

Pathological Changes in the Lungs of Calves Following Intratracheal Exposure to *Pasteurella multocida* B:2

M.N. Khin, M. Zamri-Saad* and M.M. Noordin

Department of Veterinary Pathology and Microbiology,
Faculty of Veterinary Medicine, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia

*E-mail: zamri@vet.upm.edu.my

ABSTRACT

Pasteurella multocida B:2 is an etiological agent of hemorrhagic septicemia in cattle and buffaloes. It is commonly fatal and considered as one of the most economically important cattle diseases in Southeast Asia. This study describes the pathological changes in the lungs of calves following intra-tracheal challenge with wild-type *P. multocida* B:2. For this purpose, six calves of 8-month old were selected and divided into two groups of 3 calves. Calves of Group 1 were challenged with intra-tracheal 5ml inoculum containing 10^9 cfu/ml of wild-type *P. multocida* B:2, while the calves of Group 2 were similarly administered with PBS. All the challenged calves of Group 1 showed slight dullness and were found to be inactive within 72 hours after inoculation, but none died. Meanwhile, their lungs showed petechiations and patches of acute pneumonia affecting few lobules. Histological examinations revealed the presence of haemorrhages into the alveoli, whereas some sections showed thickened inter-alveolar septa due to congestion and the presence of neutrophils. However, pulmonary oedema was absent. *P. multocida* B:2 was successfully isolated from all the calves of Group 1.

Keywords: Pathology, lungs, calves, *Pasteurella multocida* B:2, intra-trachea

INTRODUCTION

Pasteurella multocida B:2 is the etiological agent of hemorrhagic septicemia in cattle and buffaloes (Bain *et al.*, 1982; Verma and Jaiswal, 1998). This peracute disease is manifested by a short clinical course involving severe depression, pyrexia, submandibular edema and dyspnea, followed by recumbent and death (Graydon *et al.*, 1993). It is commonly fatal and is considered as one of the most economically important cattle diseases in Southeast Asia (Benkirane and De Alwis, 2002). Moreover, it is also endemic in most parts of the tropical Asia, particularly India and Southeast Asia leading to high mortality (Bain *et al.*, 1982; Verma and Jaiswal, 1998).

One of the routes of the infection is through the respiratory tract, where *P. multocida* B:2 causes mild lesions in the lungs before they enter the blood circulation via the pulmonary capillaries to cause severe septicaemia (Zamri-Saad and Shafarin, 2007). This report describes the pathological changes in the lungs of calves, following the intra-tracheal administration of wild-type *P. multocida* B:2.

MATERIALS AND METHODS

Animals

In this experiment, six clinically healthy local calves of approximately 8 months of age were

Received: 16 November 2009

Accepted: 11 January 2010

*Corresponding Author

used. Upon arrival to the experimental house, anthelmintic (Ivomectin) was administered subcutaneously at the rate of 0.2 mg/kg body-weight for three consecutive days to control internal parasitism, which had been shown to influence disease development (Zamri-Saad *et al.*, 1994). Concurrently, nasal swabs were collected from all the calves at the time of arrival to ensure that the calves were free of *P. multocida* prior to the start of the experiment. All the calves were observed on a daily basis for the development of clinical signs of any disease and the experiment were started only when all calves were found negative from carrying *P. multocida* and clinically appeared healthy for a period of 2 weeks. The calves were fed daily with cut grasses and supplemented with pellets at the rate of 1 kg/animal/day. Drinking water was available ad libitum.

Bacterial Strain

Wild-type *P. multocida* B:2, which was isolated earlier from cattle that died during an outbreak of haemorrhagic septicaemia, was used. The organism was grown on blood agar overnight at 37 °C and kept stored at the room temperature.

Preparation of the Inoculums

To prepare the inoculums, the stock culture of wild-type *P. multocida* B:2 was selected and inoculated into 100 ml brain-heart infusion broth. These inoculums were then incubated at 37 °C for 18 h with gentle shaking before viable count of the bacterial concentration was determined using the plate count technique method proposed by Alcamo *et al.* (1997). The concentration was readjusted to give the required final concentration of $\times 10^9$ colony-forming unit (cfu)/ml using sterile phosphate-buffered saline (PBS). These inoculums were freshly prepared for use on the experimental calves.

