Veterinary Research Communications, 24 (2000) 153–167

Ultrastructural Observation of Nasal and Pulmonary Intracellular *Pasteurella multocida* A:3 in Rabbits

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Al-Haddawi, M.H., Jasni, S., Zamri-Saad, M., Mutalib, A.R., Son, R. and Sheikh-Omar, A.R., 2000. Ultrastructural observation of nasal and pulmonary intracellular *Pasteurella multocida* A:3 in rabbits. *Veterinary Research Communications*, **24(3)**, 153–167

ABSTRACT

Sixteen 8- to 9-week-old *Pasteurella multocida*-free rabbits were divided into two equal groups. Eight rabbits in one group were inoculated intranasally with *P. multocida* type A:3. The other eight were inoculated intranasally with phosphate-buffered saline and used as controls. Nasal swabs taken before and after inoculation were cultured for bacterial isolation. Post-mortem nasal swabs and lung samples were cultured for bacteriological isolation. Nasal mucosa and lung samples were collected and processed for transmission electron microscopy. *Pasteurella multocida* was isolated from the nasal cavity of all infected rabbits and from the lungs of four infected rabbits. Degenerative ultrastructural changes in epithelial cells and endothelial cells were seen in the infected rabbits. Deciliation of the cilated epithelium and hyperplasia of the goblet cells in the nasal mucosa were noted. Thickening of the alveolar septa due to hyperplasia of type II pneumocytes, swelling of the endothelial lining of capillaries and infiltration of inflammatory cells were also observed. Intracellular invasion of the nasal epithelial cells and of type II pneumocytes by the organism was observed. Coccobacilli were observed in membrane-bound vacuoles in the cytoplasm of these cells. The vacuoles were adjacent to the host-cell mitochondria and some of these vacuoles appeared to be fused to the mitochondrial membrane. Some type I pneumocytes with intracellular membrane-bound vacuoles containing bacterial cells showed protrusions, which appeared to detach into the alveolar lumina. These results indicated that *P. multocida* serotype A:3 in rabbits can invade the epithelial cell and cause structural changes in the interstitium, epithelium and endothelium. Heterophils and macrophages appear to play important roles in tissue injury.

Keywords: alveolar septa, endothelium, epithelium, lung, *Pasteurella multocida*, pneumocytes, rabbits, ultrastructure

Abbreviations: AM, alveolar macrophages; BHI, brain–heart infusion; cfu, colony-forming units; F, fibrin; H, heterophil; LPS, lipopolysaccharide; NP, necrotic pneumocytes; PBS, phosphate-buffered saline; PIM, pulmonary intravascular macrophage; PMN, polymorphonuclear leukocyte; rER, rough endoplasmic reticulum; TEM, transmission electron microscopy

INTRODUCTION

Respiratory disease is a major cause of morbidity and mortality in rabbits (Deeb, 1997). Upper respiratory tract infections (rhinitis and sinusitis) are recognized as the most frequent clinical manifestations, often leading to pneumonia and septicaemia

(Rush *et al.*, 1981). *Pasteurella multocida* is the most important bacterial pathogen causing pasteurellosis in laboratory and commercial breeders' rabbits (Manning, 1982). *P. multocida* infections are often subclinical. Use of rabbits for studies involving the respiratory system may be compromised because of the predilection of this bacterium for the respiratory organs (Lu and Pakes, 1981). Exposure to the causative organism, *P. multocida*, occurs by way of the upper respiratory tract, and the infection then spreads to other organs (Campbell, 1983). It is not known whether *P. multocida* is actually able to invade epithelial cells in rabbits or whether invasion of deep tissues is a result of pulmonary damage or dissemination by phagocytes. Despite the high incidence of pasteurellosis, particularly in the respiratory system (DiGiacomo *et al.*, 1983) and a recent ultrastructural study of upper respiratory infection with this microorganism (Al-Haddawi *et al.*, 1997), only limited ultrastructural studies on the lungs of rabbits in response to *P. multocida* infection have been reported.

The objective of this study was to associate the ultrastructural changes in the lung and nasal mucosa of rabbits exposed intranasally to *P. multocida* type A:3 and to demonstrate colonization and invasion by *P. multocida* in rabbits.

MATERIALS AND METHODS

Animals

Sixteen 8- to 9-week-old New Zealand White rabbits, of both sexes, were obtained from the Animal Resource Center, Universiti Putra Malaysia. Nasal swabs from these rabbits were negative for *P. multocida* on three occasions during 14 days before the experiment began. The rabbits were housed individually in stainless-steel cages in separate animal care facilities and provided with water and rabbit pellets *ad libitum*.

