

Clinico-pathological Responses of Calves Associated with Infection of *Pasteurella multocida* Type B and the Bacterial Lipopolysaccharide and Outer Membrane Protein Immunogens

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Abstract: The current study aims to investigate the Clinico-pathological responses of calves associated with the infections of *Pasteurella multocida* type B and the bacterial lipopolysaccharide and outer membrane protein immunogens. Alterations in the behavior of animals and pathological lesions observed following innate or experimental infections usually divulge extensive and detrimental changes in the clinical signs, organs and tissues of the animals afflicted with the disease. These alterations are imperative for Veterinary evaluation of herd health. Eight clinically healthy, non-pregnant and non-lactating Brangus cross heifers weighing 150±50 kg were used in the study. The heifers (n = 8) were divided into 4 groups of 2 calves per group. The control calves in group 1 were inoculated intramuscularly with 10 mL of sterile Phosphate Buffered Saline (PBS). Calves in group 2 were inoculated intramuscularly with 10 mL of 10¹² colony forming unit (cfu) of wild-type *P. multocida* and calves in group 3 were inoculated intravenously with 10 mL of LPS broth extract. Calves in group 4 were inoculated intramuscularly with 10 mL of OMP broth extract. All animals were observed for 48 h for clinical signs, changes in behavior and mortality pattern, including the time of death. The results divulged significant differences in the Clinico-pathological alterations. Calves inoculated with whole cell *P. multocida* type B: 2 showed a significant (p<0.05) increased in rectal temperature. The affected calves showed significant severe dullness (p<0.000) and significant rumen hypomotility (p<0.000) was also exhibited. The calves showed signs of hypersalivation at 14 h. There is no significant difference (p = 0.240) in pulmonary oedema in the Calves of group 2 compared to control group 1. Calves of group 4 also showed no significant difference in pulmonary oedema (p = 0.612) compared to control group 1. Calves of group 3 showed significantly moderate pulmonary oedema (p<0.000). All the three treatment groups showed significant (p<0.05) differences in the presence of inflammatory cells in the lung. All the three treatment groups showed significant (p<0.05) in the presence of degeneration and necrosis of cells in the lung. Calves of group 2 showed significantly severe haemorrhage (p<0.000) in the lung including groups 3 and 4 (p<0.000) respectively. Calves in group 2 showed significantly (p<0.000) mild thrombus formation. There is no significant thrombus formation in the lung of calves in groups 3 (p = 0.352) and 4 (p = 0.184) respectively. In conclusion, the pathophysiological changes in cattle will assist in the improvement of the vaccines and the vaccination methods that are currently employed in controlling this important disease in Malaysia.

Keywords: Bacterial lipopolysaccharide, calves, clinico-pathological, outer membrane protein immunogens, *Pasteurella multocida* type B

INTRODUCTION

The production of ruminants in Malaysia is progressively shifting from subsistence to intensive operations (Jamaluddin, 1992). It comprises of large ruminants, largely cattle and buffaloes and small ruminants, predominantly goats and sheep. The ruminant industry plays an imperative role in the food industry through the provision of milk, meat and the by

products such as hide for the leather industry. In Malaysia, the large ruminant sector is endangered by Haemorrhagic Septicaemia (HS) caused by a Gram negative bacterium *Pasteurella multocida* type B. The disease is an acute and fatal infectious disease which causes colossal losses to farmers and the country.

Pasteurella multocida is of substantial economic significance in the livestock industry (Collins, 1977). Infections by *Pasteurella multocida* have been reported

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in mammals and fowls (Soltys, 1979). It is an important principal animal pathogen for over a century and is becoming crucial as human pathogen (Biberstein, 1979) leading to a disease process termed Pasteurellosis. Yet, Pasteurellae have been shown to be a common microflora of the upper respiratory tract in normal animals (Campbell, 1983). The organisms more often than not act as secondary invaders in animals with concurrent diseases or suffering from debilitating stressful conditions (Benirschke *et al.*, 1978).

The serotype 6: B has been recovered from HS-affected animals in Southern Europe, Central and South America, the Middle East and Asia, including Malaysia. The infection causes substantial morbidity and mortality in cattle, buffaloes, sheep and goats (Bain and Knox, 1961). In Malaysia, the stressful condition is during the raining season where most outbreaks occurred (Saharee *et al.*, 1993).

The pathological modifications incorporated generalized lymphadenopathy, submandibular and brisket edema, acute fibrinous pneumonia, proctitis, acute colitis and hemorrhagic typhilitis. There were also severe clinical signs characteristic of HS infections which includes increased body temperature, respiratory rate, salivation, depression and anorexia (Mohammad and Mohd, 2011).