Experimental Procedure

At the start of the experiment, the calves were divided into two groups comprising of 3 calves per group, and each group was kept separated.

The 3 calves of Group 1 were exposed intratracheal to 5 ml of the inoculum containing live wild-type *P. multocida* B:2, whereas the calves of Group 2 were similarly exposed to PBS. Following the exposures, all the calves were observed for clinical signs before surviving calves were killed on day 3 of post-exposure. Post-mortem examinations were carried out before the right apical lobe of the lungs were fixed in 10 % buffered formalin for histological examinations, while the lung tissue, heart swab, small intestine, and lung lavage fluid were subjected to bacterial isolation.

Bacterial Isolation

Post-mortem examinations were carried out immediately with special attention given to the changes in the respiratory tract. Following the post-mortem examination, the lungs were lavaged by introducing 1 litre of cold, sterile phosphate buffered saline into the lungs through the trachea. This was followed by gentle massaging of the lung before the fluid was re-collected into a beaker container. The lung lavage fluid was then centrifuged at 1,000 xg for 15 min to remove the debris.

Specimens from the lungs, heart blood, lung lavage fluid, and small intestine were collected for bacterial isolation. These samples were taken aseptically, cultured onto blood agar and incubated at 37 °C for 24 h. Suspected colonies of *P. multocida* B:2 were confirmed by multiplex PCR assay.

The Multiplex PCR assay was conducted using two primer sets which were designed from the sequence of the clones of Zamri-Saad *et al.* (2006); KMTI (KMT1T7-5'- ATCCGCT ATTTACCCAGTGG-3' and KMT1SP6-5'-GCTGTAAACGAACTCGCCAC-3') and 6b (KTT2-5'-AGGCTCGTTTGGATTATGAAG-3' and KTSP61-5'-ATCCGCTAACACAC TCTC-3'). Briefly, 25 µl reaction mixture containing 1 x PCR buffer, 2.0 mmol/L MgCl₂, 200 µmol/L of each dNTP, 20 pmol of each primer, and 1 U *Taq* DNA polymerase was prepared and one colony was picked from the blood agar plate as a template and re-

suspended in the PCR mixture. The reaction mixture was subjected to amplification in a thermal cycler (Eppendorf) according to the following program: initial denaturation at 95 °C for 4 min, denaturation at 95 °C for 45s, annealing at 55 °C for 45s, extension at 72 °C for 45s, which was repeated for 30 cycles, and a final extension of 72 °C for 6 min. The amplified products were separated by agarose gel electrophoresis (1.0 % agarose in 1 x TBE) at 70 V for 1 h 30 min and stained with ethidium bromide. The DNA band was observed under UV transillumination and photographed (Alpha Imager).

RESULTS

Clinical Observations

On the 3-day of the experimental period, the challenged calves of Group 1 appeared slightly dull and inactive. However, no other clinical signs associated with haemorrhagic septicaemia were observed. All the control calves appeared healthy and alert prior to and after infection.

Pathology Changes

While none of the control calves had gross lung lesions (Fig. 1A), those of Group 1 showed petechiations, particularly in the apical lobes with occasional small dark red discoloration affecting few lobules (Fig. 1B).

Histological examinations on the lungs of calves of Groups 1 showed mild to moderate congestion of the capillaries with evidence

of hemorrhages into the alveolus (Fig. 2A). Neutrophils were observed within the alveoli of many lung sections, while some areas showed thickened inter-alveolar septa (Fig. 2B). Similar exudate was observed in some bronchi (Fig. 2C). On the contrary, the lungs of the control calves showed no significant changes.

Bacterial Isolation

Pasteurella multocida B:2 was successfully isolated from the lung, heart swabs, small intestine, and lung lavage fluid of the infected calves of Group 1 (Fig. 3). However, none of the control uninfected calves had *P. multocida* B:2.

