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Acute Phase Protein Profiles in Calves Following Infection with Whole cell, Lipopolysaccharide and Outer Membrane Protein Extracted from *Pasteurella multocida* Type B: 2

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ABSTRACT

Acute Phase Protein (APP) investigations of serum or plasma following natural or experimental infection frequently reveal substantial alterations in the APPs, several of which are of veterinary importance in the assessment of herd health. The present study of the experimental nature was conducted to evaluate the acute phase protein responses; haptoglobin, Serum Amyloid A (SAA) and serum albumin in relation to infection with *Pasteurella multocida* type B and its immunogens; lipopolysaccharide (LPS) and Outer Membrane Protein (OMP) in calves. Eight clinically healthy, non-pregnant and non-lactating Brangus cross calves weighing 150±50 kg were used in this study. The calves (n = 8) were divided into 4 groups of 2 calves in each group. The control group was inoculated with sterile Phosphate Buffered Saline (PBS) whereas group 2 were inoculated with wild-type *P. multocida* type B:2 and group 3 and 4 were inoculated with LPS and OMP respectively. Blood samples were collected via jugular vein-puncture at 3 h intervals for APPs analysis. APPs were quantified by commercially available ELISA methods. Moribund animals were euthanized while the surviving animals were killed after 48 h. The results revealed that there were statistically significant differences (p<0.05) between APPs concentrations throughout the experimental period in challenged groups compared to control groups. Over 7-fold increase was observed in Hp concentrations with mean maximum levels of 1.316±0.558 ng mL⁻¹, 1.521±0.687 ng mL⁻¹ after experimentally induced with OMP and LPS respectively. SAA increased less than 3-fold with mean levels of 2.187±0.880 ng mL⁻¹, 2.421±0.432 ng mL⁻¹, 2.657±0.099 ng mL⁻¹ in almost all challenged groups; *Pasteurella Multocida*, OMP and LPS respectively. In contrast, for the negative APP, the albumin levels of groups LPS and OMP did not show significant difference (p>0.05) with mean levels of 32.677±1.556 and 36.185±2.239 U L⁻¹, respectively. While *P. multocida* group (22.193±2.727 U L⁻¹) showed statistically significant difference (p<0.05) than the negative control group (34.233±6.900 U L⁻¹). In conclusion, the findings of this study indicated that APPs; SAA and haptoglobin are sensitive biomarkers to explore host response in relation to Haemorrhagic Septicaemia infections in clinical settings.

Key words: Haemorrhagic septicaemia, *Pasteurella multocida* type B, lipopolysaccharides, outer membrane protein, calves, haptoglobin, serum amyloid A, albumin

INTRODUCTION

In Malaysia, the large ruminant sector is mainly threatened by fatal, infectious disease with regular outbreaks causing huge impact on the national economic conditions of the country. As such this infectious disease is Haemorrhagic Septicaemia (HS), a major disease of cattle and buffaloes with high morbidity and mortality in southern and south east Asia, including Malaysia and a number of countries in all regions of Africa (De Alwis, 1992). It is mainly caused by a Gram⁻ bacterium; *Pasteurella multocida* type B: 2.

For controlling the infectious diseases, the Department of Veterinary Services (DVS), Malaysia, has embarked on large scale National Immunization Plan (NIP) with strategic support of the Ministry of Agriculture and Agro-Based Industry. Within the current NIP; specifically in the context of controlling Haemorrhagic Septicaemia (HS), killed whole cell vaccines have been adopted. These vaccines have certain limitations, such as shorter duration of immunity and swelling at the site of inoculation. Moreover, outbreaks of HS have been reportedly documented to re-occur despite vaccinations (Carter and de Alwis, 1989). Previous studies have suggested that capsular antigens, lipopolysaccharide (LPS) or LPS-protein complex and the outer membrane proteins (OMPs), including the iron-regulated OMPs as effective immunogens for sero-groups B and E (Muniandy *et al.*, 1993). Other studies of experimental nature reported successful demonstration of infection with *Pasteurella multocida* type B:2 (Jesse *et al.*, 2013). However, quantitative evidence of host responses in relation to the acute phase proteins induced by whole cell, Outer Membrane Protein (OMP) and lipopolysaccharide (LPS) extracted from *P. multocida* type B: 2 is still scarce. Furthermore, there is a need to improve disease surveillance systems as well as to identify new indicators of health and disease. Thus, Acute Phase Proteins (APPs) have been recently developed and used widely as biological markers in veterinary medicine. Haptoglobin and Serum Amyloid A (SAA) are important bovine APPs, which increase in serum for example during viral and bacterial diseases (Murata *et al.*, 2004; Petersen *et al.*, 2004), but are absent, or present in very low levels, in healthy animals (Heegaard *et al.*, 2000; Ganheim *et al.*, 2003). Therefore, quantification of their serum concentration is clinically useful in both diagnosis and prognosis. Although, in the last two decades, many advances in monitoring of the APPs response in animals for clinical and experimental purposes have been achieved; yet there is no documentation of acute phase protein responses in experimental studies with infections of *P. multocida* type B:2 and its immunogens, OMP and LPS. Therefore, this study was conducted with objective of determining the levels of haptoglobin, Serum Amyloid A (SAA) and albumin in calves of experimentally infected with *Pasteurella multocida* type B and its immunogens. Different patterns of APP concentration changes were also compared to explore host response differences in relation to the character of inocula between these groups. This will assist in the improvement of the vaccine production to control this important disease (HS) in Malaysia.