Haemorrhagic septicaemia in Malaysia is generally controlled by the use of killed whole cell vaccines. These vaccines have certain limitations, such as shorter duration of immunity and swelling at the site of inoculation. Furthermore, outbreaks of HS have been reported to occur despite vaccinations (Chandrasekaran *et al.*, 1994). Previous studies have suggested those capsular antigens, Lipopolysaccharide (LPS) or LPS-protein complex and the Outer Membrane Proteins (OMPs), including the iron-regulated OMPs as effective immunogens for serogroups B and E (De Alwis *et al.*, 1975). Studies on immunization in mice with LPS of *P. multocida* (6: B) has shown that the protection observed was associated with LPS-associated proteins (Muniandy *et al.*, 1998). Nevertheless, knowledge of host responses towards the outer membrane protein and Lipopolysaccharide (LPS) of *P. multocida* in HS is still deficient. There is no documentation of clinical responses during the infection by *P. multocida* B: 2. there is also no report on the pathological changes in the host inoculated with the immunogens from *P. multocida*. This study is designed to evaluate the possible pathophysiological changes that can occur in cattle. This will assist in the improvement of the vaccines and the vaccination methods that we have currently employed in controlling this important disease in Malaysia.

MATERIALS AND METHODS

Eight clinically healthy, non-pregnant and non-lactating Brangus cross heifer's weighing 150±50 kg

were used in the study. Upon arrival at the Animal Experimental House, Faculty of Veterinary Medicine, Universiti Putra Malaysia, anthelmintic (Ivermectin) was administered subcutaneously at the rate of 1 mL/50 kg body weight to control internal parasitism, which has been shown to influence disease development (Zamri-Saad *et al.*, 2006). Nasal swabs were collected from all calves at the time of arrival to ensure that the calves were free of *P. multocida* prior to the experiment. The animals were placed in individual pen and were fed cut grass supplemented with pellets at the rate of 1 kg/animal/day. Water was available *ad libitum*.

Inoculum: Throughout the experiments, three types of inoculums were used; Wild-type *P. multocida* B: 2 used in this study were obtained from stock culture. It was isolated from a previous outbreak of HS in the state of Kelantan, Malaysia. Identification of *P. multocida* was made using the Gram-staining method and biochemical characterization of oxidase, urea broth, Sulphur Indole Motility (SIM), Triple Sugar Iron (TSI) and citrate tests. The isolate was confirmed to be type B: 2 by the Veterinary Research Institute (VRI) Ipoh, Perak. Pure stock culture that was stored on nutrient agar slants was sub-cultured onto 5% horse blood agar and incubated at 37°C for 18 h. A single colony of *P. multocida* was selected and grown in Brain Heart Infusion broth (BHI), incubated in shaker incubator at 37°C for 24 h before the concentration was determined by McFarland Nephelometer Barium Sulfate Standards.

The Lipopolysaccharide (LPS) *P. multocida* B: 2: The LPS Extraction Kit (Intron Biotechnology) was used to prepare the inoculums of LPS. For this experiment, LPS was extracted from 10¹² cfu. The whole cells were centrifuged for approximately 30 sec at 13,000 rpm at room temperature. Then the supernatant was removed before 1 mL of lysis buffer was added and vortexed vigorously to lyse the bacterial cells. This was followed by adding 200 µL of chloroform and vortexed vigorously. The mixture was incubated for 5 min at room temperature before centrifuged at 13,000 rpm for 10 min at 4°C. Then, 400 µL of the supernatant was transferred into a new 1.5 mL centrifuge tube and 800 µL of purification buffer was added. The mixture was incubated for 10 min at -20°C. This was followed by another centrifugation at 13,000 rpm for 15 min at 4°C. Lastly, the LPS pellet was washed with 1ml of 70% ethanol and dried completely. Following that, 70 µL of 10 mM Tris-HCl (pH 8.0) (Sigma®) was added into the LPS pellet and was dissolved by boiling for 1 min. The LPS extraction obtained was subjected to SDS-PAGE to confirm that no protein was present in the extracted LPS.

The Outer Membrane Proteins (OMP) of *P. multocida* B: 2: The Qproteome™ Bacterial Protein Extraction kit was used to prepare the inoculums of

Table 1: The microscopic lesions scored for the organs examined

Organ/lesion	Oedema	Inflammatory cells	Degeneration and necrosis	Haemorrhage	Thrombus	Kupffer cells
Lung	√	√	√	√	√	-
Liver	-	√	√	√	-	√
Heart	-	√	√	√	-	-

√: Lesions observed in the organs

OMP. For this experiment, the OPM was extracted from 10^{12} cfu. Firstly, freshly harvested cell pellets were frozen using liquid nitrogen for 24 h prior to the extraction. The cell pellets were then thawed for 15 min on ice and were re-suspended in 10 mL of native lysis buffer. Then the cells were incubated on ice for 30 min followed by centrifugation at 14,000 rpm for 30 min at 4°C. Lastly, the supernatant containing the soluble fraction of the bacterial outer membrane proteins was retained and subjected to SDS-PAGE to locate the range of protein bands present in the extraction.