Pasteurella multocida inoculum

The *P. multocida* serotype A:3 used in this experiment was isolated from a naturally infected rabbit that had signs of mucopurulent rhinitis. The isolate was maintained on a nutrient agar slant (Oxoid) at 25°C and was subcultured onto 5% horse blood agar before a single colony was grown in brain–heart infusion broth (BHI) (Oxoid) at 37°C for 18 h. A mouse was inoculated intraperitoneally with 0.1 ml of the cultured broth and the microorganism was re-isolated from the heart blood of the mouse after 24 h. The isolate was identified as type A by the staphylococcal hyaluronidase decapsulation test (Carter and Rundell, 1975) and somatic serotyping was conducted by Dr Thula Wijewardena at the Veterinary Research Institute, Sri Lanka. The bacteria were quantified by diluting a 1.0 ml sample of the broth (BHI growth) culture tenfold in sterile phosphate-buffered saline (PBS), pH 7.4. Each dilution was streaked onto 5% horse blood agar and the colonies were counted after incubation for 24 h at 37°C. Samples for inoculation were diluted in PBS to 1.75×10^8 colony-forming units per ml (cfu/ml).

Detection of fimbriae

The presence of fimbriae in the *P. multocida* isolate used was observed in negatively stained bacteria under a transmission electron microscope (Joel 6400, Tokyo, Japan) as described by Trigo and Pijoan (1988).

Experimental procedure

The 16 rabbits which were free of *P. multocida*, were divided into two equal groups. The rabbits in group 1 were inoculated intranasally with 1.75×10^8 cells of *P. multocida* in 1 ml of PBS by inserting a syringe, without a needle, intranasally and gently expressing the inoculum to avoid any injury to the nasal mucosa. The control rabbits in group 2 were similarly inoculated intranasally with 1 ml PBS, pH 7.4. On days 14 and 21 post inoculation (pi), four rabbits from each group were killed by severing the jugular vein following anaesthesia with 0.5 ml of Ketavet (ketamine hydrochloride 100 mg/ml; Delta Vet Laboratories Pty Ltd, Australia) and 0.2 ml Romazine (xylazine 20 mg/ml; Jurox Pty Ltd, Australia), given intramuscularly.

Bacteriology

Nasal swabs were collected for bacterial isolation at 3-day intervals throughout the experiment. Swabs from the nasal mucosa and samples from the lungs were collected for bacterial isolation at post-mortem. Nasal swabs were collected from live rabbits by inserting small sterile swabs about 3–4 cm deep into both sides of the nasal cavity. At post-mortem, swabs from the nasal mucosa and lung samples were collected from the dead rabbits. Each swab or sample was immediately cultured onto blood agar containing 5% horse blood and MacConkey agar (Oxoid) and incubated at 37°C for 24 h. The indentification of *P. multocida* was based on the criteria described by Carter (1990).

Pathology

The rabbits were examined for gross and histological lesions. Samples from three portions of nasal mucosa (both sides) and from all the lobes of lungs of all the rabbits in groups 1 and 2 were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm, stained with haematoxylin–eosin and examined for microscopic lesions. Samples from the same locations in the nasal mucosa from six rabbits and from all the lobes of lungs from four rabbits in group 1, which had all yielded heavy to pure growth of *P. multocida*, were processed for transmission electron microscopy (TEM). Samples from four rabbits in group 2 were also collected and processed for TEM. The nasal mucosal samples were fixed for 8 h with 2.5% glutaraldehyde and the samples from the lungs were fixed for 8 h with 4%

glutaraldehyde in 0.1 mol/L sodium cacodylate buffer. The samples were post-fixed for 1 h with 1% osmium tetroxide. Fixed samples were rinsed, dehydrated in a graded acetone series and embedded in resin (Agar Scientific, UK). Ultrathin sections were cut on an Ultracut E (Reichert-Jung, Vienna, Austria) microtome and mounted on 200-mesh-copper grids. The sections were stained with uranyl acetate and lead citrate and examined using a transmission electron microscope H7100 (Hitachi, Tokyo, Japan).

RESULTS

Detection of fimbriae

Negative staining showed that fimbriae were present in the isolate of *P. multocida* used.

Bacterial isolation

P. multocida was isolated from the nasal cavity of all the rabbits from group 1 that were killed 14 and 21 days pi and from the lungs of two rabbits in group 1 killed 14 days pi and two rabbits killed 21 days pi. Heavy or pure growth was obtained from the nasal swabs of six rabbits killed 14 and 21 days pi (three rabbits each) and from all *P. multocida*-positive lungs of rabbits in group 1. In cases of heavy growth, other bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* were isolated as well as *P. multocida*. *P. multocida* was not isolated from the nasal swabs or lung samples from rabbits from group 2 killed 14 and 21 days pi.

Pathology and histopathology

Nasal mucosa: The gross findings in the rabbits of group 1 killed 14 days pi were congestion of the nasal mucosa with mucopurulent exudate in the nasal cavity of one rabbit and catarrhal exudate mixed with yellowish threads of pus in three cases. There was a catarrhal exudate on the nasal mucosa of all the rabbits killed 21 days pi.

Histologically, the lesions in the rabbits of group 1 killed 14 days pi were chronic suppurative rhinitis in one rabbit and mild rhinitis in three rabbits. Chronic catarrhal rhinitis was observed in the nasal mucosa of three rabbits in group 1 killed 21 days pi. The fourth rabbit had ulceration of the mucosal surface of the nasal mucosa and infiltration of mononuclear cells, predominantly macrophages and a few lymphocytes and plasma cells, in the *lamina propria*. In addition, there was proliferation of fibroblasts and production of collagen in the ulcerated area.

