



**UNIVERSITI PUTRA MALAYSIA**

***BUFFALOES' CLINICO-PATHOLOGICAL RESPONSES TO *Pasteurella multocida* TYPE B:2 AND THEIR IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN***

**ERIC LIM TEIK CHUNG**

**FPV 2016 28**



**BUFFALOES' CLINICO-PATHOLOGICAL RESPONSES TO *Pasteurella multocida* TYPE B:2 AND THEIR IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN**

**By**

**ERIC LIM TEIK CHUNG**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**October 2016**

## **COPYRIGHT**

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



## **DEDICATION**

My father (Lim Khong Chiu)  
My mother (Ng Yoke Oi)  
My wife (Lim Ai Phing)  
My siblings (Felicia, Alvin and Andy)  
And my friends

For their care, love, great source of motivation, inspiration, encouragement and endless support.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**BUFFALOES' CLINICO-PATHOLOGICAL RESPONSES TO *Pasteurella multocida* TYPE B:2 AND THEIR IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN**

By

**ERIC LIM TEIK CHUNG**

**October 2016**

**Chairman: Associate Professor Faez Firdaus Jesse Abdullah, PhD**  
**Faculty : Veterinary Medicine**

Sudden death is usually the main finding in field during haemorrhagic septicaemia (HS) outbreaks among livestock in Malaysia. HS is an acute fatal disease, caused by particular serotypes of *Pasteurella multocida*. Epidemic haemorrhagic septicaemia in Asian countries including Malaysia is caused by *P. multocida* serotype B:2. This organism causes acute, highly fatal septicaemia with high morbidity and mortality in cattle and more susceptible in buffaloes. *P. multocida* is a gram negative, short, ovoid, bipolar staining coccoid forms. It is an extracellular parasite, and immunity is primarily humoral. In most cases, the clinical findings are either acute or peracute, resulting death within 8 to 24 hours after onset. The two immunogens of wild-type *Pasteurella multocida* that have the virulence factors are the outer membrane protein (OMP) and lipopolysaccharide (LPS). Little is known about the effect of endotoxin of *P. multocida* type B:2 and its immunogens LPS and OMP towards host cell responses in buffaloes. Thus, this study was designed to investigate the clinico-pathology, haemato-biochemistry changes, the acute phase responses (APP), antibody titres and cytokine concentrations in buffaloes infected by *P. multocida* type B:2 and its immunogens.

A total of twenty one buffalo heifers were divided equally into 7 treatment groups. Group 1 was inoculated orally with 10 mL of phosphate buffer saline (PBS) as negative control; Groups 2 and 3 were infected with 10 mL of  $10^{12}$  colony forming unit (cfu) *P. multocida* type B:2 subcutaneously and orally respectively; Groups 4 and 5 were inoculated with 10 mL of LPS broth intravenously and orally respectively; and OMP broth were inoculated subcutaneously and orally into Groups 6 and 7 buffaloes respectively. During the post infection period, all the buffaloes were observed for clinical signs and clinical response for 21 days. Blood samples were also collected throughout the 21 days for determination of blood and biochemistry changes, concentration of proinflammatory cytokines, APP and antibody titres. At the end of the study, buffaloes that exhibited typical HS signs and buffaloes that survived throughout the 21 days study period were euthanized for post mortem and histopathological examination.

All buffaloes from Groups 1, 3, 4, 5, and 7 were able to survive throughout the stipulated experimental period of 21 days. Group 2 and 6 buffaloes were only able to survive for 12 hours and 3 days respectively. Group 2 buffaloes showed severe HS clinical responses and were only able to survive for 12 hours post infection. The blood and biochemistry results showed erythrocytosis, leukopaenia, neutropaenia and lymphopaenia. All vital organs, gastrointestinal and immune organs were severely affected with severe histopathology lesions. However, Group 3 buffaloes were able to survive throughout the experiment for 21 days despite showing mild clinical responses that only lasted for 4 days. The blood and biochemistry results showed leukocytosis for the first 5 days. Only the lung and liver organs were moderately affected with moderate histopathology lesions. On the other hand, Group 4 buffaloes demonstrated mild clinical responses and survived throughout the stipulated time of 21 days. All buffaloes had leukocytosis, lymphocytosis and monocytosis throughout 21 days experiment. All vital organs, gastrointestinal and immune organs were moderately affected with mild histopathology lesions. Similarly, Group 5 buffaloes demonstrated very mild clinical responses and able to survive for 21 days. Leukocytosis, monocytosis and eosinophilia were observed in this buffalo group. Only the lung and liver had mild gross lesions. Besides, mild histopathology findings were observed in all organs. Similar to Group 2, Group 6 buffaloes also exhibited severe HS clinical responses and were only able to survive for 72 hours post infection. The blood and biochemistry results showed monocytosis and elevated gamma glutamyl transferase (GGT) during the first 72 hours of the experiment prior euthanasia. All vital organs, gastrointestinal and immune organs were severely affected with moderate histopathology changes. In contrast, there were no significant clinical responses in Group 7 buffaloes. All buffaloes only had monocytosis throughout the 21 days of experiment. Gross lesions were only observed in the lung and liver of Group 3 buffaloes with moderate histopathological lesions. Buffaloes in all treatment groups of *P. multocida* type B:2 and its immunogens LPS and OMP showed significant increase in haptoglobin (Hp) and serum amyloid A (SAA) concentrations throughout the study period. There were significant differences ( $p < 0.05$ ) in all treatment groups compared to control group of buffaloes. In addition, buffaloes in all treatment groups showed significant increase in IgM, IgG and IgA concentrations throughout the study period. There were significant different ( $p < 0.05$ ) in all treatment groups compared to control group. Similarly, all treatment groups showed significant increase ( $p < 0.05$ ) in IL-2 and IL-6 concentration; but no significant difference ( $p > 0.05$ ) in IL-12 concentration observed compared to control group.

Therefore, from the present study, it can be concluded that buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP demonstrated different host cell responses. Information on this will play a significant role in understanding the host cell response during HS outbreaks. Buffaloes inoculated orally with OMP demonstrated as a promising candidate to be developed into oral vaccine based on the clinical response, haemato-biochemistry, gross lesion, histopathology finding, and most importantly antibody level. With this, alternative route for HS where oral route of vaccination can be considered compared to intramuscular route in future. Besides that, the development of biomarkers using cytokines in future will be able to control the outbreak of HS which causes major losses with great economic impact to the country.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**TINDAK BALAS SEL BADAN KERBAU TERHADAP *Pasteurella multocida* B:2 DAN IMUNOGEN LIPOPOLISAKARIDA DAN MEMBRAN PROTIN LUAR**

Oleh

**ERIC LIM TEIK CHUNG**

**Oktober 2016**

**Pengerusi : Professor Madya Faez Firdaus Jesse Abdullah, PhD**  
**Fakulti : Perubatan Veterinar**

Kematian mengejut merupakan penemuan pertama pada haiwan semasa penyakit hawar berdarah di Malaysia. Penyakit ini disebabkan oleh serotype bakteria tertentu *P. multocida* yang mengakibatkan kematian dan septisemia dengan kadar jangkitan dan kematian yang sangat tinggi pada lembu dan terutamanya kerbau. Penyakit hawar berdarah di negara Asia termasuklah Malaysia disebabkan oleh *P. multocida* jenis B:2. *P. multocida* adalah bakteria gram negatif, pendek, bujur telur, bipolar apabila diwarnai. Ia adalah parasit luar sel dan imuniti terutamanya humoral. Dalam kebanyakan kes, tanda-tanda klinikal adalah sangat awal dan boleh menyebabkan kematian dalam masa 8 hingga 24 jam selepas dijangkiti. Membran protin luar dan lipopolisakarida merupakan imunogen terdapat pada bakteria *P. multocida* yang mempunyai faktor merbahaya. Tidak banyak yang diketahui tentang kesan endotoksin daripada *P. multocida* jenis B:2 dan imunogennya lipopolisakarida dan membran protin luar kepada sel badan kerbau. Oleh itu, kajian ini telah direka untuk menyiasat perubahan klinikal, darah, bedah siasat, histopatologi, kepekatan akut protein fasa, antibodi dan sitokin dalam kerbau selepas dijangkiti oleh *P. multocida* jenis B:2, lipopolisakarida dan membran protin luar.

21 ekor kerbau dibahagikan sama rata kepada 7 kumpulan rawatan. Kumpulan 1 disuntik dengan 10 mL air suling sebagai kawalan negatif; Kumpulan 2 dan 3 dijangkiti dengan  $10^{12}$  *P. multocida* jenis B:2 masing-masing secara injeksi subkutaneus dan oral; Kumpulan 4 dan 5 disuntik dengan 10ml lipopolysaccharide masing-masing secara intravena dan oral; dan 10 mL protein membran luar disuntik masing-masing secara subkutaneus dan oral untuk kerbau kumpulan 6 dan 7. Dalam tempoh jangkitan, semua kerbau diperhatikan tanda-tanda klinikal selama 21 hari. Sampel darah juga diambil sepanjang 21 hari bagi analisis darah dan untuk menentukan kepekatan akut protein fasa, antibodi dan sitokin. Pada akhir kajian ini, kerbau yang masih hidup selepas 21 hari disembelih dimana sampel organ pernafasan, jantung, hati, ginjal, organ imunisasi dan organ pencernaan diambil untuk pemeriksaan histopatologi.