MATERIALS AND METHODS

Animals: All procedures and experiments described were undertaken under a project license approved by Animal Utilization Protocol Committee. Eight clinically healthy, non-pregnant and non-lactating Brangus cross heifers weighing 150±50 kg were used. Upon arrival at the Animal Experimental House (AEH), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), anthelmintic (Ivermectin) was administered subcutaneously (S/C) at the rate of 1 mL/50 kg body weight to control internal parasitism, which has been shown to influence disease development (Kushner, 1982). 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 start of 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 preparation: Throughout the experiments, three types of inocula were used; the whole cell, lipopolysaccharide (LPS) and Outer Membrane Protein (OMP) extracted from of *P. multocida* type B: 2.

Wild-type *P. multocida* type B: The wild-type *P. multocida* type B, used in this study, was 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 microscopic examination, Gram-staining method and was biochemically identified. Thereafter, molecular characterization was performed and confirmed as *P. multocida* type B:2 at 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.

Lipopolysaccharide (LPS) extracted from *P. multocida* type B: The lipopolysaccharide (LPS) Extraction Kit (Intron Biotechnology) was used to prepare the inoculum of LPS. LPS was extracted from 10¹² Colony Forming Unit (CFU) of *P. multocida* type B:2. 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 then incubated for 5 min at room temperature before centrifuged at 13,000 rpm for 10 min at 4°C. Following this, 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 -20°C. This was followed by another centrifugation at 13,000 rpm for 15 min at 4°C. Finally, the LPS pellet was washed with 1 mL 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.

Outer Membrane Protein (OMP) of *P. multocida* type B: The Qproteome™ Bacterial Protein Extraction kit was used to prepare the inoculum of OMP. Outer Membrane Protein (OMP) was extracted from 10¹² Colony Forming Unit (CFU) of the organism (*P. multocida* type B:2). Briefly, 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. Supernatant containing the soluble fraction of the bacterial Outer Membrane Protein (OMP) was retained and subjected to SDS-PAGE to locate the range of protein bands present in the extraction.

Animals, sampling procedure and clinical examination: A group of 8, Brangus cross calves were used in this study. Calves were housed in single group fences with an automatic milk feeding

system. The calves (n = 8) were divided into 4 groups of 2 calves in each group. The negative 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* type B 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. The detailed information of the individuals used in the experiment was outlined in Table 1.

Calves were initially included in the study to investigate the effects of the inocula on the physiological concentration of APPs and were blood sampled at 3 h intervals without any other manipulation. All animals were observed for 48 h for clinical signs, changes in behavior and mortality pattern. The clinical signs monitored were temperature, rumen motility, movement and dullness. Clinical scoring was done and the data was analyzed using R software. Blood samples were taken at 3 h intervals from the jugular vein using an 18 gauge, one inch precision glide vacutainer needle inserted into 10×10 mL vacutainer tubes containing 0.054 mL of 15% Tri-potassium ethylene diaminetetraacetic acid (K3EDTA) and sodium citrate containing tubes; serum was separated by centrifugation, frozen in portions and stored at -20°C to await further analysis. Calves were clinically examined in conjunction with each blood sampling. Clinical score system used in this study was summarized in Table 2. Moribund animals were euthanized while the surviving animals were killed after 48 h (experimental period). Post-mortems examinations were carried out where lung, liver and heart samples were fixed for microscopic observation and cellular changes were scored (data excluded).