There were no significant lesions in the nasal mucosa of the animals in group 2.

Lung: The lungs of two rabbits killed 14 days pi exhibited lobular consolidation in the left and right cardiac lobes in one individual and the presence of a small depressed grey area on the surface of the right apical lobe in a second. In one rabbit killed on day 21

pi, the entire left apical lobe was consolidated and there was a yellowish firm nodule on its surface. There were also small consolidated areas on the right and left cardiac lobes and the right apical lobe. Consolidation of the right and left apical and cardiac lobes, with adjacent emphysematous areas, was present in a second rabbit, which was also killed on day 21 pi. A third rabbit showed small areas of consolidation in the right and left cardiac lobes, with yellowish-white firm nodules on the surface of these lobes, which extended deeply into the parenchyma.

Histologically, two of the four rabbits from group 1 killed 14 days pi did not show any significant lesions in the lungs, while the third had thickening of the interalveolar septa due to congestion of alveolar capillaries and infiltration of mononuclear cells with perivascular lymphocytic cuffing; the lesion was characterized as subacute interstitial pneumonia. The fourth rabbit had chronic suppurative bronchopneumonia, which was characterized by infiltration of inflammatory cells in the alveoli and bronchiolar lumina, consisting of heterophils and macrophages. Chronic suppurative bronchopneumonia with pleuritis was observed in two of four rabbits in group 1 killed 21 days pi. In the third rabbit, there were multifocal areas of thickening of the interalveolar septa caused by congestion of the alveolar capillaries and mononuclear cell infiltration, with proliferation of fibroblasts. Heterophils and a few mononuclear cells also infiltrated the alveoli. The lesion was characterized as chronic interstitial pneumonia and alveolitis. Hyperplasia of the bronchial-associated lymphoid tissue was also observed. The fourth rabbit did not show any significant lung lesions.

The rabbits from group 2 killed 14 and 21 days pi did not show any significant lung lesions.

Electron microscopy

Nasal mucosa: The nasal mucosa of three of the rabbits that gave rise to a heavy or pure culture of *P. multocida* and that were killed 14 days pi had severely degenerated epithelial cells, which were swollen and had vacuolated cytoplasm. These cells had degenerative changes in their mitochondria and rough endoplasmic reticulum. The mitochondria had no cristae or disorganized or distended and osmiophilic cristae. The rough endoplasmic reticulum was dilated and appeared as irregular-shaped cytoplasmic vacuoles. Loss of cilia was frequently observed in the degenerated cells, whereas necrotic cells had both loss of cilia and breakdown of the cell membrane. Deformations were observed in the cilia and microvilli of degenerated epithelial cells, such as balloon-like structures and rupture of their walls. Bacterial cells, which were ovoid in shape and with a trilaminar membrane, were seen attached to the microvilli and deformed cilia (Figure 1). Some degenerate cells contained bacteria within membrane-bound vacuoles inside the cytoplasm adjacent to the mitochondria. The intracytoplasmic bacteria had a coccobacillary shape (Figure 2). The internal structure consisted of numerous granules, presumably the bacterial ribosomes. Polymorphonuclear leukocytes (PMNs) and macrophages were seen infiltrating between the degenerated epithelial cells and the subepithelial layer. Intravascular fibrin deposition was found in the nasal mucosa of rabbits from group 1 killed 14 days pi. Additionally, in the rabbits from group 1 that

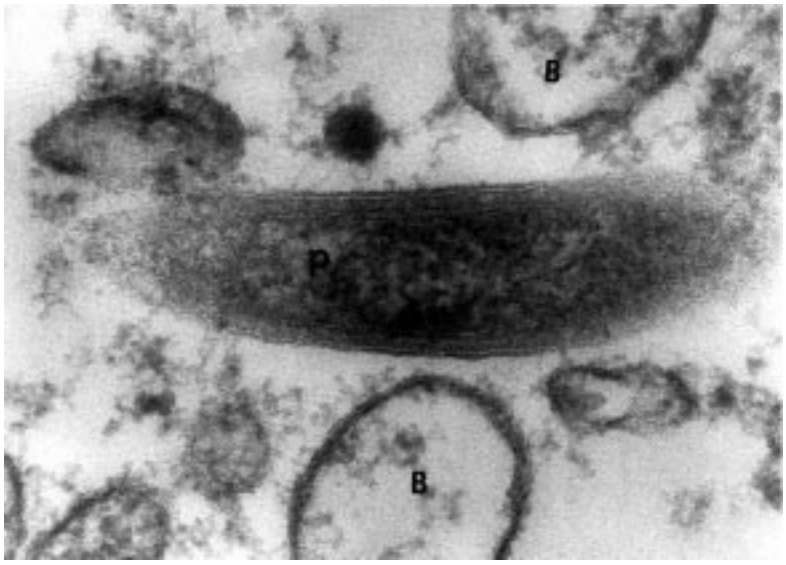

Figure 1. Transmission electron micrograph. Nasal epithelium from a rabbit in group 1 infected with *Pasteurella multocida* and killed 14 days pi. Note *P. multocida* (p) between balloon-like (B) structures of cilia with the presence of mucus. Original magnification, $\times 126\,000$