Semua kerbau dari Kumpulan 1, 3, 4, 5, dan 7 berjaya hidup sepanjang tempoh 21 hari eksperimen. Kerbau Kumpulan 2 dan 6 masing-masing hanya mampu bertahan selama 12 jam dan 3 hari. Kerbau Kumpulan 2 menunjukkan respon penyakit hawar berdarah

dan hanya mampu hidup selama 12 jam selepas jangkitan. Keputusan darah dan biokimia menunjukkan peningkatan eritrosit dan penurunan sel darah putih. Semua organ yang diambil terjejas teruk dengan perubahan histopatologi yang teruk. Walau bagaimanapun, kerbau daripada Kumpulan 3 dapat bertahan sepanjang tempoh kajian iaitu selama 21 hari walaupun menunjukkan sedikit tanda klinikal untuk 4 hari yang pertama. Keputusan darah dan biokimia menunjukkan peningkatan sel darah putih bagi 5 hari yang pertama. Hanya organ paru-paru dan organ hati terjejas dengan perubahan histopatologi yang sederhana. Sebaliknya, kerbau daripada Kumpulan 4 menunjukkan sedikit tanda klinikal dan berjaya hidup sepanjang masa yang ditetapkan iaitu 21 hari. Semua kerbau menunjukkan peningkatan sel darah putih, limfosit dan monosit sepanjang 21 hari eksperimen. Semua organ-organ penting, pencernaan dan organ-organ imun terjejas dengan sedikit perubahan histopatologi. Begitu juga untuk kerbau daripada Kumpulan 5 yang menunjukkan perubahan kecil pada tanda klinikal dan mampu bertahan untuk 21 hari. Keputusan darah dan biokimia menunjukkan peningkatan sel darah putih, monosit dan eosinofil dalam kumpulan kerbau ini. Hanya paru-paru dan hati terjejas dengan sedikit perubahan histopatologi. Sama dengan Kumpulan 2, kerbau daripada Kumpulan 6 mempamerkan respon klinikal penyakit hawar berdarah dimana kerbau-kerbau tersebut hanya mampu bertahan selama 72 jam selepas jangkitan. Keputusan darah dan biokimia menunjukkan peningkatan monosit dan gamma glutamyl transferase yang tinggi. Semua organ-organ penting, pencernaan dan imunisasi terjejas teruk dengan perubahan histopatologi yang sederhana. Sebaliknya, tidak ada tanda klinikal yang ketara dalam kerbau daripada Kumpulan 7. Semua kerbau menunjukkan peningkatan monosit sepanjang 21 hari kajian. Hanya paru-paru dan hati terjejas dengan perubahan histopatologi yang sederhana. Kerbau dalam semua kumpulan rawatan *P. multocida* jenis B:2, lipopolisakarida dan membrane protin luar menunjukkan peningkatan dalam kepekatan haptoglobin dan serum amyloid A sepanjang tempoh kajian. Terdapat perbezaan yang signifikan ( $p < 0.05$ ) dalam semua kumpulan rawatan berbanding kumpulan kawalan. Di samping itu, kerbau dalam semua kumpulan rawatan juga menunjukkan peningkatan yang ketara dalam kepekatan IgM, IgG dan IgA sepanjang tempoh kajian. Terdapat perbezaan yang signifikan ( $p < 0.05$ ) dalam semua kumpulan rawatan berbanding kumpulan kawalan. Begitu juga, semua kumpulan rawatan menunjukkan peningkatan yang signifikan ( $p < 0.05$ ) dalam kepekatan IL-2 dan IL-6; tetapi tidak menunjukkan signifikansi ( $p > 0.05$ ) dalam kepekatan IL-12 berbanding kumpulan kawalan.

Daripada kajian ini, dapat disimpulkan bahawa kerbau dijangkiti dengan bakteria *P. multocida* jenis B:2, lipopolisakarida dan membran protin luar menunjukkan tindak balas sel yang berbeza. Maklumat mengenai ini akan memainkan peranan penting dalam memahami tindak balas sel badan semasa wabak hawar berdarah. Berdasarkan perubahan klinikal, darah, bedah siasat, histopatologi, dan kepekatan antibodi; kerbau disuntik secara oral dengan membran protin luar menunjukkan response yang positif dimana ia mungkin boleh dikembangkan untuk menjadi vaksin secara oral. Dengan ini, kita akan dapat mencari jalan alternatif untuk membuat vaksinasi secara menyeluruh untuk penyakit hawar berdarah. Selain itu, pembangunan penanda bio menggunakan sitokin pada masa akan datang dapat membantu mengawal penyakit hawar berdarah yang member kesan yang besar kepada ekonomi negara secara lebih efektif.



## ACKNOWLEDGEMENTS

I would like to offer my sincere thanks to the individuals who have directly and indirectly contributed to the completion of this work. First and foremost, I would like to express my deepest gratitude to my worthy supervisor, Associate Professor Dr Faez Firdaus Jesse Abdullah, for his professional insight, skilful guidance, encouragement, suggestion, time and patience throughout my study period. Thanks a lot for his kind and helpful supervision. I would also extend my sincere appreciation to my co-supervisors, Professor Dr Abd Wahid Haron, Professor Dr Mohd Zamri-Saad and Professor Dr Abdul Aziz Saharee for their valuable assistance and advice throughout the whole study. This research was funded by Ministry of Higher Education, Grant No. 5524417. I sincerely acknowledge the support from Graduate Research Fellowship Scholarship awarded by Universiti Putra Malaysia. Part of this thesis is already published. My appreciation is extended to the anonymous reviewers who gave valuable suggestions, comments and positive feedbacks prior to publication. I would also like to thank the faculty and laboratory members, Mr. Jefri Norsidin, Mr. Yap, Mr. Murthi, Dr Ali Dhiaa Marza, Dr Hayder Hamzah Ibrahim, and many more for their assistance, supports and friendship throughout this study. My heartfelt thanks and love goes to my family especially my parents, Lim Khong Chiu and Ng Yoke Oi, not forgetting my siblings, Felicia, Alvin and Andy for their endless love and strong mental supports throughout the journey of my study. Last but not least, my deepest appreciation to my beloved wife Lim Ai Ping for her sincere encouragement, support, advice and understanding throughout this moment.

I certify that a Thesis Examination Committee has met on 13 October 2016 to conduct the final examination of Eric Lim Teik Chung on his thesis entitled "Buffaloes' Clinico-Pathological Responses to *Pasteurella multocida* Type B:2 and their Immunogens Lipopolysaccharide and Outer Membrane Protein" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Noordin bin Mohamed Mustapha, PhD**

Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Hazilawati binti Hamzah, PhD**

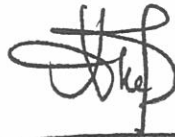
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Imad Ibrahim Al-Sultan, PhD**

Professor  
Universiti Malaysia Kelantan  
Malaysia  
(External Examiner)

**Peter David Eckersall, PhD**

Professor  
University of Glasgow  
United Kingdom  
(External Examiner)



---

**NOR AINI AB. SHUKOR, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 27 December 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory committee were as follows:

**Faez Firdaus Jesse Abdullah, PhD**

Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Abd Wahid Haron, PhD**

Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Mohd Zamri-Saad, PhD**

Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Abdul Aziz Saharee, PhD**

Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

---

**ROBIAH BINTI YUNUS, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Name and Matric No: Eric Lim Teik Chung, GS38967

## Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: \_\_\_\_\_  
Name of  
Chairman of  
Supervisory  
Committee: \_\_\_\_\_

Signature: \_\_\_\_\_  
Name of  
Member of  
Supervisory  
Committee: \_\_\_\_\_

Signature: \_\_\_\_\_  
Name of  
Member of  
Supervisory  
Committee: \_\_\_\_\_

Signature: \_\_\_\_\_  
Name of  
Member of  
Supervisory  
Committee: \_\_\_\_\_