Experimental design: The heifers were divided into 4 groups of 2 calves in each group. The control calves in group 1 were inoculated intramuscularly with 10 mL of sterile Phosphate Buffered Saline (PBS). Calves in group 2 were inoculated intramuscularly with 10^{12} colony forming unit (cfu) of wild-type *P. multocida* and calves in group 3 were inoculated intravenously with 10 mL of LPS broth extract. Calves in group 4 were inoculated intramuscularly with 10 mL of OMP broth extract. All animals were observed for 48 h for clinical signs, changes in behavior and mortality pattern, including the time of death. The clinical signs monitored were temperature, rumen motility, movement and dullness. Clinical signs scoring were done and the data was analyzed using JMP9 SAS. The observed patterns of behaviour were recorded using Sony Handycam 25x Optical Zoom-DCR DVD 708 (Sony Corporation) and Nikon Digital Camera 7.1 Megapixels (Coolpix) (Nikon Corporation, Japan). Post-mortems examinations were carried out and the lung, liver and heart samples were fixed for microscopic examination and cellular changes were scored and analyzed using JMP9 SAS.

Clinical signs scoring: Clinical signs scoring for rumen motility in calves were divided into 4, namely score 0: normal (normal rumen motility 2-3 rolls/minute), score 1: mild (2 rolls/min), score 2: moderate (1 roll/min), score 3: severe (0 roll/min).

Clinical signs scoring for dull and depression in calves were divided into 4, namely score 0: normal (normal movement and appetite), score 1: mild (reduced movement and appetite by 30%), score 2: moderate (reduced movement and appetite by 60%), score 3: severe (reduced movement and appetite more than 60%).

Lesions scoring: Cellular changes were scored by examining twenty slides for each organ where 6 fields per slide were observed at 200×magnification.

Cellular changes scoring were divided into 4 scores, namely: score 0: normal (normal tissue), score 1: mild (less than 1/3 of field involved), score 2: moderate (between 1/3 and 2/3 of field involved), score 3: severe (more than 2/3 of the field involved). Lesions observed and scored for each organ were as stated Table 1.

Histopathology analysis: Lung, liver and heart samples were preserved in 10% buffered formalin. The samples were fixed for 24 h before they were processed using the routine histology slide preparation technique and stained with Hematoxylin and Eosin (H&E).

Statistical analysis: All the data's were analyzed using Anova and Tukey-Kramer test. This was done using JMP® 9. NC: SAS Institute Inc. software Version. The data were considered significant at $p < 0.05$.

RESULTS

Clinical signs: Calves inoculated with whole cell *P. multocida* B: 2 showed a significant ($p < 0.05$) 2 times increase in rectal temperature ($39.8 \pm 0.75^\circ\text{C}$) as early as 2 hours post-infection (p.i), while the affected calves showed severe dullness ($p < 0.000$) with mean score of 2.52 ± 0.51 (Table 2). The calves also exhibited rumen hypomotility at 4 h (p.i) that persisted until the calves died at 18 h (p.i). Significant rumen hypomotility ($p < 0.000$) with mean score of 2.76 ± 0.44 (Table 2) was exhibited. The calves showed signs of hypersalivation at 14 h (p.i) (Table 3).

Calves of group 3 showed a significant ($p < 0.024$) 1 time increase in rectal temperature ($40.0 \pm 0.45^\circ\text{C}$) at 2 hours (p.i) but lasted for only 2 h when the temperature returned to normal at 4 h (p.i). However, the calves showed significant mild to moderate dullness ($p < 0.000$) with mean score of 1.50 ± 0.51 (Table 2) and these calves appeared dull and depressed at 2 h (p.i), while rumen hypomotility was detected at 9 h (p.i) and persisted until the end of 48 h experimental period. All the calves in group 3 showed significant rumen hypomotility ($p < 0.000$) with mean score of 2.69 ± 0.47 (Table 2). All calves in this group survived the inoculation (Table 3).