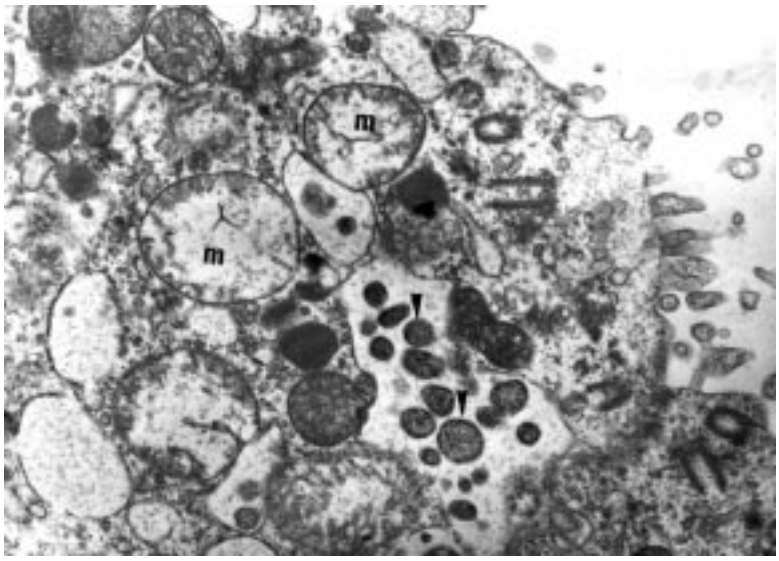

Figure 2. Transmission electron micrograph. Nasal mucosa from a rabbit infected with *P. multocida* and killed 14 days pi. Note the degenerated epithelial cells with swollen mitochondria (m) and intracellular bacterial cells (arrow heads) within membrane-bound vacuoles inside the cytoplasm. Original magnification, $\times 17\,700$

were killed 21 days pi, sloughed degenerated cells were observed in the nasal cavity, leaving only one layer of basal cells. Hyperplasia and hypertrophy of goblet cells were evident. These cells contained numerous mucous granules, causing them to appear more elongated and larger than the normal cells. Excessive secretion of mucus was also seen. Bacterial cells were also observed attached to the secreted mucus in the nasal cavity. Collagen fibres were found in the subepithelial area and beneath the detached cells. The rabbits in group 1 killed either 14 or 21 days pi had enlargement of endothelial cells and precipitation of dense materials in the wall of the blood vessels. The endothelial cells had large nuclei, foamy cytoplasm and intracytoplasmic osmiophilic dense particles. Destruction of the endothelial cell membrane was also noted.

Lung: The ultrastructural changes seen in the lungs of rabbits from group 1 killed 14 or 21 days pi were thickening of the alveolar septa due to congestion of the alveolar capillaries, swelling of the capillary endothelium, hyperplasia of type II pneumocytes, swelling of type I pneumocytes and inflammatory cell infiltration. Numerous PMNs and macrophages were present in the interstitium and located near the proliferating type II cells (Figure 3). Some PMNs had migrated across the endothelial basement membrane into the alveolar lumen. Some interstitial PMNs contained cytoplasmic granules near the plasma membrane (Figure 4), which is typical of the degranulation stage of activated PMNs. Macrophages and plasma cells were infiltrating the

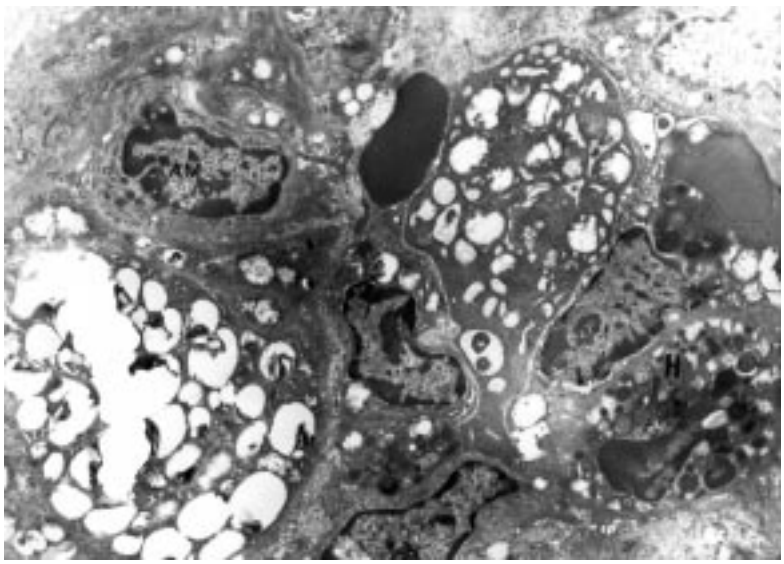

Figure 3. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 14 days pi. Note the infiltrated heterophils (H) and proliferated alveolar macrophages (AM) between proliferating pneumocyte type II cells. Original magnification, × 5000