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	iii
<b>ACKNOWLEDGEMENTS</b>	v
<b>APPROVAL</b>	vi
<b>DECLARATION</b>	viii
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xiv
<b>LIST IF ABBREVIATIONS</b>	xxi
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	
1.1 Introduction	1
1.2 Problem Statement	3
1.3 Hypotheses	3
1.4 Objectives	3
<b>2 LITERATURE REVIEW</b>	
2.1 Haemorrhagic septicaemia	5
2.2 Haemorrhagic septicaemia in Malaysia	5
2.3 Aetiology	7
2.3.1 Lipopolysaccharide	7
2.3.2 Outer membrane protein	8
2.4 Transmission of haemorrhagic septicaemia	9
2.5 Pathogenesis of haemorrhagic septicaemia	10
2.6 Clinical signs	10
2.7 Post mortem changes	11
2.8 Diagnosis of haemorrhagic septicaemia	11
2.9 Treatment and prevention of HS	11
2.10 Histopathology	12
2.11 Haematology and biochemistry	13
2.12 Acute phase proteins	13
2.13 Antibody	14
2.14 Cytokines	16
<b>3 MATERIALS AND METHODS</b>	
3.1 Ethical approval	18
3.2 Animal model	18
3.3 Inoculum preparation of PM B:2	18
3.4 Inoculum preparation of lipopolysaccharide	18
3.5 Inoculum preparation of OMP	19
3.6 SDS-PAGE analysis	19
3.7 Experimental design	19
3.8 Clinical signs scoring	22
3.9 Haematology and biochemistry analysis	22
3.10 Histopathology analysis and lesion scoring	22
3.11 Acute phase protein analysis	23
3.12 Antibodies analysis	24
3.13 Cytokines analysis	24

3.14	Statistical analysis	24
<b>4</b>	<b>CLINICAL RESPONSES IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B:2 AND ITS IMMUNOGEN LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
4.1	Introduction	25
4.2	Materials and methods	26
4.3	Results	26
	4.3.1 Temperature	30
	4.3.2 Heart rate	32
	4.3.3 Respiratory rate	34
	4.3.4 Rumen motility	36
4.4	Discussion and conclusion	38
<b>5</b>	<b>HAEMATOLOGY AND SERUM BIOCHEMISTRY CHANGES IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B:2 AND ITS IMMUNOGEN LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
5.1	Introduction	40
5.2	Materials and methods	41
5.3	Results	41
	5.3.1 Erythrocytes	42
	5.3.2 Haemoglobin	43
	5.3.3 Packed cell volume	44
	5.3.4 Mean corpuscular volume	45
	5.3.5 MCHC	46
	5.3.6 Leukocytes	47
	5.3.7 Band neutrophils	48
	5.3.8 Segmented neutrophils	49
	5.3.9 Lymphocytes	50
	5.3.10 Monocytes	51
	5.3.11 Eosinophils	52
	5.3.12 Basophils	53
	5.3.13 Thrombocytes	54
	5.3.14 Plasma protein	55
	5.3.15 Icterus index	56
	5.3.16 Gamma glutamyl transferase	57
	5.3.17 Total protein	58
	5.3.18 Albumin	59
	5.3.19 Globulin	60
	5.3.20 Albumin:Globulin ration	61
5.4	Discussion and conclusion	62
<b>6</b>	<b>POST MORTEM AND HISTOPATHOLOGICAL CHANGES IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B:2 AND ITS IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
6.1	Introduction	65

6.2	Materials and methods	65
6.3	Results	66
	6.3.1 Post mortem lesions	66
	6.3.2 Histopathological lesions	72
6.4	Discussion and conclusion	89
<b>7</b>	<b>ACUTE PHASE PROTEINS CONCENTRATION IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B:2 AND ITS IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
7.1	Introduction	93
7.2	Materials and methods	94
7.3	Results	94
	7.3.1 Haptoglobin	94
	7.3.2 Serum amyloid A	97
7.4	Discussion and conclusion	99
<b>8</b>	<b>ANTIBODIES CONCENTRATION IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B:2 AND ITS IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
8.1	Introduction	102
8.2	Materials and methods	103
8.3	Results	103
	8.3.1 IgM	103
	8.3.2 IgG	106
	8.3.3 IgA	108
8.4	Discussion and conclusion	110
<b>9</b>	<b>CYTOKINES CONCENTRATION IN BUFFALOES INFECTED WITH <i>PASTEURELLA MULTOCIDA</i> TYPE B: 2 AND ITS IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN VIA DIFFERENT ROUTES OF INOCULATION</b>	
9.1	Introduction	112
9.2	Materials and methods	113
9.3	Results	113
	9.3.1 Interleukin-2	113
	9.3.2 Interleukin-6	116
	9.3.3 Interleukin-12	118
9.4	Discussion and conclusion	120
<b>10</b>	<b>GENERAL DISCUSSION AND CONCLUSION</b>	123
	<b>REFERENCES</b>	129
	<b>APPENDICES</b>	141
	<b>BIODATA OF STUDENT</b>	152
	<b>LIST OF PUBLICATIONS</b>	153



## LIST OF TABLES

Table		Page
3.1	Microscopic lesions observed in different organs in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP infection via different routes of inoculation.	23
6.1	Histopathological alterations in Group 2 buffaloes after 12 hours of subcutaneous inoculations of <i>P. multocida</i> type B:2.	83
6.2	Haemorrhage and congestion lesion in Group 1, 3, 4, 5, and 7 buffaloes after 21 days of <i>P. multocida</i> type B:2 and its immunogens LPS and OMP infection via different routes of inoculation.	84
6.3	Necrosis and degeneration lesion in Group 1, 3, 4, 5, and 7 buffaloes after 21 days of <i>P. multocida</i> type B:2 and its immunogens LPS and OMP infection via different routes of inoculation.	85
6.4	Inflammatory cells infiltration lesion in Group 1, 3, 4, 5, and 7 buffaloes after 21 days of <i>P. multocida</i> type B:2 and its immunogens LPS and OMP infection via different routes of inoculation.	86
6.5	Oedema lesion in Group 1, 3, 4, 5, and 7 buffaloes after 21 days of <i>P. multocida</i> type B:2 and its immunogens LPS and OMP infection via different routes of inoculation.	87
6.6	Histopathological alterations in Group 6 buffaloes after 72 hours of subcutaneous inoculations of <i>P. multocida</i> Type B:2 immunogen OMP.	88

## LIST OF FIGURES

Figure		Page
3.1	Experimental design	21
4.1	Serous nasal discharge after 3 hours post infection in Group 2 buffaloes.	27
4.2	Congested mucous membrane after 4 hours post infection in Group 2 buffaloes.	27
4.3	Submandibular oedema after 5 hours post infection in Group 2 buffaloes.	28
4.4	Dullness after 5 hours post infection in Group 2 buffaloes.	28
4.5	Mucopurulent nasal discharge after 11 hours post infection in Group 2 buffaloes.	29
4.6	Recumbency with respiratory distress after 12 hours post infection in Group 2 buffaloes.	29
4.7	Mean rectal temperatures for Group 2 buffaloes after 12 hours of inoculation with <i>P. multocida</i> type B:2.	30
4.8	Mean rectal temperatures for Group 3, 4, 5, and 7 after 21 days of inoculation with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP.	31
4.9	Mean rectal temperatures for Group 6 after 72 hours of inoculation with <i>P. multocida</i> type B:2 immunogen OMP.	31
4.10	Heart rate for Group 2 buffaloes after 12 hours of inoculation with <i>P. multocida</i> type B:2.	32
4.11	Heart rate for Group 3, 4, 5, and 7 after 21 days of inoculation with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP.	33
4.12	Heart rate for Group 6 after 72 hours of inoculation with <i>P. multocida</i> type B:2 immunogen OMP.	33
4.13	Respiratory rate for Group 2 buffaloes after 12 hours of inoculation with <i>P. multocida</i> type B:2.	34
4.14	Respiratory rate for Group 3, 4, 5, and 7 after 21 days of inoculation with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP.	35

4.15	Respiratory rate for Group 6 after 72 hours of inoculation with <i>P. multocida</i> type B:2 immunogen OMP.	35
4.16	Rumen motility for Group 2 buffaloes after 12 hours of inoculation with <i>P. multocida</i> type B:2.	36
4.17	Rumen motility for Group 3, 4, 5, and 7 after 21 days of inoculation with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP.	37
4.18	Rumen motility for Group 6 after 72 hours of inoculation with <i>P. multocida</i> type B:2 immunogen OMP.	37
5.1	Erythrocytes in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	42
5.2	Haemoglobin in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	43
5.3	Packed cell volume in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	44
5.4	MCV in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	45
5.5	Mean MCHC in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	46
5.6	in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	47
5.7	Band neutrophils in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	48
5.8	Segmented neutrophils in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	49
5.9	Lymphocytes in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	50

5.10	Monocytes in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	51
5.11	Eosinophils in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	52
5.12	Basophils in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	53
5.13	Thrombocytes in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	54
5.14	Plasma protein in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	55
5.15	Icterus index in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	56
5.16	GGT in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	57
5.17	Total protein in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	58
5.18	Albumin in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	59
5.19	Globulin in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	60
5.20	A:G ratio in buffaloes infected with <i>P. multocida</i> type B:2 and its immunogens LPS and OMP via different routes of inoculation throughout 21 days of experiment.	61
6.1	Normal organs in-situ in a Group 1 buffalo.	68
6.2	Generalized congested and hemorrhagic (arrow) lung lobes in a Group 2 buffalo.	68
6.3	Generalized congested and hemorrhagic (arrows) in the left and right kidneys in a Group 2 buffalo.	69