Table 1: Detailed information about the animals used in the experiment

Calves	Sex	Breed	Status	Inoculums character	Dose	Administration route
1	M	Brangus cross	Non-pregnant and non-lactating	^a PBS	10 mL	I.m.
2	F	Brangus cross	Non-pregnant and non-lactating	^a PBS	10 mL	I.m.
3	M	Brangus cross	Non-pregnant and non-lactating	^b Pm	10 ¹² CFU	I.m.
4	F	Brangus cross	Non-pregnant and non-lactating	^b Pm	10 ¹² CFU	I.m.
5	M	Brangus cross	Non-pregnant and non-lactating	^c LPS	10 ¹² CFU	I.v.
6	F	Brangus cross	Non-pregnant and non-lactating	^c LPS	10 ¹² CFU	I.v.
7	M	Brangus cross	Non-pregnant and non-lactating	^d OMP	10 ¹² CFU	I.m.
8	F	Brangus cross	Non-pregnant and non-lactating	^d OMP	10 ¹² CFU	I.m.

^a: *Pasteurella multocida* type B; ^b: Phosphate buffered saline, ^c: Lipopolysaccharide and ^d: Outer membrane protein

Table 2: Clinical score procedure used in the assessment of the tested parameters

Parameters	Clinical scoring					
	0	1	2	3	4	5
Rectal temperature (°C)	<39	39.0-39.5	39.6-39.9	40.0-40.4	>40.5	n/a*
Respiratory rate (min ⁻¹)	<40	40-49	50-59	60-69	70-79	>79
Nasal discharge	Normal	Serous	Mucopurulent	Purulent	n/a*	n/a*
Lung auscultation	No abnormal sound	Wheezing sounds	Crackle sound	n/a*	n/a*	n/a*
Cough	No cough	Occasional sporadic cough	More than one spontaneous cough	n/a*	n/a*	n/a*
Demeanour	Bright	Mildly depressed	Moderately depressed	Severely depressed	n/a*	n/a*

*n/a: Not applicable

Acute Phase Proteins (APPs) analysis: The test kits were highly sensitive, two sites enzyme linked immunoassay (ELISA) obtained from Life Diagnostics Inc. (West Chester) info@lifediagnosics.com and from Tridelta Development Ltd (Ireland) info@trideltaltd.com. The APPs used in this study included bovine serum amyloid A (SAA) and bovine haptoglobin (Hp).

Bovine Serum Amyloid A (SAA): Serum SAA concentrations (ng mL^{-1}) were determined using commercial ELISA kits (Tridelta PHASE™, Tridelta Development, Ltd., Wicklow, Ireland). The assay was performed according to the manufacturer's instructions. Serum samples were initially diluted 1:1000. Samples obtained from challenged groups and the standards, were tested in duplicate. Control samples were measured in triplicate and a blank (assay buffer only) measured in quadruplicate. Samples reading outside the range of the standard curve were diluted further and reassayed. The absorbance was measured at 450 nm using automatic plate reader (BioRad). The mean absorbance for each sample, control or calibrator was then calculated.

Bovine Haptoglobin (Hp): Serum Hp concentration (ng mL^{-1}) was measured using commercial ELISA kits (Life Diagnostic Inc.) and performed according to the manufacturer's instructions with all steps carried out at room temperature. Serum samples were initially diluted 1:10,000. Samples were tested neat and all samples including the standards, were run in duplicate. Samples with an optical density outside the range of the standard curve were diluted further and reanalyzed. Optical densities were read on an automatic plate reader (BioRad) at 450 nm. The mean absorbance for each sample, control or calibrator was then calculated.

Statistical analysis: The statistical package R software (version Rx64 2.15.0) for Windows was used. Results are expressed as means \pm standard deviation ($M \pm SD$). APP values were summarized and subjected to Analysis of Variance (ANOVA). An error level of 0.05 was used.

RESULTS

In general, the finding of the current study indicated significant differences ($p < 0.05$) in the terms of producing clinical signs (data excluded) and alterations in the serum levels of positive and negative acute phase proteins. All the acute phase protein responses (APR) were significantly ($p < 0.05$) associated with type of acute phase protein and the character of inoculum.