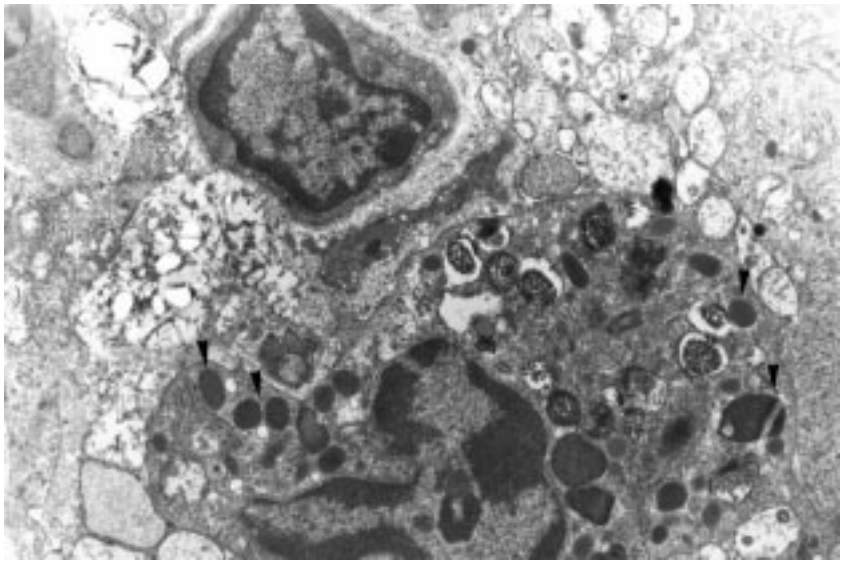

Figure 4. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 14 days pi. Note the heterophil with granules (arrow heads) near the plasma membrane, indicating its activity. Original magnification, × 10 000

interstitium and were also observed inside the alveolar lumen. Secretory plasma cells were numerous, with extensive cisternae of rough endoplasmic reticulum (rER) connected to vacuoles containing precipitates of globulin. Some of these cells appeared to have exhausted their ability to produce protein and displayed single-strand fragmented cisternae of rER. Macrophages containing remnants of degenerated cells (Figure 5) were seen in the alveolar lumen of infected rabbits. Proliferation of alveolar macrophages and some that had engulfed bacteria inside their cytoplasm (Figure 6) were also noted. Alveolar type I cells were swollen and had vacuolated cytoplasm. Some of these cells protruded from and appeared about to detach from the basement membrane. These cells had swollen mitochondria with loss of their cristae. Bacterial cells were also seen within membrane-bound vacuoles in the cytoplasm of these cells, adjacent to the mitochondria (Figure 7). The bacterial cells were of a similar shape and size to those found in the cytoplasm of the nasal epithelial cells. The mitochondrial membrane appeared to be fused to the membrane of the vacuoles containing bacterial cells.

Alveolar type II cells were often seen dividing and forming clusters on the alveolar septa, so contributing to the thickening of the alveolar walls. The intracellular vacuoles that contained lamellar material were enlarged and often coalesced with other vacuoles. The lamellar material was disrupted and reduced in electron density. Some of these cells were necrotic and had sloughed off into the alveolar lumen (Figure 8). Proliferation of fibroblasts and production of collagen fibres were prominent in the

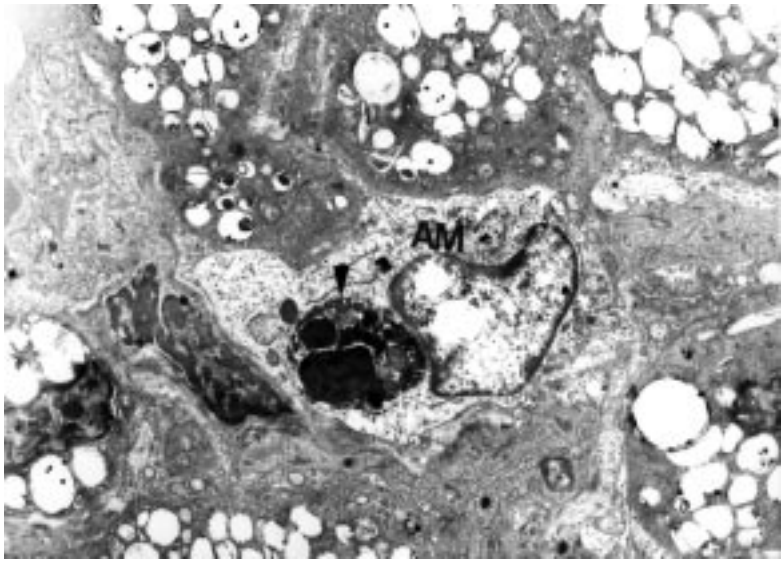

Figure 5. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 14 days pi. Note the alveolar macrophage (AM) that has engulfed remnants of degenerated heterophils (arrow head). Original magnification, ×4100