Table 2: Mean score of clinical signs observed in calves for 48 h post-inoculation with immunogens

Parameters	Group 1 (control)	Group 2 (<i>P. multocida</i>)	Group 3 (LPS)	Group 4 (OMP)
Dull and depressed	0.00±0.00	*2.52±0.51	*1.50±0.51	*2.63±0.49
Rumen motility	0.00±0.00	*2.76±0.44	*2.69±0.47	*2.80±0.41

*: Significant value at $p < 0.05$ (ANOVA and Tukey-Kramer was used for the analysis) for the comparison between immunogens groups and negative control group calves

Table 3: Clinical signs exhibited by inoculated calves

Hours	10^{12} whole cells	LPS	OMP
2	Increased temperature dull and depressed	Increased temperature dull and depressed	Increased temperature dull and depressed
4	Increased temperature dull and rumen hypomotility-0/min	Increased temperature Dull and depressed	Increased temperature Dull and depressed
9	Dull and depressed rumen hypomotility-0/min	Same as above rumen hypomotility-0/min	Dull and depressed
14	Dull, depressed and recumbent rumen hypomotility-0/min hyper salivation	Same as above rumen hypomotility-0/min	Dull and depressed rumen hypomotility-0/min
18	Euthanized due to severe clinical signs	Same as above rumen hypomotility-0/min	Same as above rumen hypomotility-0/min
24		Dull and depressed rumen hypomotility-0/min	Same as above rumen hypomotility-0/min
36		Dull and depressed rumen hypomotility-0/min	Dull and depressed rumen hypomotility-1/min
42		Dull and depressed rumen hypomotility-1/min	Dull and depressed rumen hypomotility-0/min
48		Dull and depressed rumen hypomotility-1/min	Dull and depressed rumen hypomotility-0/min

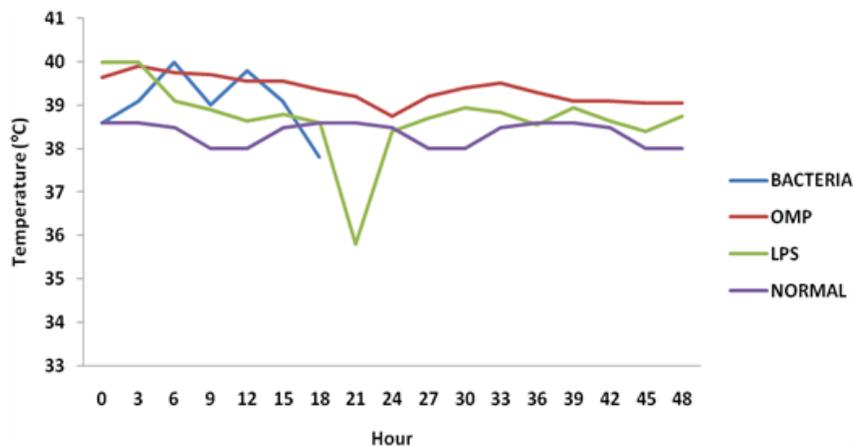


Fig. 1: Mean body temperature (°C) of calves following inoculation with different immunogens of *P. multocida* B: 2



Fig. 2: Photograph of the lung of a calf inoculated with whole cell *P. multocida* B: 2 showing mosaic and congested appearances involving all lung lobes

Calves inoculated with OMP (group 4) showed a significant ($p < 0.000$) 1 time increased in rectal temperature ($39.9 \pm 0.54^\circ\text{C}$) at 2 h (p.i) but lasted for 2 h when the temperature returned to normal at 4 h (p.i) Similarly, the affected calves showed significant severe dullness and depression ($p < 0.000$) with mean score of 2.63 ± 0.49 (Fig. 1) whereas rumen hypomotility signs

was observed at 9 h (p.i) and persisted until the end of 48 h of experimental period. All the calves in group 4 showed significant rumen hypomotility ($p < 0.000$) with mean score of 2.80 ± 0.41 (Table 2). All calves survived the inoculation (Table 3).

Pathological changes: The lungs of the calves in groups 2, 3 and 4 appeared mosaic and congested, involving all lung lobes (Fig. 2). Microscopically, the lesions appeared as severe congestion of alveolar capillaries (Fig. 3), thickening of inter-alveolar septa with oedema (Fig. 4) and inflammatory cells and haemorrhages into the alveoli lumen. There were also thrombus formations in major blood vessels of the lungs of calves of group 2.