6.4	Generalized congested and hemorrhagic (arrow) liver in a Group 2 buffalo.	69
6.5	Congested and hemorrhagic (arrow) gastrointestinal tract in Group 2 buffalo.	70
6.6	Presence of straw coloured fluid (arrow) in the thoracic cavity in a Group 2 buffalo.	70
6.7	Generalized congested and fibrinous (arrows) cranial lung lobes in a Group 3 buffalo.	71
6.8	Multifocal haemorrhage (arrow) with fibrin deposition around the liver in a Group 3 buffalo.	71
6.9	Photomicrograph of the lung, H&E, 200X. a) congestion of the blood vessel; b) haemorrhage in the interalveolar septum; c) oedema in the alveoli spaces.	74
6.10	Photomicrograph of the heart, H&E, 200X. a) congestion of the blood vessel; b) necrosis and degeneration; c) oedema in the cardiac muscle.	74
6.11	Photomicrograph of the trachea, H&E, 200X. a) macrophages infiltration in the perichondrium.	75
6.12	Photomicrograph of the kidney, H&E, 200X. a) congestion of the blood vessel; b) necrosis and degeneration of the tubules; c) haemorrhage in the glomeruli.	75
6.13	Photomicrograph of the liver, H&E, 200X. a) congestion of the portal vein; b) haemorrhage in the Disse's space; c) proliferation of Kupffer cells.	76
6.14	Photomicrograph of the tonsil, H&E, 200X. a) congestion of the blood vessel; b) necrosis and degeneration in the medullary sinuses.	76
6.15	Photomicrograph of the prescapular lymph node, H&E, 200X. a) congestion of the blood vessels; b) depopulation of the lymphoid in the white pulp; c) necrosis and degeneration of the trabecule.	77
6.16	Photomicrograph of the femoral lymph node, H&E, 200X. a) haemorrhage; b) necrosis and degeneration in the medullary sinuses.	77
6.17	Photomicrograph of the mesenteric lymph node, H&E, 200X. a) congestion of the blood vessels; b) depopulation of the lymphoid in the white pulp.	78

6.18	Photomicrograph of the spleen, H&E, 200X. a) congestion of the blood vessel; b) haemorrhage in the red pulp.	78
6.19	Photomicrograph of the esophagus, H&E, 200X. a) congestion of the blood vessel in the submucosa layer.	79
6.20	Photomicrograph of the rumen, H&E, 200X. a) congestion; b) necrosis and degeneration in the muscularis layer.	79
6.21	Photomicrograph of the abomasum, H&E, 200X. a) congestion of the blood vessel in the submucosa layer.	80
6.22	Photomicrograph of the duodenum, H&E, 200X. a) congestion of the blood vessel in the muscularis layer.	80
6.23	Photomicrograph of the jejunum, H&E, 200X. a) haemorrhage; b) necrosis and degeneration; c) macrophages infiltration in the mucosa layer.	81
6.24	Photomicrograph of the ileum, H&E, 200X. a) severe haemorrhage in the mucosa layer.	81
6.25	Photomicrograph of the rectum, H&E, 200X. a) congestion of the blood vessel; b) macrophages infiltration in the mucosa layer.	82
7.1	APP of Hp in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	95
7.2	APP of Hp in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	95
7.3	APP of Hp in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	96
7.4	APP of SAA in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	97
7.5	APP of SAA in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	98
7.6	APP of SAA in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	98
8.1	IgM antibody concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	104
8.2	IgM antibody concentration in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	104

8.3	IgM antibody concentration in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	105
8.4	IgG antibody concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	106
8.5	IgG antibody concentration in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	107
8.6	IgG antibody concentration in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	107
8.7	IgA antibody concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	108
8.8	IgA antibody concentration in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	109
8.9	IgA antibody concentration in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	109
9.1	IL-2 concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	114
9.2	IL-2 concentration in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations	114
9.3	IL-2 concentration in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	115
9.4	IL-6 concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	116
9.5	IL-6 concentration in buffaloes infected with <i>P. multocida</i> and its immunogens LPS and OMP via different route of inoculations.	117
9.6	IL-6 concentration in buffaloes infected with <i>P. multocida</i> immunogen OMP via subcutaneous route of inoculation.	117
9.7	IL-12 concentration in buffaloes infected with <i>P. multocida</i> via subcutaneous route of inoculation.	118

- 9.8 IL-12 concentration in buffaloes infected with *P. multocida* and its immunogens LPS and OMP via different route of inoculations. 119
- 9.9 IL-12 concentration in buffaloes infected with *P. multocida* immunogen OMP via subcutaneous route of inoculation. 119





## LIST OF ABBREVIATIONS

HS	Haemorrhagic septicaemia
<i>P. multocida</i>	<i>Pasteurella multocida</i>
LPS	Lipopolysaccharide
OMP	Outer membrane protein
cfu	Colony forming unit
PCV	Packed cell volume
MCV	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin concentration
GGT	Gamma glutamyl transferase
A:G	Albumin:Globulin ration
Hp	Haptoglobin
SAA	Serum amyloid A
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IgA	Immunoglobulin A
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-12	Interleukin-12
PM	Post mortem lesion
APP	Acute phase proteins

## CHAPTER 1

### INTRODUCTION

#### 1.1 Introduction

*P. multocida* was first shown to be the causative agent of fowl cholera by Louis Pasteur in 1881. Since then, this Gram-negative bacterium had been identified as the causative agent of many other economically important diseases in a wide range of hosts. HS is a disease, caused by particular serotypes of *P. multocida* that causes acute, highly fatal septicaemia with high morbidity and mortality in cattle and more susceptible in buffaloes. Epidemic HS is caused by 1 or 2 serotypes of *P. multocida*, designated B:2 and E:2. E:2 causes outbreaks only in African region; however, B:2 serotype causes disease in Asian region (OIE, 2008, OIE, 2009). The disease is distinctly different from some other pasteurelloses when pasteurella play only a secondary role. HS is thus defined as a specific form of pasteurellosis in cattle and buffaloes (DeAlwis, 1992).

*P. multocida* is a gram negative, short, ovoid, bipolar staining coccoid forms. It is an extracellular parasite, and its immunity is primarily humoral. LPS, OMP, capsule, frimbriae, iron and toxin are the few virulence factors of *P. multocida* type B:2 (DeAlwis, 1992). The cell envelope of Gram-negative bacteria consists of an inner cytoplasmic membrane, a thin peptidoglycan cell wall, and LPS containing OMP that surrounds the peptidoglycan layer (Quan et al., 2013). LPS can be found on the outer cell wall of the organism (Bain et al., 1982; Srivastava, 2013). LPS is released during multiplication which leads to inflammatory reaction. It represents the endotoxin of *P. multocida* type B:2 and responsible for toxicity in HS which plays an important role in the pathogenesis of the disease (Rhoades, 1967; Rebers, 1988; DeAlwis, 1999). After inhalation or oral ingestion, LPS causes endothelial damage enabling the entry of the *P. multocida* organism into the organs of susceptible cattle and buffaloes (DeAlwis, 1999; Zamri, 2013). The bacteria will then move into the circulation causing bacteraemia, septicaemia and then toxemia. However, this theory has never been proven before. Experimental inoculation of LPS and OMP were found to cause clinico-pathological and haemato-biochemical changes in mice and cattle (Jesse et al., 2013; Jesse et al., 2013a; Ali et al., 2014).

*P. multocida* organism can survive for long hours and probably days in moist soil and water. This organism is transmitted by direct or indirect contact (Kahn and Line, 2005). In susceptible animals, septicaemia develops rapidly and causes death due to endotoxaemia within 8-24 hours after the development of the first sign. According to Abubakar et al. (2011), the heaviest loss occurs during stressor factors which include poor weather, humid conditions, high animal population density, extensive grazing system and poor husbandry practice in Southeast Asia. In most cases, the clinical findings are either acute or peracute, resulting death within 8 to 24 hours after onset (Kahn and Line, 2005). In an experiment done by DeAlwis (1992), buffaloes infected through aerosol and direct contact with diseased buffaloes showed clinical signs after 24 and 48 hours respectively. Animals first show sign of dullness, then reluctant to move, fever, salivation, and serous nasal discharge. Oedematous swelling is frequently seen, beginning at the throat region and then spreads to the parotid, neck and brisket region. Mucous membrane will then be congested leading to respiratory distress where

animals will usually die within hours (DeAlwis, 1999). For post mortem lesions, the most obvious lesions in affected animals are the oedema, widely distributed haemorrhages, and general hyperaemia. In most cases, there will also be clear or straw coloured oedematous fluid at the head, neck, brisket, and musculature region. Petechial haemorrhages are particularly prominent in the pharyngeal and cervical lymph nodes. Besides, blood tinge fluid is often found in the pericardial sac, thoracic and abdominal cavity (Kahn and Line, 2005; Abubakar et al., 2012).