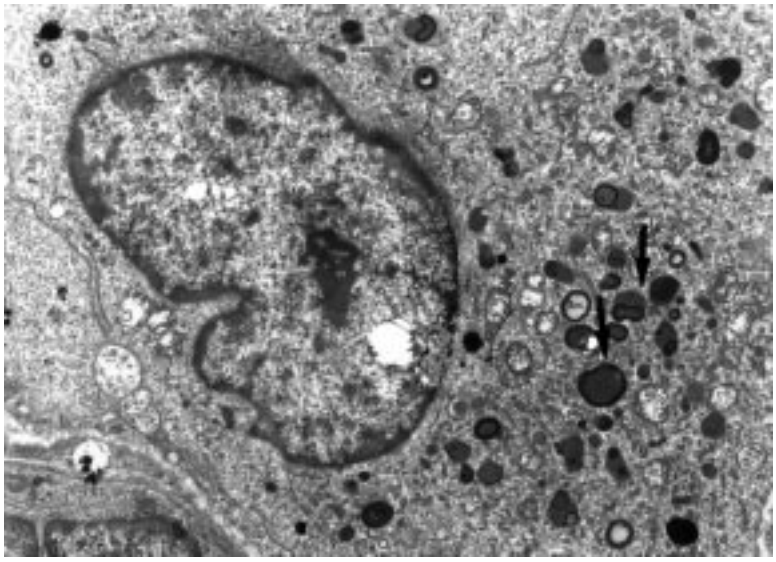

Figure 6. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 21 days pi. Note the phagosomes (arrows) inside the cytoplasm of an alveolar macrophage. Original magnification, ×7350

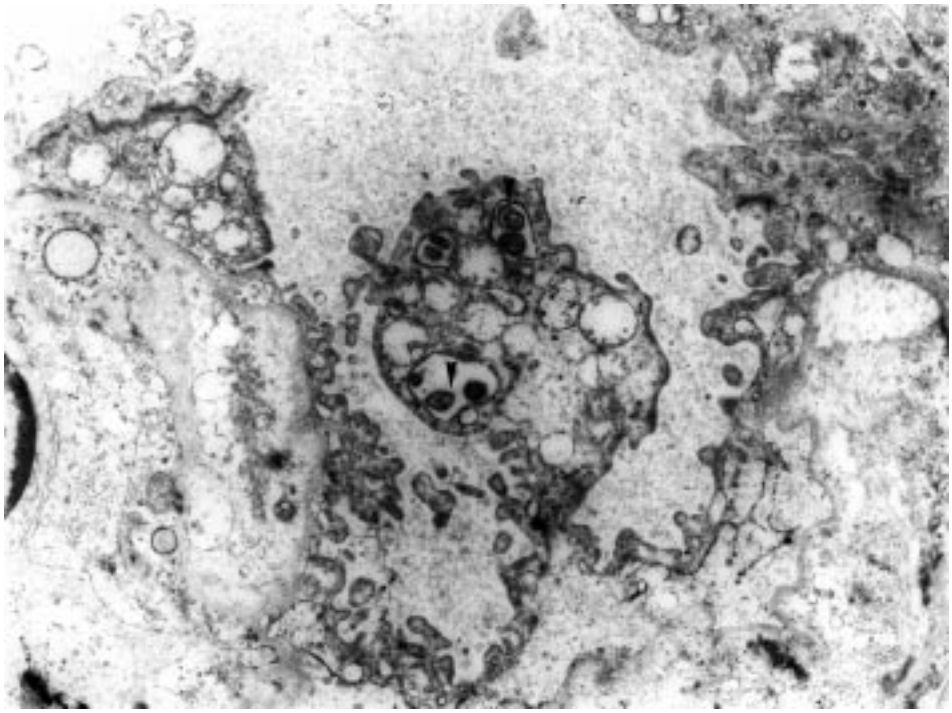

Figure 7. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 14 days pi. Note the protrusion of type I pneumocytes in the alveolar lumen and the presence of intracellular bacterial cells inside the cytoplasm (arrow heads). Original magnification, × 13 200

lungs of rabbits that were killed 21 days pi.

Deposition of fibrin was seen intravascularly, engulfed by macrophages (Figure 9), and also in the interstitium.

The walls of the alveolar capillaries were thickened and irregular in shape. The endothelial cells were swollen owing to the presence of vacuoles in their cytoplasm. Some of the endothelial cells had dense osmiophilic deposits in their cytoplasm.

There were no significant changes in the nasal cavity or lungs of any of the rabbits from group 2 killed 14 and 21 days pi.