Calves of group 2 showed no significant of pulmonary oedema ($p = 0.240$) with mean score lesion of 0.16 ± 0.05 compared to control group 1. Calves of

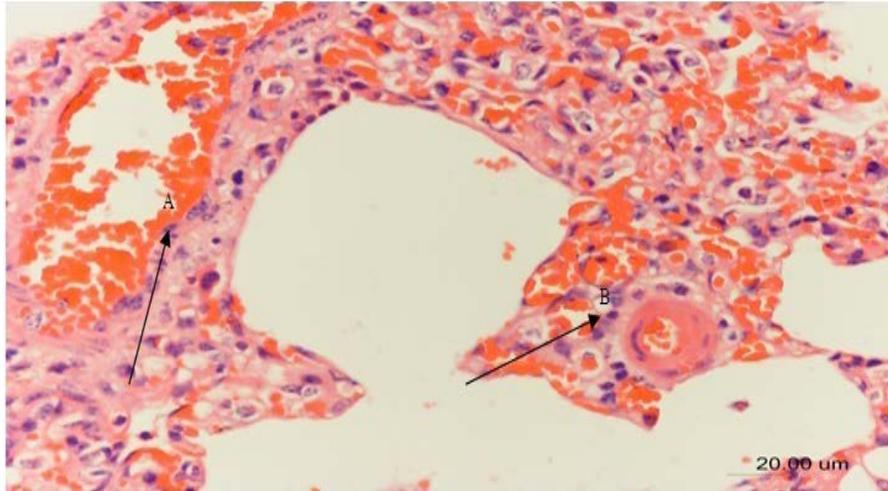


Fig. 3: Photomicrograph of a section of a lung of a calf inoculated with whole cell *P. multocida* B: 2. Note congestion of the alveolar capillaries (A) and haemorrhage into the alveolar lumen (B) H&E×40

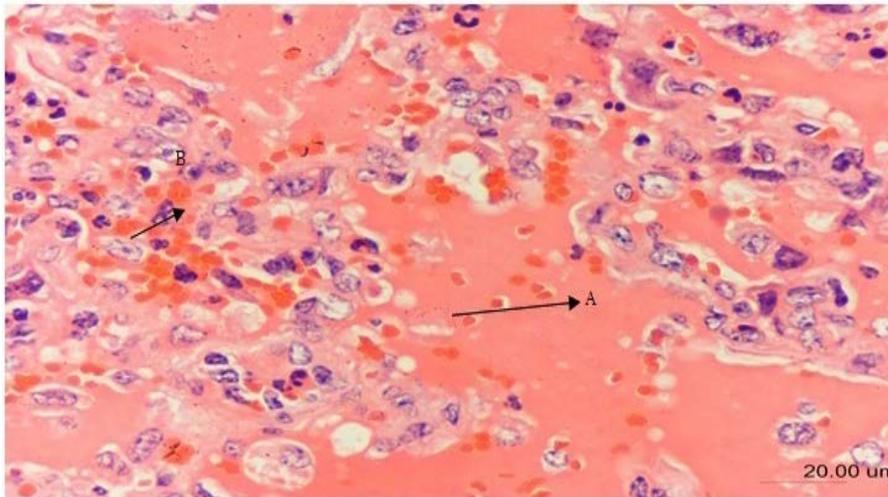


Fig. 4: Photomicrograph of a section of the lung of a calf inoculated with LPS. Note congestion of alveolar capillaries (B), while alveolar spaces are filled with oedema fluid (A) H&E×400

group 4 also showed no significant pulmonary oedema ($p = 0.612$) with mean score lesion of 0.04 ± 0.01 compared to control group 1. Calves of group 3 showed significant moderate pulmonary oedema ($p < 0.000$) with mean score of 2.10 ± 0.99 (Table 4). All the three treatment groups showed significant ($p < 0.05$) in the presence of inflammatory cells in the lung. Calves of group 2 showed significant moderate presence of inflammatory cells in the lung ($p < 0.000$) with mean score lesion of 2.27 ± 0.67 compared to control group 1. Calves of groups 3 and 4 showed similar significant mild to moderate presence of inflammatory cells in the lung ($p < 0.000$) for group 3 ($p < 0.000$) and for group 4 with mean score of 1.63 ± 0.53 and 1.98 ± 0.59 respectively (Table 4). All the three treatment groups showed significant ($p < 0.05$) in the presence of degeneration and necrosis of cells in the lung. Calves of groups 2, 3 and 4 showed similar significant ($p < 0.000$)

mild degeneration and necrosis of cells in the lung. Calves from group 2 showed mean score of 1.57 ± 0.65 , group 3 mean score of 1.00 ± 0.35 and group 4 mean score of 1.33 ± 0.47 (Table 4). For haemorrhage in the lung, calves of group 2 showed significant severe haemorrhage ($p < 0.000$) with the mean score of 2.76 ± 0.43 . For calves in groups 3 and 4, significant moderate haemorrhage ($p < 0.000$) with mean score of 2.14 ± 0.35 and 2.02 ± 0.14 respectively were observed (Table 4). For thrombus formation in the lung, only calves in group 2 showed significant ($p < 0.000$) mild thrombus formation with mean score of 0.51 ± 0.74 (Table 3). There is no significant thrombus formation in the lung of calves in groups 3 ($p = 0.352$) and 4 ($p = 0.184$) with mean score of 0.02 ± 0.01 and 0.04 ± 0.02 respectively (Table 4).

