erler and Schimmel, 1993; Cutlip *et al.*, 1998). Endotoxin of *P. haemolytica* and *P. multocida* has been associated with changes in blood vessels (Erler and Schimmel, 1993; Scholes and Kelly, 1997), and all lung lesions observed in the present study followed changes in blood vessels. Even though the lung lesions in both *P. haemolytica* and *P. multocida* infections seemed to develop in similar manner, *P. haemolytica* did not have the ability to cross the barrier into blood vessels compared to *P.*

multocida, which was readily available in the blood. Thus, acute infection by *P. haemolytica* involves toxemia compared to the septicaemic nature of *P. multocida* infections.

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RINGKASAN

PERUBAHAN DALAM PEPARU KAMBING MENGIDAP PNEUMONIA AKUT BERIKUTAN CABARAN UJIKAJI DENGAN PASTEURELLA HAEMOLYTICA DAN PASTEURELLA MULTOCIDA

Empat puluh ekor kambing, berumur lebih kurang 7 bulan, dibahagikan kepada 4 kumpulan mengandungi 10 ekor setiap satu. Kambing kumpulan 1 dicabar secara intratrakea dengan 4 mL inokulum mengandungi 1.0×10^8 cfu/mL Pasteurella haemolytica A2 yang dipencil daripada paru-paru pneumonia seekor kambing. Kambing kumpulan 2 dicabar seumpama juga dengan P. haemolytica A2 yang dipencil daripada rongga nasum seekor kambing sihat dan kambing kumpulan 3 dan 4 masing-masing dicabar dengan P. multocida tip A dan D dipencil daripada paru-paru pneumonia. Perubahan kasar termasuk edema pulmonari, pempetekiaan dan tompokan pneumonia akut, sambil perubahan histologi pula termasuk pengsekuesteran leukosit dalam kapilari, trombosis dan penyahjanaan endotelium. Ini adalah tipikal untuk perubahan disebabkan oleh endotoksin pada endotelium vesel darah pulmonari. Kesimpulan yang diambil ialah, lesi akut untuk jangkitan P. haemolytica dan P. multocida pada kambing melibatkan endotoksin dan kedua-duanya berkembang dengan cara yang sama. Jangkitan P. haemolytica, bagaimanapun, mengakibatkan toksemia dan bukan septisemia seperti dalam jangkitan P. multocida.