Experiments done by Jesse et al. (2013d); Jesse et al. (2013e); and Abubakar et al. (2011), showed some interesting histopathology parameters which included pulmonary oedema, presence of inflammatory cells, haemorrhage and necrosis in animals' organs after infecting *P. multocida* type B:2 via different routes of inoculations in mice, cattle and buffaloes. However, the signs observed following oral exposure were much milder compared to intratracheal or respiratory routes (Abubakar et al., 2012). This could be proof that oral route transmission could manipulate the *P. multocida* organism better compared to the other routes of inoculation. This indicated that the oral route could perhaps be a readily available route for effective vaccine administration and heightened the animals' immunity. Therefore, this study was done to know more about the clinico-pathological changes in buffaloes infected with *P. multocida* and its immunogens LPS and OMP infections via different routes of inoculations. Thus, this experiment will play a significant role in getting a better understanding and knowledge of the *P. multocida* organism and its immunogens.

According to Jesse et al. (2013a), inoculation of *P. multocida* type B:2 and its immunogens LPS and OMP stimulate response to the host humoral immunity and specific cellular response. During bacterial infection, haematological and biochemical changes are first detected during routine blood sampling. However, animals' defence mechanism can react quite differently and there is no singular pattern in complete blood count that indicates a bacterial infection. Jesse et al. (2013b), had reported that there were some haematological and biochemical markers that could be used for early detection in animals infected with wild type of *Pasteurella multocida*. On the other hand, acute phase response will be the first line of immune defence mechanism against preliminary infections (Eckersall et al., 2008; Khaleel et al., 2013). APP such as fibrinogen, C-reactive, Hp, albumin and SAA are proteins found in the blood. These APP will either increase or decrease in cases of infection and inflammation (Jesse, et al., 2013c). Hepatic production of APP is stimulated by pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor alpha that are released into the circulation during acute phase response (Eckersall et al., 2008).

Immunity is a process where the body protects against foreign organism or antigens (Blood et al., 2011). The immune system against HS is made of two important components which are the innate and humoral immunity. Innate immunity is also known as the first line of defence that prevents the entry of pathogens. Humoral immunity or the antibody-mediated immune system involves the production of specific antibodies against *P. multocida* which is an extracellular organism (Kumar, 2013). Morbidity and mortality due to HS in an endemic or epidemic area are largely dependent on the proportion of immune to non-immune animals. According to DeAlwis, (1999) there is also relation between cells mediated immunity and the

pathogenesis of *P. multocida* but is not explored yet. On the other hand, the production of humoral antibodies and cell mediated immunity are stimulated by the production of cytokines (Eckersall et al., 2008). IL-2 and IL-6 produced by macrophages and T-cells respectively are found to stimulate the production of humoral antibodies. Besides, macrophages are also found to produce IL-12 that will activate cell mediated immunity (Kindt et al., 2007).

## 1.2 Problem statement

1. Experimental infections of LPS and OMP via different routes of inoculations in the real host buffaloes have not been carried out before.
2. Information on clinical responses, post mortem, histopathological changes, haemato-biochemistry, APP, cytokines, as well as antibody titre in the real host buffalo after inoculation of LPS and OMP is still very scarce.
3. Outbreaks of HS were still reported despite vaccination and sudden death is usually the main finding in field animals during disease outbreak.

## 1.3 Hypotheses

It is hypothesized that there will be host cell response in buffaloes following experimental-infection with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.

1. Buffaloes inoculated with *P. multocida* type B:2 will show more severe clinico-pathological changes compared to buffaloes inoculated with LPS and OMP via different routes of inoculation.
2. There will be changes in haemato-biochemistry, APP, IL, and antibody concentrations in the experimental animals infected with *P. multocida* type B:2 and its immunogens with LPS and OMP via different routes of inoculation.

## 1.4 Objectives

The main objective of this study was to determine the best candidate of *P. multocida* type B:2 and its immunogens LPS and OMP to be used as oral vaccination in buffaloes and cattle; together with the development of biomarkers to control outbreak of HS widely and more effectively. Thus, this study was further divided into 6 main objectives:

1. To determine the severity of the clinical response in experimental buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.
2. To determine and compare the blood and biochemistry changes in experimental buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.
3. To determine the severity of post mortem and histopathological changes in buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.

4. To determine and compare the concentration of APP in experimental buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.
5. To determine and compare the antibody titre in experimental buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.
6. To determine and compare the concentration of proinflammatory cytokines in experimental buffaloes infected with *P. multocida* type B:2 and its immunogens LPS and OMP via different routes of inoculation.



## REFERENCES

- Abubakar, M.S., and Zamri-saad, M. (2011). Clinico-pathological changes in buffalo calves following oral exposure to *Pasteurella multocida* B2. *Basic and Applied Pathology*, 4: 130-135.
- Abubakar, M.S., Zamri-saad, M., and Jasni, S. (2012). Ultrastructural changes and bacterial localization in buffalo calves following oral exposure to *Pasteurella multocida* B2. *Pakistan Veterinary Journal*, 33(1): 101-106.
- Adorini, L. (1999). Interleukin-12, a key cytokine in Th1-mediated autoimmune diseases. *Cellular and Molecular Life Sciences*, 55: 1610-1625.
- Agha, I., Mahoney, R., Beardslee, M., Liapis, H., Cowart, R.G., and Juknevičius, I. (2002). Systemic amyloidosis associated with pleomorphic sarcoma of the spleen and remission of nephrotic syndrome after removal of the tumor. *American Journal of Kidney Diseases*, 40: 411-415.
- Ahmad, A., Rahman, S., Ahmed, Z., Bilal, M., Rabbani, M., and Muhammad, K. (2011). Passive immunization of *Pasteurella multocida* infected rabbit. *Pakistan Journal of Zoology*, 43(4): 623-628.
- Ali, O.S., Adamu, L., Abdullah, F.F.A., Ilyasu, Y., Abba, Y., Hamzah, H.B., Mohd-Azmi, M.L., Haron, A.W.B., and Zamri, M. (2014). Alterations in Interleukin-1 $\beta$  and Interleukin-6 in Mice Inoculated Through the Oral Routes Using Graded Doses of *P. multocida* Type B2 and its Lipopolysaccharide. *American Journal of Animal and Veterinary Sciences*, 9(4): 177-184.
- Allam, N.G., and Elslik, S.E. (2011). Evaluation of lipopolysaccharide, exopolysaccharide and mutant strain as subunit vaccines against *Serratia marcescens* W225. *African Journal of Microbiology Research*, 5(12): 1389-1397.
- Annas, S., Zamri-Saad, M., Abubakar, M.S., Jesse, F.F.A., and Zunita, Z. (2014a). Distribution of *Pasteurella multocida* B:2 in the respiratory, gastrointestinal and urinary tracts of buffaloes following experimental subcutaneous inoculation. *Journal of Veterinary Science and Technology*, 5(3): 1-6.
- Annas, S., Zamri-saad, M., Jesse, F.F.A., and Zunita, Z. (2014b). New sites of localization of *Pasteurella multocida* B2 in buffalo surviving experimental haemorrhagic septicaemia. *BMC Veterinary Research*, 10(88): 1-7.
- Atkinson, S.H., Mwangi, T.W., Uyoga, S.M., Ogada, E., Macharia, A.W., Marsh, K., Prentice, A.M., and Williams, T.N. (2007). The haptoglobin2-2 genotype is associated with a reduced incidence of *Plasmodium falciparum* malaria in children on the coast of Kenya. *Clinical Infectious Disease*, 44: 802-809.

- Bachmann, M.F., and Oxenius, A. (2007a). Interleukin 2: from immunostimulation to immunoregulation and back again. *European Molecular Biology Organization*, 8(12): 1142-1148.
- Bachmann, M.F., Wolint, P., Walton, S., Schwarz, K., Oxenius, A. (2007b). Differential role of IL-2R signaling for CD8(+) T cell responses in acute and chronic viral infections. *European Journal of Immunology*, 37:1502–1512.
- Bain, R.V.S, DeAwis, M.C.L., Carter, G.R., and Gupta, B.K. (1982). *Haemorrhagic septicaemia FAO Animal Reproduction and health paper*. No.33. Food and Agriculture Organisation on the United Nations, Rome.
- Benditt, E.P., Eriksen, N. (1971). *Amyloid protein SAA is associated with high density lipoprotein from human serum*. Proceeding of the National Academy of Sciences of the United States of America, 74(9): 4025-4028.
- Benkirane, A., and DeAlwis, M.C.L. (2002). Haemorrhagic septicaemia, its significance, prevention and control in Asia. *Veterinary Medicine – Czech*, 2002(8): 234-240.
- Bhatti, A.R., Tasneem, K., Gill, Z.J., and Raza, A. (2005). Potency and safety testing of haemorrhagic septicaemia oil adjuvant vaccine in Swiss albino mice. *Pakistan Veterinary Journal*, 25(3): 153-154.
- Biswas, A., Shivachandra, S.B., Saxena, M.K., Kumar, A.A., Singh, V.P., and Srivastava, S.K. (2004). Molecular variability among strains of *Pasteurella multocida* isolated from an outbreak of haemorrhagic septicaemia in India. *Veterinary Research Communications*, 28(4): 287-298.
- Blood, D.C., Studdert, V.P., and Gay, C.C. (2011). *Saunders Comprehensive Veterinary Dictionary 4<sup>th</sup> Edition*. London:Saunders.
- Bruna, M.J. (1994). Interleukin-12. Review. *Journal of Leukocyte Biology*, 55: 280-288.
- Carrongean, M. (1902). Bovine pasteurellosis in Malay Peninsular. *Veterinary Journal*, 55: 321-327.
- Carter, G.R. (1962). Further observation on typing *Pasteurella multocida* by the indirect hemagglutination test. *Canadian Journal of Comparative Medicine and Veterinary Science*, 26: 238-240.
- Chandrasekaran, S.L., Kennett, P.C., Yeap, N., Muniandy, B., Rani, and Mukkur, T.K.S. (1994). Relationship between active protection in vaccinated buffaloes against haemorrhagic septicaemia and passive mouse protection test or serum antibody titer. *Veterinary Microbiology*, 41: 303-309.