The three treated group showed significant ($p < 0.05$) in the presence of inflammatory cells in the

Table 4: Mean score of cellular changes in the lung of calves after 48 h of post-inoculation with immunogens

Parameters	Group1 (control)	Group 2 (<i>P. multocida</i>)	Group 3 (LPS)	Group 4 (OMP)
Oedema	0.02±0.01	0.16±0.05	*2.10±0.99	0.04±0.21
Inflammatory cells	0.00±0.00	*2.27±0.67	*1.63±0.53	*1.98±0.59
Degeneration/necrosis	0.00±0.00	*1.57±0.65	*1.00±0.35	*1.33±0.47
Haemorrhage	0.00±0.00	*2.76±0.43	*2.14±0.35	*2.02±0.14
Thrombus	0.00±0.00	*0.51±0.74	0.02±0.14	0.04±0.20

*: Significant value $p < 0.05$ (ANOVA and Tukey-Kramer was used for the analysis) for the comparison between immunogens groups and negative control group calves

Table 5: Mean score of cellular changes in the liver of calves after 48 h of post-inoculation with immunogens

Parameters	Group 1 (control)	Group 2 (<i>P. multocida</i>)	Group 3 (LPS)	Group 4 (OMP)
Inflammatory cells	0.00±0.00	*2.12±0.48	*1.51±0.51	*1.98±0.59
Degeneration/necrosis	0.00±0.00	*1.82±0.67	*2.06±0.56	*1.59±0.54
Haemorrhage	0.00±0.00	*2.06±0.59	*1.65±0.59	*1.16±0.75
Kupffer cell	0.00±0.00	*1.47±0.58	*2.47±0.65	*0.35±0.06

*: Significant value $p < 0.05$ (ANOVA and Tukey-Kramer was used for the analysis) for the comparison between immunogens groups and negative control group calves

Table 6: Mean score of cellular changes in the heart of calves after 48 h post-inoculation with immunogens

Parameters	Group1 (Control)	Group 2 (<i>P. multocida</i>)	Group 3 (LPS)	Group 4 (OMP)
Inflammatory cells	0.00±0.00	*1.90±0.55	*1.80±0.46	*1.80±0.29
Degeneration/necrosis	0.00±0.00	*2.29±0.84	*2.65±0.63	*1.88±0.93
Haemorrhage	0.00±0.00	*1.88±0.53	*1.84±0.51	*1.37±0.53

*: Significant value $p < 0.05$ (ANOVA and Tukey-Kramer was used for the analysis) for the comparison between immunogens groups and negative control group calves



Fig. 5: Photograph of the liver of a calf inoculated with the OMP group showing moderate congestion

liver. Calves of group 2 showed significant moderate presence of inflammatory cells in the liver ($p < 0.000$) with mean lesion score of 2.12 ± 0.48 compared to control group 1. Calves of groups 3 ($p < 0.000$) and 4 ($p < 0.000$) showed significant mild presence of inflammatory cells in the liver with mean score of 1.51 ± 0.51 and 1.57 ± 0.54 respectively (Table 5). The three treated group showed significant ($p < 0.05$) presence of degeneration and necrosis of hepatocytes. Calves of groups 2 and 3 showed significant ($p < 0.000$) moderate degeneration and necrosis of hepatocytes with mean score of 1.82 ± 0.67 and 2.06 ± 0.56 respectively. Calves of group 4 showed significant ($p < 0.000$) mild degeneration and necrosis of hepatocytes with mean score of 1.59 ± 0.54 (Table 5).

For haemorrhage in the liver, calves of group 2 showed significant moderate haemorrhage in the liver ($p < 0.000$) with mean score of 2.06 ± 0.59 . For calves in groups 3 and 4 significant mild haemorrhage in liver ($p < 0.000$) with mean score of 1.65 ± 0.59 and 1.16 ± 0.75 respectively was observed (Table 5). For presence of Kupffer cells in the liver, calves in group 3 showed significant ($p < 0.000$) moderate presence of Kupffer cell with mean score of 2.47 ± 0.65 (Table 5). There is a significant ($p < 0.000$) mild presence of Kupffer cells in the liver of calves in group 2 with mean score of

1.47 ± 0.58 whereas calves in group 4, there was significant ($p < 0.000$) very mild presence of Kupffer cells in liver of calves in group 4 with mean score of 0.35 ± 0.06 (Table 5).