Concentrations of haptoglobin (HP), serum amyloid A (SAA) and serum albumin: Calves in all treated groups showed significant ($p < 0.05$) changes in the acute phase proteins concentration (Table 3). Haptoglobin concentration increased markedly in all infected calves (Table 3). Among the challenged group, Haptoglobin levels of calves induced with LPS showed significant differences ($p < 0.05$) with maximum mean concentration of $1.521 \pm 0.687 \text{ ng mL}^{-1}$. OMP challenged group

Table 3: Acute phase proteins concentration following inoculation with different immunogens (Mean \pm SD)

Parameters	Groups			
	Control	<i>Pasteurella multocida</i> type B:2	*OMP	^b LPS
Haptoglobin (ng mL^{-1})	0.202 \pm 0.054	0.600 \pm 0.419*	1.316 \pm 0.558*	1.521 \pm 0.687*
Serum amyloid A (ng mL^{-1})	1.571 \pm 0.795	2.187 \pm 0.880	2.421 \pm 0.432*	2.657 \pm 0.099*
Albumin (U L^{-1})	34.233 \pm 6.900	22.193 \pm 2.727*	36.185 \pm 2.239	32.677 \pm 1.556

^a: Outer membrane protein, ^b: Lipopolysaccharide and *Significant value $p < 0.05$

(1.316 ± 0.558 ng mL⁻¹) showed also highly significant levels ($p < 0.05$) of haptoglobin compared to control group. Moreover, *P. multocida* treated group (0.600 ± 0.419 ng mL⁻¹) showed considerably elevated levels of haptoglobin in comparison to the control group (0.202 ± 0.054 ng mL⁻¹). Similarly, the Serum Amyloid A (SAA) protein of LPS and OMP groups showed a significant differences ($p < 0.05$) with mean levels of 2.657 ± 0.099 and 2.421 ± 0.432 ng mL⁻¹, respectively. *P. multocida* group (2.187 ± 0.880 ng mL⁻¹), however, did not show significant difference in SAA protein concentration compared to the control group. In contrast, for the negative APP the albumin concentration of groups LPS and OMP did not show significant differences ($p > 0.05$) with mean levels of 32.677 ± 1.556 U L⁻¹ and 36.185 ± 2.239 U L⁻¹, respectively. While *P. multocida* group (22.193 ± 2.727 U L⁻¹) differed significantly ($p < 0.05$) than the control group (34.233 ± 6.900 U L⁻¹).

DISCUSSION

In the present study, the APPs response were significantly different ($p < 0.05$) in all treated groups of calves. These results were in agreement with other studies (Kent, 1992; Baumann and Gauldie, 1994; Rokke *et al.*, 1988). For this study, all treated groups of calves showed significant increases ($p < 0.05$) in Hp and SAA concentrations. The concentrations of SAA and Hp were the highest in calves infected with LPS extracted from *Pasteurella multocida* type B:2, followed by the calves challenged with OMP where the calves challenged with *Pasteurella multocida* type B:2 showed considerable increase in concentration. It is believed that the longer duration and severity of tissue damage had led to the highest concentration of Hp and SAA in LPS and OMP treated groups. The LPS challenged calves showed increase production of APP but none of the calves showed clinical signs of illness in comparison to the calves infected with *Pasteurella multocida* type B:2. It is possible that the dose of LPS inoculated was insufficient to produce its endotoxic effects and subsequent clinical manifestation. A continuous infusion of LPS would allow its prolonged presence in the blood that would mimic more closely to clinical septicaemia (Jacobsen *et al.*, 2004). However, the LPS dose given to the calves was able to cause increased in Hp and SAA concentration where the calves were pyrexia (Gerros *et al.*, 1993). The LPS dose inoculated was able to initiate concentrations APP responses which therefore suggest LPS dose dependent APP response (Eckersall and Conner, 1988). The evidence of APP production in the OMP group calves is a new contribution to the available database. The Hp and SAA levels increased significantly in calves to which *Pasteurella multocida* type B:2 has been induced, but, however, the increased levels were not so high as LPS and OMP immunogen groups. This could be explained that the calves infected with *Pasteurella multocida* type B:2 group had died earlier as a result of the infection in comparison to those infected with LPS and OMP immunogen groups. The concentrations and kinetics of systemic APPs during inflammatory response appeared to be related to the severity of tissue damage and time course of the inflammation process (Kent, 1992). Increased concentration of serum Hp was detected in these calves which suggest an acute disease such as HS in cattle where high serum Hp levels have been reported in gram negative bacterial infection including HS (Conner *et al.*, 1989; Alsemgeest *et al.*, 1994; Hirvonen *et al.*, 1996; Horadagoda *et al.*, 1999). However, these biomarkers (Hp and SAA) were sensitive APPs for *P. multocida* type B infection in response to the bacterial immunogens.

CONCLUSION

Serum concentration of acute phase proteins is affected by several factors ranging from physiological to management conditions. With the aim of minimizing such confounding factors, this

study showed significant differences ($p < 0.05$) in levels of the haptoglobin, serum amyloid A and albumin in calves of experimentally infected. Hence, it is concluded that these quantitative indicators can be used as a potential biomarkers for detection of Haemorrhagic Septicaemia (HS) infections in susceptible animals.