DISCUSSION

Intra-tracheal exposure of calves to live wild-type *P. multocida* B:2 resulted in mild to moderate lesions in the lungs. They were, nevertheless, peracute lesions in the form of slight congestion with petechiations and mild pneumonia observed, following both gross and histological examinations. Similar lesions have been observed in goats following subcutaneous and intra-tracheal exposures (Zamri-Saad and Shafarin, 2007), and in calves following subcutaneous exposure to *P. multocida* B:2 (Graydon *et al.*, 1993).

Nevertheless, the severe pulmonary and subcutaneous odema observed both in calves and goats were not observed in this study. This was due to the inability of wild-type *P.*

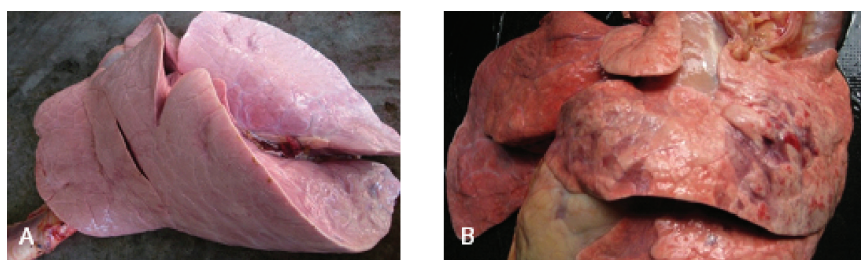


Fig. 1: Lung of a control calf (A) showing relatively normal pink colour without lesion while that of the Group 1 (B) appeared slightly congested with petechiations of the apical lobes and dark-red discolorations affecting several lobules

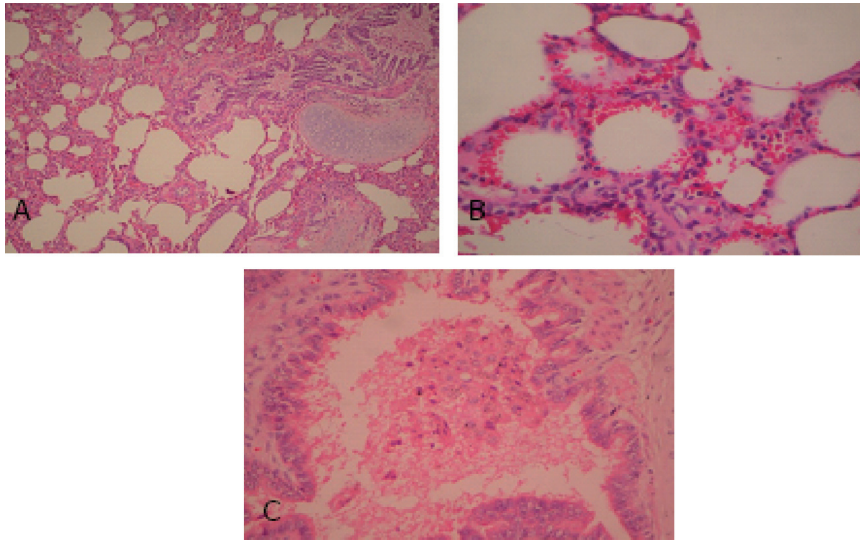


Fig. 2: Photomicrograph of lung of Group 1 calf exhibited congestion, haemorrhages (A; HE x100) and thickened interalveolar septa due to congestion and infiltration by neutrophils (B; HE x40). Photomicrograph of a bronchiole of Group 1 calf showing the presence of exudates consisting of desquamated epithelium, fibrin and few neutrophils.(C; HE x100)