All three treated groups showed significant ($p < 0.05$) presence of inflammatory cells in the heart. Calves of groups 2, 3 and 4 showed significant moderate presence of inflammatory cells in the heart ($p < 0.000$) with mean lesion score of 1.90 ± 0.55 , 1.80 ± 0.46 and 1.80 ± 0.29 respectively (Table 6). All three treated groups showed significant ($p < 0.05$) presence of degeneration and necrosis of cardiomyocytes. Calves of group 3 showed significant ($p < 0.000$) severe degeneration and necrosis of cardiomyocytes with mean score of 2.65 ± 0.63 . Calves of groups 2 and 4 showed similar significant ($p < 0.000$) moderate degeneration and necrosis cardiomyocytes with mean score of 2.29 ± 0.84 and 1.88 ± 0.93 respectively (Table 6). For haemorrhage in the heart, calves of groups 2 and 3 showed significant moderate haemorrhage of the heart ($p < 0.000$) with mean score of 1.88 ± 0.53 and 1.84 ± 0.51 respectively. For calves in group 4, significant mild haemorrhage of the heart ($p < 0.000$) with mean score of 1.37 ± 0.53 was evident (Table 6).

The liver appeared congested, which was severe in group 2 but moderate in groups 3 and 4 (Fig. 5). The congestion affected the sinusoids with necrosis of the hepatocytes (Fig. 6). There was presence of numerous Kupffer cells in the liver of calves of groups 3 and 4 (Fig. 7).

DISCUSSION AND CONCLUSION

Haemorrhagic Septicaemia (HS) is a disease with clinical course of very short duration involving severe

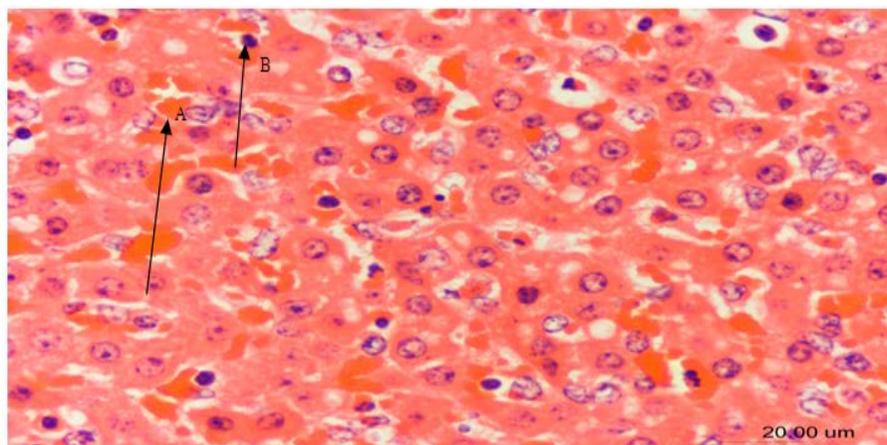


Fig. 6: Photomicrograph of a section of the liver of a calf inoculated with whole cell *P. multocida* B: 2. Note severe congestion of sinusoids (A) and necrosis (pyknosis) of hepatocytes (B); H&E×200

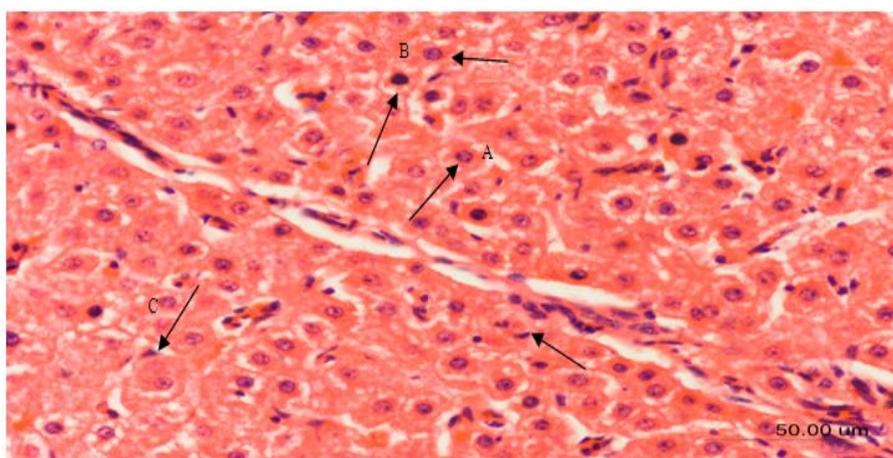


Fig. 7: Photomicrograph of a section of the liver of a calf inoculated with LPS. Note the hydropic degeneration, necrosis (A&B) and presence of kupffer cells (C) H&E×100