- Darwish, R.K., Ramadan, D.I., Mohy, A.M., Raafat, H.A., Youssef, H.E.A., and El-Kateb, S.M. (2014). Interleukin-1 $\beta$  gene polymorphisms in Egyptian patients with rheumatoid arthritis. *Comparative Clinical Pathology*, 23(3): 689-694.
- Dawkin, H.J.S., Ramdani, Johnson, R.B., and Spencer, T.L. (1991). HS: correlation of vaccinal antibody titre in mice with protection against *Pasteurella multocida* strain M 1404. *Veterinary Microbiology*, 27, 307-326.
- DeAlwis, M.C.L. (1981). Mortality among cattle and buffaloes in Sri Lanka due to Haemorrhagic septicaemia. *Tropical Animal Health and Production*, 13: 195-202.
- DeAlwis, M.C.L. (1982). Immune status of buffalo calves exposed to natural infection with haemorrhagic septicaemia. *Tropical Animal Health and Production*, 14: 29-30.
- DeAlwis, M.C.L. (1992). Haemorrhagic septicaemia- A general review. *British Veterinary Journal*, 148: 99-112.
- DeAlwis, M.C.L. (1999). *Haemorrhagic Septicaemia*. Canberra: ACIAR.
- Deed, B.J., DiGiacomo, R.J., Bernard, B.L., and Silbernagel, S.M. (1990). *Pasteurella multocida* and *Bordetella bronchiseptica* infections in rabbits. *Journal of Clinical Microbiology*, 1(1990): 70-75.
- Delanghe, J., Langlois, M., Duprez, D., De Buyzere, M., and Clement, D. (1999). Haptoglobin polymorphism and peripheral arterial occlusive disease. *Arteriosclerosis*, 145: 287-297.
- Dinarello, C.A. (2010). Anti- inflammatory agents: present future. *Journal of Cell*, 140(6): 935-950.
- Dinarello, C.A. (2007). Historical review of cytokines. *European Journal of Immunology*, 37 (1):34-45.
- Dinarello, C.A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annual Review of Immunology*, 27: 519-550.
- Dionissopoulos, L., Steele, M.A., AlZahal, O., Plaizier, J.C., Li, S., and McBride, B.W. (2011). Subacute ruminal acidosis (SARA) in dairy cows leads to increase in ruminal lipopolysaccharide (LPS), plasma LPS binding protein and correlates with the rumen epithelial interleukin-6 (IL-6) response. *Canadian Journal of Animal Science*, 91(3): 503-503.
- Eaton, J.W., Brandt, P., Mahoney, J.R., and Lee Jr, J.T. (1982). Haptoglobin: A natural bacteriostat. *Science*, 215: 691-693.



- Eckersall, P.D. (2000). Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue de Medecine Veterinaire*, 151(7): 577-584.
- Eckersall, P.D., and Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal*, 185(1): 23-27.
- Eckersall, P.D., and Conner, J.G. (1988). Bovine and canine acute phase protein. *Veterinary Research Communications*, 12(2-3): 169-178.
- Eckersall, P.D., Lawson, F.P., Kyle, C.E., Waterson, M., Bence, L., Stear, M.J., and Rhind, S.M. (2008). Maternal undernutrition and the ovine acute phase response to vaccination. *BMC Veterinary Research*, 4(1): 1-10.
- Eckersall, P.D., Saini, P.K., and McComb, C. (1996). The acute phase responses of acid soluble glycoprotein,  $\alpha$ 1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. *Veterinary Immunology and Immunopathology*, 53(3-4):377-385.
- El-Eragi, A.M.S., Mukhtar, M.M., and Babiker, S.H. (2001). Specific antibodies of *Pasteurella multocida* in newborn calves of vaccinated dams. *Tropical Animal Health and Production*, 33(2001): 275-283.
- FAO (1979): *Proceeding of the third international workshop on Haemorrhagic septicaemia*. FAO-APHCA, December 1979, Colombo, Sri Lanka.
- Farooq, U., Saeed, Z., Khan, M.A., Ali, I., and Qamar, M.F. (2011). Sero-surveillance of haemorrhagic septicaemia in buffaloes and cattle in Southern Punjab, Pakistan. *Pakistan Veterinary Journal*, 31(3): 254-256.
- Fieschi, C., and Casanova, J. (2003). The role of interleukin-12 in human infectious diseases: only a faint signature. *European Journal of Immunology*, 33: 1461-1464.
- Fomsgaard, A., Freudenberg, M.A., and Galanos, C. (1990). Modification of the silver staining technique to detect lipopolysaccharide in polyacrylamide gels. *Journal of Clinical Microbiology*, 28(12): 2627-2631.
- Gazzinelli, R.T., Hieny, S., Wynn, T.A., Wolf, S., and Sher, A. (1993). *Interleukin 12 is required for the T-lymphocyte-independent induction of interferon  $\gamma$  by an intracellular parasite and induces resistance in T-cell-deficient host*. Proceeding of the National Academy Sciences, 90:6115-6119.
- Goodman, M., Cabral, L., and Cassileth, P. (1998). Interleukin-2 and leukemia. *Leukemia*, 12: 1671-1675.
- Gronlund, U., Hulten, C., Eckersall, P.D., Hogarth, C., and Waller, K.P. (2003). Haptoglobin and serum amyloid A in milk and serum during acute and

- chronic experimentally induced *Staphylococcus aureus* mastitis. *Journal of Dairy Research*, 70: 379-386.
- Hack, C.E., Groot, E.R.D., Felt-Bersma, R.J.F., Nuijens, J.H., Schijndel, S.V., Eerenberg, A.J.M., Thijs, L.G., and Aarden, L.A. (1989). Increased plasma level of interleukin-6 in sepsis. *Blood*, 74(5): 1704-1710.
- Hamza, T., Barnett, J.B., and Li, B. (2010). Interleukin 12 a key immunoregulatory cytokine in infection applications. *International Journal of Molecular Sciences*, 11: 789-806.
- Harper, M., Cox, A.D., Adler, B., and Boyce, J.D. (2011). *Pasteurella multocida* lipopolysaccharide: the long and short of it. *Veterinary Microbiology*, 153(1-2): 109-115.
- Heddleston, K.L., Gallagher, J.E., and Rebers, P.A. (1972). Fowl Cholera: Gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. *American Association of Avian Pathologists*, 14(4): 925-936.
- Horadagoda, N.U., Hodgson, J.C., moon, G.M., Wijewardana, T.G., and Eckersall, P.D. (2001). Role of endotoxin in the pathogenesis of haemorrhagic septicaemia in the buffalo. *Microbial Pathogenesis*, 30: 171-178.
- Iovane, G., Pagini, P., Galdiero, M., L'Ero, G.C., Vitiello, M., D'Isanto, M., and Marcatili, A. (1998). Role of *Pasteurella multocida* porin on cytokine expression and release by murine splenocytes. *Veterinary Immunology and Immunopathology*, 66: 391-404.
- Jalal A, Jazayeri A, Graeme J, Carroll B, Ann B, and Vernallis: Interleukin-6 subfamily cytokines and rheumatoid arthritis: role of antagonists. *International Immunopharmacology*, 2010, 10: 1-8.
- Janeway, C., Travers, P., and Walport, M. (2011). *Immunobiology: The immune System in Health and Disease*. New York: Garland Science.
- Jesse, F.F.A, Affandi, S.A., Osman, A.Y., Adamu, L., Zamri-saad, M., Wahid, A., Rahman, A., Sabri, J., and Saharee, A.A. (2013b). Clinico-pathological Features in Mice Following Oral Exposure to *Pasteurella multocida* B:2. *IOSR Journal of Agriculture and Veterinary Science*, 3(4): 35-39.
- Jesse, F.F.A., Adamu, L., Osman, A.Y., Wahid, A., Saharee, A.A., Abdullah, R., Zamri-saad, M., and Zakaria, Z. (2013d). Biochemical and haematological alterations in mice inoculated with outer membrane protein, lipopolysaccharides and whole cells of *Pasteurella multocida* type B2. *American Journal of Animal and Veterinary Science*, 8(3): 152-158.
- Jesse, F.F.A., Adamu, L., Osman, A.Y., Zakaria, Z., Abdullah, R., Zamri-saad, M., and Saharee, A.A. (2013c). Clinico-pathological responses of calves associated with infection of *Pasteurella multocida* type B2 and the

bacterial lipopolysaccharide and outer membrane protein immunogens. *International Journal of Animal and Veterinary Advances*, 5(5): 190-198.