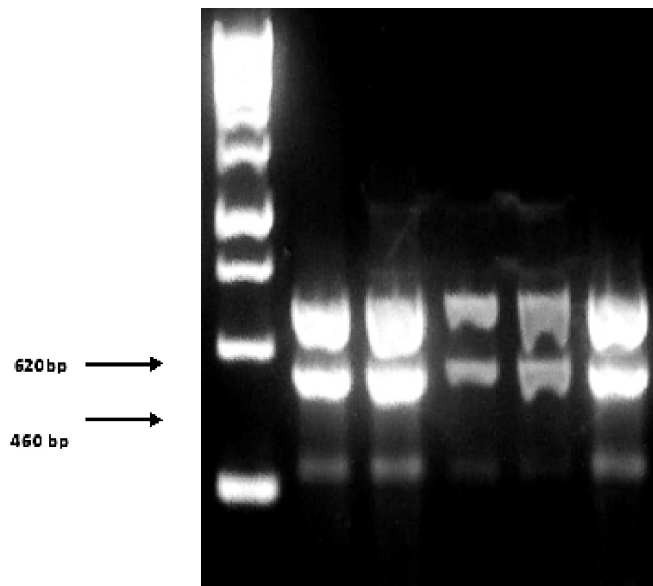


Fig. 3: Photograph of a multiplex PCR products showing bands characteristic of P. multocida B:2. The presence of double bands in between ~460bp and ~620 bp are indicative of a positive result. Lane 1 = 1kb DNA ladder marker; Lane 2 = positive control; Lane 3 = lung sample; Lane 4 = small intestinal sample; Lane 5 = heart swab sample; Lane 6 = lung lavage fluid sample

multocida B:2 to cause severe disease in this study. Dexamethasone administration prior to infection has been shown to cause much severe lesions (Shafarin *et al.*, 2009). Stressful condition causing immuno-suppression has been strongly associated with bacterial infection and recognized as the most important factor which can lead to outbreaks of haemorrhagic septicaemia in cattle and buffaloes (Saharee *et al.*, 1993). Immunosuppression, through dexamethasone treatment, enhances the ability of *P. multocida* B:2 to prolong colonization and significantly cause more severe lung lesions. Therefore, the failure to cause stress in the calves, through the usage of dexamethasone in this study led to development of only mild and moderate lesions.

REFERENCES

- Alcama, I.E. (1997). *Fundamentals of Microbiology* (5th edn.). Menlo Park, California: Addison Wesley Longman.
- Bain, R.V.S., De Alwis, M.C.L., Carter, G.R. and Gupta, B.K. (1982). Haemorrhagic septicaemia, In *FAO Animal Production and Health Paper 33* (pp.11-13). Food and Agriculture Organization of the United Nations, Rome, Italy.
- Benkirane, A. and De Alwis, M.C.L. (2002). Haemorrhagic septicaemia, its significance, prevention and control in Asia. *Veterinary Medicine*, 47, 234-240.
- Graydon, R.J., Patten, B.E. and Hamid, H. (1993). The pathology of experimental haemorrhagic septicaemia in cattle and buffalo. *Pasteurellosis in production animals. ACIAR Proceedings* (pp. 89-91) No. 43.
- Saharee, A.A., Salim, N.B., Rasedee, A. and Jainudeen, M.R. (1993). Haemorrhagic septicaemia carriers among cattle and buffalo in Malaysia. *Pasteurellosis in production animals. ACIAR Proceedings* (pp. 89-91) No. 43.
- Shafarin, M.S., Zamri-Saad, M., Siti Khairani, B. and Saharee, A.A. (2009). Pathological changes in the respiratory tract of goats infected by *Pasteurella multocida* B:2. *Journal of Comparative Pathology*, 140, 194-197.
- Verma, R. and Jaiswal, T.N. (1998). Haemorrhagic septicaemia vaccines. *Vaccine*, 16, 1184-1192.
- Zamri-Saad, M., Subramaniam, P., Sheikh-Omar, A.R., Sani, R.A. and Rasedee, A. (1994). The role of concurrent haemonchosis in the development of pneumonic pasteurellosis in goats. *Veterinary Research Communication*, 18, 119-122.
- Zamri-Saad, M. and Shafarin, M.S. (2007). Response of goats to different routes of infection by *Pasteurella multocida* B:2. *Journal of Animal and Veterinary Advance*, 6, 340-343.