depression, pyrexia, submandibular oedema and dyspnea (Wijewardana, 1992; Carter and De-Alwis, 1989; Mohammad and Mohd, 2011). Horadagoda *et al.* (2002) demonstrated the development of clinical disease resembling haemorrhagic septicaemia in the buffaloes following intravenous inoculation of endotoxin of *P. multocida* B: 2, while Jacobsen *et al.* (2004) showed that all cows developed clinical syndrome after inoculation with LPS. The characteristic clinical features include depression, anorexia, pyrexia, tachypnea, tachycardia, ruminal hypomotility and diarrhea. However, fever is the most significant clinical indicator of microbial infection of haemorrhagic septicaemia. It is one of the mechanisms that immune system utilizes to rid the body of invading pathogens (Tatro, 2000). Similarly, Thompson (2005) stated that fever is a normal adaptive systemic response to an immune stimulus while Jiang (2000) mentioned that fever is the body's natural response to illness that has been shown to improve survival rates and shorten the duration of disease.

Substances that induce fever are termed pyrogens and they can be exogenous, such as pathogens, bacterial toxins, antigen-antibody complexes, or endogenous such as interleukin and interferon (Tatro, 2000). This study found that all *P. multocida* B: 2 immunogen treated calves developed significant increased in body temperature, a result that is consistent with those of Wijewardana (1992), Carter and De-Alwis (1989), Jiang (2000), Tatro (2000), Horadagoda *et al.* (2002), Jacobsen *et al.* (2004) and Thompson (2005). Calves inoculated with whole cell *P. multocida* B: 2 showed most severe clinical signs that lasted longer than other groups. Nevertheless, diarrhea that was observed by Jacobsen *et al.* (2004) was absent in this study.

Gross post mortem lesions in cattle during outbreak of HS were fibrinous deposits, marbling and adhesions of the lungs to the rib case. The lungs were severely consolidated with liver-like consistency. All visceral organs exhibited petechial to ecchymotic haemorrhages on the serosal surfaces. In some animals, hydrothorax, pleurisy and hydropericardium were also prominent.

The heart revealed epicardial and endocardial haemorrhages indicating septicaemia in most cases but in some cases either epicardial or endocardial haemorrhages were recorded (Kumar *et al.*, 2006). Kumar *et al.* (2006) indicated that the lung is the worst affected organs during outbreak of HS seen by histopathological examination. The pleura were thickened due to deposition of fibrin and infiltration of inflammatory cells. The alveolar septae were thickened due to fibrinous oedema giving marbling appearance to lungs and the blood vessels were highly congested with evidence of thrombosis of some blood vessels. Alveolar haemorrhages and marked infiltration of inflammatory cells were also noticed. Similar findings have been reported by earlier workers (Schiefer *et al.*, 1978; Gibbs *et al.*, 1984; Allan *et al.*, 1985; Lane *et al.*, 1992; Priadi and Natalia, 2000). Rhoades *et al.* (1967) stated that intramuscular inoculation of *P. multocida* in calves showed interstitial pneumonia with hyperemia and oedema in the lungs and increased in number of lymphocytes and macrophages in the thickened alveolar septae, along with a few neutrophils. Rhoades *et al.* (1967) also observed that the liver was hyperemic and the hepatocytes showed cloudy swelling and fatty degenerative changes. For the heart, marked hyperemia along with subepicardial and subendocardial haemorrhages (Rhoades *et al.*, 1967) were evident. Examination of the lungs from the calf which died following intravenous administration of *P. multocida* endotoxin revealed marked hyperemia and numerous areas of haemorrhage. Oedema was extensive in the lung parenchyma and numerous erythrocytes and haemorrhage were observed in the lungs. In the liver, cloudy swellings were observed (Rhoades *et al.*, 1967).

In this study, the gross lesions of positive control group calves were similar with that observed by Kumar *et al.* (2006) where the lungs were congested and showed mosaic appearance. The liver was congested and the heart revealed moderate haemorrhage at the endocardial region. In this study, the positive control group calves showed cellular changes similar to that observed by earlier workers (Rhoades *et al.*, 1967; Schiefer *et al.*, 1978; Gibbs *et al.*, 1984; Allan *et al.*, 1985; Lane *et al.*, 1992; Priadi and Natalia, 2000; Kumar *et al.*, 2006). For LPS immunogen groups, the results of gross and cellular changes in this study revealed similarities with the findings of Rhoades *et al.* (1967) except for the presence of numerous number of Kupffer cells in the liver. For the OMP group calves, these results added new knowledge to limited previous works on the effects of OMP. From this study, the gross and cellular changes in the lungs, heart and liver in the OMP immunogen group calves showed similar lesions as in positive control group calves and there were also presences of Kupffer cells in the liver.

Therefore, it can be concluded that the positive control group calves, exhibited host cell response. The severity of host cell response differs between each group. The findings from the current study will assist in the improvement of the vaccines and the vaccination

methods currently employed in controlling this important disease in Malaysia.

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