DISCUSSION

It was clear that *P. multocida* caused damage to the epithelial cells, pneumocytes and endothelial cells in the nasal mucosa and lung and resulted in inflammatory responses, which may subsequently cause tissue damage. Hyperplasia of goblet cells with

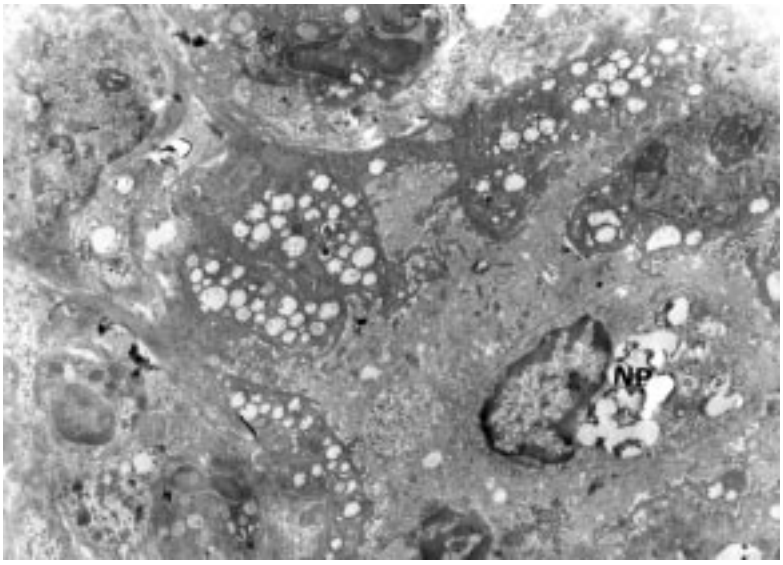

Figure 8. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 14 days pi. Note the necrotic type II pneumocytes (NP) sloughed into the alveolar lumen. Original magnification, × 5000

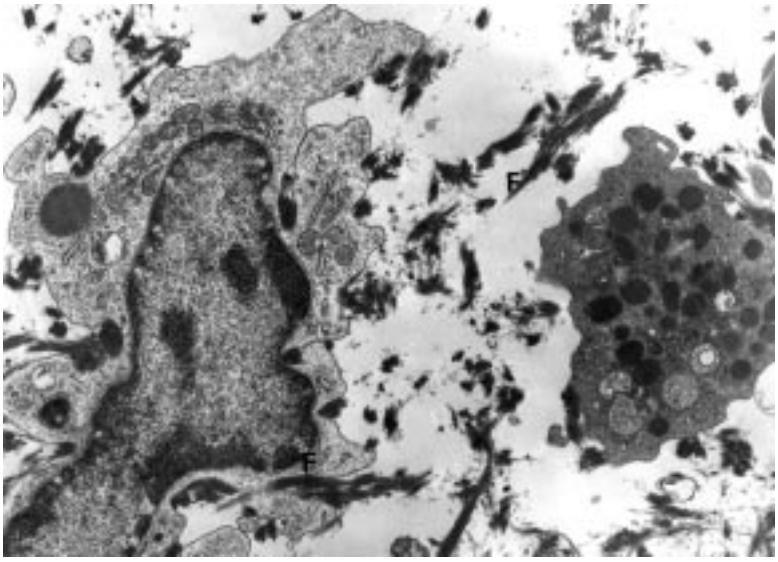

Figure 9. Transmission electron micrograph. Lung from a rabbit infected with *P. multocida* and killed 21 days pi. Note the intravascular fibrin (F) deposition being engulfed by pulmonary intravascular macrophages (PM). Original magnification, × 9000

excessive secretion of mucus in the nasal mucosa was observed. The cilial damage, deciliation and mitochondrial and rER changes caused by the organism in the nasal epithelial cells may cause impairment of the mucociliary clearance mechanism. Deciliation and stagnation of mucus can promote attachment and colonization of *P. multocida* by induction of ciliostasis (Al-Haddawi *et al.*, 1997).

This impairment appeared to have enhanced the rapid and heavy multiplication of *P. multocida* in the nasal cavity, with subsequent severe damage to the tissues. The ultrastructural changes in mitochondria and rER of the epithelial cells are believed to be either a result of the toxic effects of components of *P. multocida* or due to an inflammatory reaction.

The TEM showed the intracellular presence of microorganisms in the nasal epithelium and type I pneumocytes, indicating the possibility of invasion of epithelial cells by *P. multocida*. Invasion of epithelial cell monolayers by turkey strains of *P. multocida* type A:3 has been reported by Lee and colleagues (1994), who also reported that rabbit epithelial cells were resistant to invasion by turkey isolates of *P. multocida*. These differences in results may be due to host-specific factors and *P. multocida* serotype A:3 from rabbit isolates may have the ability to invade the nasal epithelial cells and pneumocytes of rabbits. These results suggest that invasion by *P. multocida* is involved in the mechanism of pathogenesis. Colonization or invasion of certain host tissues by the microorganisms might be mediated by bacterial products such as enzymes (Lee *et al.*, 1994). *P. multocida* has been reported to produce fimbriae under certain conditions and then to adhere to porcine and rabbit respiratory tract epithelium (Henriksen and Froholm, 1975; Glorioso *et al.*, 1982; Jacques *et al.*, 1988). It seems that adherence and the production of enzymes may be essential for this strain of *P. multocida* to be invasive. Further evidence for the possibility of bacterial invasion of epithelial cells in this experiment is the prolonged isolation of *P. multocida* from the nasal cavity and lungs of infected rabbits up to 21 days pi.