- Jesse, F.F.A., Adamu, L., Osman, A.Y., Zamri-saad, M., Zakaria, Z., Abdullah, R., and Saharee, A.A. (2013f). Acute phase protein profile and clinic-pathological changes in mice associated with the infection of *Pasteurella multocida* type B and the bacterial lipopolysaccharide and outer membrane protein immunogens. *Journal of Animal and Veterinary Advances*, 12(2): 186-193.
- Jesse, F.F.A., Khaleel, M.M., Adamu, L., Osman, A.Y., Wahid, A., Zamri-saad, M., and Rahman, A. (2013a). Polymerase chain reaction detection of *Pasteurella multocida* type B2 in mice infected with contaminated river water. *American Journal of Animal and Veterinary Sciences*, 8(3): 146-151.
- Jesse, F.F.A., Osman, A.Y., Adamu, L., Zakaria, Z., Abdullah, R., Zamri-saad, M., and Saharee, A.A. (2013e). Haematological and biochemical alterations in calves following infection with *Pasteurella multocida* type B2, bacterial lipopolysaccharide and outer membrane protein immunogens. *Asian Journal of Animal and Veterinary Advances*, 8(6): 806-813.
- Jesse, F.F.A., Osman, A.Y., Adamu, L., Zakaria, Z., Abdullah, R., Zamri-saad, M., and Saharee, A.A. (2013g). Acute phase protein Profiles in calves following infection with whole cell, lipopolysaccharide and outer membrane protein extracted from *Pasteurella multocida* type B2. *Asian Journal of Animal and Veterinary Advances*, 8(4): 655-662.
- Kahn, C.M., and Line, S. (2005). *The Merck Veterinary Manual 9<sup>th</sup> Edition*. USA: Merial.
- Khaleel, M.M., Jesse, F.F.A., Osman, A.Y., Wahid, A., Zamri-saad, M., and Rahman, A. (2013). Acute phase protein responses in mice affected with river water contaminated by *Pasteurella multocida* type B2. *American Journal of Animal and Veterinary Sciences*, 8(3): 159-164.
- Kindt, T.J., Goldsby, R.A., and Osborne, B.A. (2007). *Immunology 6<sup>th</sup> Edition*. New York: W. H. Freeman and Company.
- Koebnik, R., Locher, K.P., and G, P.V. (2000). Structure and function of bacterial outer membrane protein: barrels in a nutshell. *Molecular Microbiology*, 37(2): 239-253.
- Krishnan, J., Chu, W.S., Elrod, J.P., and Frizzera, G. (1993). Tumoral presentation of amyloidosis (amyloidomas) in soft tissue. A report of 14 cases. *American Journal of Clinical Pathology*, 100: 135-144.
- Kumar, A. (2013). *Textbook of Immunology*. New Delhi: TERI.

- Kumar, H., Sharma, S., Mahajan, V., Verma, S., Arora, A.K., Kaur, P., and Sandhu, K.S. (2006). Pathology and PCR based confirmation of Haemorrhagic septicaemia outbreaks in bovines. *Indian Journal of Veterinary Pathology*, 30:5-8.
- Lane, E.P., Kock, N.D., Hill, F.W.G., and Mohan, K. (1992). An outbreak of haemorrhagic septicaemia in cattle in Zimbabwe. *Tropical Animal Health and Production*, 24: 97-102.
- Lee, J., Kim, Y.B., and Kwon, M. (2007). Outer membrane protein H for protective immunity against *Pasteurella multocida*. *Journal of Microbiology*, 45: 179-184.
- Levin, M., Pras, M., and Franklin, E.C. (1973). Immunologic studies of the major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. *The journal of Experimental Medicine*, 138(2): 373-380.
- Levy, A.P. (2004). Haptoglobin: A major susceptibility gene for diabetic cardiovascular disease. *The Israel Medical Association Journal*, 6: 308-310.
- Lu, Y., Pakes, S.P., and Massey, L. (1987). Hyperimmune serum from rabbit immunized with potassium thiocyanate extract of *Pasteurella multocida* protects against homologous challenge. *Journal of Clinical Microbiology*, 25(11): 2173-2180.
- Macpherson, A.J., McCoy, K.D., Johansen, F.E., and Brandtzaeg. (2008). The immune geography of IgA induction and function. Mucosal immunity. *Mucosal Immunology*, 1(1): 11-22.
- Mahasreshthi, P.J., Murphy, G.I., Ill, W., Farmer, S., Hancock, R.E.W., and Confer, A.W. (1997). Purification and partial characterization of the OmpA Family of proteins of *Pasteurella haemolytica*. *Infection and Immunity*, 65(1): 211-218.
- Marion L.J. (2007). *Veterinary clinical pathology: An Introduction*. United States: Blackwell Publishing.
- Mestecky, J., and Elson, C.O. (2014). Peyer's patches as the inductive site for IgA responses. *The Journal of Immunology*, 180: 1293-1294.
- Minang, J.T., Gyan, B.A., Anchang, J.K., Troye-Blomberg, M., Perlmann, H., and Achidi, E.A. (2004). Haptoglobin phenotypes and malaria infection in pregnant woman at delivery in western Cameroon. *Acta Tropica*, 90:107-114.
- Monisha, B., and Saxena, M. (2012). Interleukin-1 (Il-1) family of cytokines: Role in type 2 diabetes. *Clinica Chimica Acta*, 413(15-16): 1163-1170.

- Morris, C.A., Towers, N.R., Hohenboken, W.D., Maqbool, B.L., Smith, and Phua, S.H. (2004). Inheritance of resistance to facial eczema: A review of research findings from sheep and cattle in New Zealand. *New Zealand Veterinary Journal*, 52:205-215.
- Moshage, H. (1997). Cytokines and the hepatic acute phase. *Journal of pathology*, 181: 257-266.
- Oh, S.K., Pavlotsky, N., and Tauber, A.I. (1990). Specific binding of haptoglobin in human neutrophils and its functional consequences. *Journal of Leukocyte Biology*, 47: 142-148.
- OIE (2008). *Haemorrhagic septicaemia*. In Chapter 2: OIE Terrestrial Manual 2008. Pp 739-751.
- OIE (2009). *Haemorrhagic septicaemia*. Chapter 2.4.12: OIE Terrestrial Manual 2008. Pp 739-750.
- Ozmen, O., Sahindurian, S., Haligur, M., and Albay, M.K. (2008). Clinicopathological studies on facial eczema outbreak in sheep in Southwest Turkey. *Tropical Animal Health and Production*, 40: 545-551.
- Paule, B.J.A., Azevedo, V., Regis, L.F., Carminati, R., Bahia, C.R., Vale, V.L.C., Moura-Costa, L.F., Freire, S.M., Nascimento, I., Goes, A.M., and Meyer, R. (2003). Experimental *Corynebacterium pseudotuberculosis* primary infection in goats: kinetics of IgG and interferon-gamma production, IgG avidity and antigen recognition by western blotting. *Veterinary Immunology and Immunopathology*, 96: 129-139.
- Pépin, M., Seow, H.F., Corner, L., Rothel, J.S., Hodgson, A.L., and Wood, P.R. (1997). Cytokine gene expression in sheep following experimental infection with various strains of *Corynebacterium pseudotuberculosis* differing in virulence. *Veterinary Research*, 28(2): 149-163.
- Pillay, J., Hietbrink, F., Koenderman, L., Leenen, L.P. (2007). The systemic inflammatory response induced by trauma is reflected by multiple phenotypes of blood neutrophils. *Injury*, 38: 1365-1372.
- Popov, D., and Pavlov, G. (2013). Sepsis model in experimental animals. *Trakia Journal of Sciences*, 1: 13-23.
- Praveena, P.E., Periasamy, S., Kumar, A.A., and Singh, N. (2010). Cytokines profiles, apoptosis and pathology of experimental *Pasteurella multocida* serotype A1 infection in mice. *Research in Veterinary Science*, 89: 332-339.
- Procianoy, R.S., and Silveira, R.C. (2004). The role of sample collection timing on interleukin-6 levels in early-onset neonatal sepsis. *Journal de Pediatria*, 80(5): 407-410.