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REFERENCES

- Alsemgeest, S.P.M., H.C. Kalsbeek, T. Wensing, J.P. Koeman, A.M. van Ederen and E. Gruys, 1994. Concentrations of Serum Amyloid-A (SAA) and Haptoglobin (HP) as parameters of inflammatory diseases in cattle. *Vet. Q.*, 16: 21-23.
- Baumann, H. and J. Gauldie, 1994. The acute phase response. *Immunol. Today*, 15: 74-80.
- Carter, G.R. and M.C.L. de Alwis, 1989. Haemorrhagic Septicaemia. In: *Pasteurella and Pasteurellosis*, Adlam, C. (Ed.). Academic Press, London, New York, ISBN-13: 9780120442744, pp: 37-73, 131-160.
- Conner, J.G., P.D. Eckersall, A. Wiseman, R.K. Bain and T.A. Douglas, 1989. Acute phase response in calves following infection with *Pasteurella haemolytica*, *Ostertagia ostertagi* and endotoxin administration. *Res. Vet. Sci.*, 47: 203-207.
- De Alwis, M.C.L., 1992. Haemorrhagic septicaemia-a general review. *Br. Vet. J.*, 148: 99-112.
- Eckersall, P.D. and J.G. Conner, 1988. Bovine and canine acute phase proteins. *Vet. Res. Commun.*, 12: 169-178.
- Ganheim, C., C. Hulten, U. Carlsson, H. Kindahl, R. Niskanen and K.P. Waller, 2003. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or *Mannheimia haemolytica*. *J. Vet. Med. B*, 50: 183-190.
- Gerros, T.C., S.D. Semrad, R.A. Proctor and A. LaBorde, 1993. Effect of dose and method of administration of endotoxin on cell mediator release in neonatal calves. *Am. J. Vet. Res.*, 54: 2121-2127.
- Heegaard, P.M.H., D.L. Godson, M.J.M. Toussaint, K. Tjørnehoj, L.E. Larsen, B. Viuff and L. Ronsholt, 2000. The acute phase response of haptoglobin and Serum Amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Vet. Immunol. Immunopathol.*, 77: 151-159.
- Hirvonen, J., S. Pyorala and H. Jousimies-Somer, 1996. Acute phase response in heifers with experimentally induced mastitis. *J. Dairy Res.*, 63: 351-360.
- Horadagoda, N.U., K.M. Knox, H.A. Gibbs, S.W. Reid, A.S.E. Horadagoda, S.E. Edwards and P.D. Eckersall, 1999. Acute phase proteins in cattle: Discrimination between acute and chronic inflammation. *Vet. Rec.*, 144: 437-441.
- Jacobsen, S., P.H. Andersen, T. Toelboell and P.M.H. Heegaard, 2004. Dose dependency and individual variability of the Lipopolysaccharide-induced bovine acute phase protein response. *J. Dairy Sci.*, 87: 3330-3339.

- Jesse, F.F., Y.O. Abdinasir, L. Adamu, M. Syamil and A.R. Omar et al., 2013. Polymerase chain reaction detection of *Pasteurella multocida* type B: 2 in mice following oral inoculation. *Asian J. Anim. Vet. Adv.*, 3: 493-501.
- Kent, J., 1992. Acute phase proteins: Their use in veterinary diagnosis (guest editorial). *Br. Vet. J.*, 148: 279-282.
- Kushner, I., 1982. The phenomenon of the acute phase response. *Ann. N. Y. Acad. Sci.*, 389: 39-48.
- Muniandy, N., J. Edgar, J.B. Woolcock and T.K.S. Mukkur, 1993. Virulence, purification structure and protective potential of the putative capsular polysaccharide of *Pasteurella multocida* type 6:B. *Proceedings of the Pasteurellosis in Production Animals, an International Workshop*, August 10-13, 1992, Bali, Indonesia, pp: 47-54.
- Murata, H., N. Shimada and M. Yoshioka, 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. *Vet. J.*, 168: 28-40.
- Petersen, H.H., J.P. Nielsen and P.M.H. Heegaard, 2004. Application of acute phase proteins in veterinary clinical chemistry. *Vet. Res.*, 35: 163-187.
- Rokke, O., A. Revhaug, B. Osterud and K.E. Giercksky, 1988. Increased plasma levels of endotoxin and corresponding changes in circulatory performance in a porcine sepsis model: The effect of antibiotic administration. *Prog. Clin. Biol. Res.*, 272: 247-262.