The observations in this study suggest that the pulmonary injury in rabbits infected with *P. multocida* results from the organism itself and/or its products. In addition, inflammatory events probably play an important role in this injury and its repair.

The failure to isolate *P. multocida* from the pneumonic lungs of four infected rabbits from group 1 may indicate that a direct association between the microorganisms and lung tissue is not necessary for the damage to occur. It seems that colonization of *P. multocida* in the lung or elsewhere with continuous release of endotoxin can cause injury to the lung. Many studies have reported pulmonary injury in calves given *Pasteurella haemolytica*-derived endotoxin (Whiteley *et al.*, 1990) and in sheep given endotoxin intravenously (Warner *et al.*, 1988). Degeneration and necrosis of type I and type II cells may be due to the direct effect of the endotoxin (Warner *et al.*, 1988).

Endotoxin has been reported to cross the alveolar wall to the blood vessels (Uragoh *et al.*, 1988) and interact with pulmonary intravascular macrophages (PIM) (Whiteley *et al.*, 1990). Endotoxin can activate macrophages directly or indirectly via activation of the complement cascade and generation of the active component of complement (C5a) (Morrison and Ulevetial, 1978). Once activated, these macrophages will produce lipid mediators, cytokines, and procoagulant factors that can mediate the inflammatory response.

In the present study, infiltration of heterophils was seen in the subepithelial layer and between degenerated epithelial cells in the nasal mucosa and lung parenchyma. It is known that endotoxin is the most potent agent capable of inducing an influx of neutrophils (Movat *et al.*, 1987). The presence of heterophils in the lesion may be due to *P. multocida* and/or its products, which act as chemotactic factors for leukocytes (Galdiero *et al.*, 1998; Latimer *et al.*, 1990). Substances that are cleaved from normal plasma peptides or tissue proteins in foci of injury, such as complement fragments and fibrinopeptides, are strong chemoattractants for heterophils (Cheville, 1983). Furthermore, the presence of heterophils at different stages of maturation may be due to the role of lipopolysaccharide (LPS) in accelerating the release of these cells from the bone marrow pools (Klut *et al.*, 1998).

Sequestration of heterophils and intravascular deposition of fibrin in association with PIM may indicate a central role for PIM in mediating the early intravascular inflammatory events. It has also been reported that endotoxin alone, derived from *P. haemolytica* A:1, is capable of initiating an inflammatory response in the bovine lung, neutrophil sequestration and aggregation of platelets in alveolar capillaries in association with PIM (Whiteley *et al.*, 1991a). Heterophils and fibrin were also found in the alveolar lumen in close association with alveolar macrophages (AM), which may be a result of endotoxin activation of the AM (Whiteley *et al.*, 1991b; Olchowy *et al.*, 1996).

The migrated interstitial heterophils in this study appeared to be either activated or fragmented and were associated with destruction of the nasal mucosa and parenchyma, suggesting that heterophils are also involved in the tissue damage. Through activation or fragmentation of inflammatory cells, including heterophils, cytotoxic products are released that can disorganize the normal architecture of the lung (Gadek and Pacht, 1990) or damage the alveolar type I cells (Cantin *et al.*, 1987). On the other hand, it has been suggested that the products from PMNs may have a mitogenic effect on type II cells that leads to hyperplasia of these cells (Klut *et al.*, 1998).

Endothelial injury was observed in this study alongside sequestration of heterophils in the affected capillaries. This injury may have been either a result of a direct effect of LPS, as reported by Gartner and colleagues (1988), or indirect through cytokines produced by monocytes, macrophages or other cells; including interleukins 1, 6 and 8, tumour necrosis factor, and platelet activating factor (Salyers and Whitt, 1994). The endothelial injury may also be caused by products of PMNs (Welsh *et al.*, 1989). The presence of osmiophilic particles inside the cytoplasm of injured endothelial cells suggests the uptake of the products of PMNs (heterophils) by the endothelium. Klut and colleagues (1998) suggested that granular proteins released from PMNs are commonly taken up by endothelial cells and that this uptake may be important in the pathogenesis of vascular injury.

This study provided morphological evidence of a complex interaction induced by *P. multocida* between the cells of the nasal mucosa and lung and leukocytes that develops early in the inflammatory process. It indicates that the *P. multocida* serotype A:3 isolate from rabbits may have an ability to invade the epithelial cell. Furthermore, when *P. multocida* enters or colonizes the nasal mucosa and lung, it activates the mucosal leukocytes and AM, which leads to the release of inflammatory mediators from these cells. These mediators and products from the bacterial wall (e.g. endotoxin) cross the

mucosal capillary walls and the alveolar wall and activate the PIM. Alveolar macrophages and PIM then seem to play an important role in the inflammatory events that occur in the lungs and lead to injury. Polymorphonuclear leukocytes also play an important role in this process of injury and repair to the nasal mucosa and lung.

ACKNOWLEDGEMENTS

The authors thank Dr Thula Wijewardena from the Veterinary Research Institute, Sri Lanka for somatic serotyping. This study was financially supported by the Malaysian Government IRPA grant no. 51173.

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(Accepted: 23 October 1999)