- Quan, S., Hiniker, A., Collet, J., and Bardwell, C.A. (2013). *Isolation of bacteria envelope protein. Chapter in Methods in molecular biology*. New York: Springer+Business Media.
- Quaye, I.K. (2008). Haptoglobin, inflammation and disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102: 735-742.
- Quaye, I.K., Abbabio, G., and Amoah, A.G. (2006). Haptoglobin 2-2 phenotype is a risk factor for type 2 diabetes in Ghana. *Journal of Atherosclerosis Thrombosis*, 13: 90-94.
- Qureshi, S., and Saxena, H.M. (2014). Estimation of titers of antibody against *Pasteurella multocida* in cattle vaccinated with haemorrhagic septicaemia alum precipitated vaccine. *Veterinary World*, 7(4): 224-228.
- Rafidah, O., and Zamri-saad, M. (2013). Effect of dexamethasone on protective efficacy of live *gdhA* derivative *Pasteurella multocida* B2 vaccine. *Asian Journal of Animal and Veterinary Advances*, 8(3): 548-554.
- Ramsay, A.J., Husband, A.J., Ramshaw, I.A., Bao, S., Matthaei, K.I., Koehler, G., and Kopf, M. (1994). The role of interleukin-6 in mucosal IgA antibody responses in vivo. *SCIENCE*, 264: 561-563.
- Rebers, P.A., Jensen, A.E., and Laird, G.A. (1988). Expression of pili and capsule by the avian strain P-1059 of *Pasteurella multocida*. *Avian Disease*, 32: 313-318.
- Remick, D.G., Bolgos, G., Copeland, S., and Siddiqui, J. (2005). Role of interleukin-6 in mortality from and physiological response to sepsis. *Infection and Immunity*, 73(5): 2751-2757.
- Rhoades, K.R., Heddleston, K.L., and Rebers, P.A. (1967). Experimental haemorrhagic septicaemia: Gross and microscopic lesions resulting from acute infections and from endotoxin administration. *Canadian Journal of Comparative Medicine*, 31: 226-233.
- Rimler, R.B., and Rhoades, K.R. (1987). Serogroup F, a new capsule serogroup of *Pasteurella multocida*. *Journal of Clinical Microbiology*, 25(4): 615-618.
- Robert H.D and Charles H.M. (2004). *Veterinary Pathophysiology*. United States: Blackwell Publishing.
- Rokke, O., Revhaugh, A., Osterud, B and Giercksky, K.E. (1988). Increase Plasma Level of Endotoxin and Corresponding Changes in Circulatory Performance in a Porcine Sepsis Model: The Effect of Antibiotic Administration. *Process in Clinical and Biological Research*, 272: 247-262.
- Saharee, A.A. (1992). *Epidemiology and pathogenesis of Haemorrhagic septicaemia in Peninsular Malaysia*. Ph.D Thesis, Universiti Putra Malaysia.

- 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 Proceeding no. 43. p89-91.
- Salminen, S., Bouley, C., Boutron-Ruault, C., Cummings, J.H., Gibson, G.R., Isolauri, E., Moreau, M.C., Roberfroid, M., and Rowland, I. (1998). Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition*, 80: 147-171.
- Samuelson, D.A. (2007). *Textbook of Veterinary Histology*. Missouri: Saunders Elsevier.
- Sastry, G.A. (2006). *Veterinary Clinical Pathology*. New Delhi: CBS Publishers & Distributors.
- Scheller, J., Chalaris, A., Schmidt-Arras, D., and Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta*, 1813: 878-888.
- Schmidt, M., Ammon, C., Schon, P.C., Manteuffel, C., and Hoffmann, G. (2014). The suitability of infrared temperature measurements for continuous temperature monitoring in gilts. *Archiv Tierzucht*, 57(21): 1-12.
- Shafarin, M.S., Zamri-saad, M., Siti-Khairani, B., and Saharee, A.A. (2008). Proliferation and transmission patterns of *Pasteurella multocida* B2 in goats. *Tropical Animal Health and Production*, 40: 335-340.
- Shah, C., Hari-Dass, R., and Rayness, J.G. (2006). Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. *Blood*, 108: 1751-1757.
- Simpson, R.J., Hammacher, A., Smith, D.K., Matthews, J.M., and Ward, L.D. (1997). Interleukin-6: Structure-function relationships. *Protein Science*, 6(5): 929-955.
- Singh, R., Gupta, P.K., and Durga, V. (2009). Expression and purification of the major outer membrane protein (OmpH) of *Pasteurella multocida* P52 from *Escherichia coli*. *Veterinarski Arhiv*, 79(6): 591-600.
- Skinner, J.G., Brown, R.A., and Roberts, L. (1991). Bovine haptoglobin responses in clinically defined field conditions. *Journal of the British Veterinary Association*, 128:147-149.
- Snick, J.V. (1990). Interleukin-6: An overview. *Annual Review of Immunology*, 8: 253-278.
- Srivastava, S.K. (2013). *Pathogenesis of bacterial infections in animals*. New Delhi: Satish Serial Publishing House.

- Steven, L.S. (2000). *Hematologic Changes due to Bacterial Infections*. In: *Schalms's Veterinary Hematology*, Bernard, F.F., Joseph, G.Z., and Neimi, C.J. 5<sup>th</sup> Edn, USA: Lippincott Williams and Wilkins.
- Stojevic, Z., Pirsljin, J., Milinkovic-Tur, S., Zdelar-Tuk, M., and Ljubic, B.B. (2005). Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. *Veterinarski Arhiv*, 75(1): 67-73.
- Susan, W., Craig, and Cebra, J.J. (1971). Payer's pathes: An enriched source of precursors for IgA producing immunocytes in the rabbit. *The Journal of Experimental Medicine*, 134: 188-200.
- Takashima, H., Sakai, H., Yanai, T., and Masegi, T. (2001). Detection of antibodies against *Pasteurella multocida* using immunohistochemical staining in an outbreak of rabbit pasteurellosis. *Journal of Veterinary Medical Science*, 63(2): 171-174.
- Thomas, J. (1972). The control of haemorrhagic septicaemia in west Malaysia. *Tropical Animal Health and Production*, 4: 95-101.
- Tizard, I.R., and Schubot, R.M. (2000). *Veterinary immunology an introduction*. Pennsylvania: W.B. Saunders Company.
- Tomer, P., Chaturvedi, G.C., Minakshi, Malik, P., and Monga, D.P. (2002). Comparative analysis of the outer membrane protein profiles of isolate of *Pasteurella multocida* (B:2) associated with haemorrhagic septicaemia. *Veterinary Research Communications*, 26(2002): 513-522.
- Trinchieri, G. (1955). Interleukin-12: A proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annual Review of Immunology*, 13: 251-276.
- Upragarin, N., Landman, W.J.M., Gaastra, W., and Gruys, E. (2005). Extrahepatic production of acute phase serum amyloid A. *Histology and Histopathology*, 20: 1295-1307.
- VanDijk, J.E., Gruys, E., and Mouwen, J.M.V.M. (2007). *Color Atlas of Veterinary Pathology 2<sup>nd</sup> Edition*. Philadelphia: Saunders Elsevier.
- Verma, R., and Jaiswal, T.N. (1998). Haemorrhagic septicaemia vaccines. *Vaccine*, 16(11/12): 1184-1192.
- Warner, A.E., DeCamp Jr, M.M., Molina, R.M., and Brain, J.D. (1988). Pulmonary Removal of Circulating Endotoxin Results in Acute Lung Injury in Sheep. *Laboratory Investigation*, 59: 219-230.
- Weiss, D.J., and Wardrop, K.J. (2010). *Schalm's Veterinary Hematology 6<sup>th</sup> Edition*. Iowa: Wiley-Blackwell.



- Wens, R., Goffin, Y., Pepys, M.B., Kutnowski, M., Van Beers, D., Decoodt, P., and Verbanck, M. (1989). Left atrial myxoma associated with systemic AA amyloidosis. *Archieve of Internal Medicine Journal*, 149:453-454.
- William, D.M., Tyznik, A.J., and Bevan, M.J. (2006). Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. *Nature*, 441: 890-893.
- Xie, Z., Li, H., Chen, J., Zhang, H., Wang, Y, Chen, Q., Zhao, Z., Cheng, C., Zhang, H., Yang, Y., Wang, H., and Gao, R. (2007). Shuffling of pigs interleukin-2 gene and its enhancing of immunity in mice to *Pasteurella multocida* vaccine. *Vaccine*, 25: 8163-8171.
- Yoo, H.S., Maheswaran, S.K., Srinand, S., Ames, T.R., and Suresh, M. (1995). Increased tumour necrosis factor- $\alpha$  and interleukin-1 $\beta$  expression in the lungs of calves with experimental pneumonic pasteurellosis. *Veterinary Immunology and immunopathology*, 49:15-28.
- Yu, S., Kuan, W., Wong, C., Li, E.K., and Tam, L. (2012). Immunopathological roles of cytokines, chemokines, signalling molecules, and pattern-recognition receptors in systemic lupus erythematosus. *Clinical and Developmental Immunology*, 2012: 1-4.
- Zamri-Saad, M. (2013) *Haemorrhagic septicaemia of cattle & buffaloes in Asia*. Selangor: Universiti Putra Malaysia Press.
- Zamri-saad, M., Effendy, A.W.M., Israf, D.A., and Azmi, M.L. (1999). Cellular and humoral responses in the respiratory tract of goats following intranasal stimulation using formalin-killed *Pasteurella haemolytica* A2. *Veterinary Microbiology*, 65: 233-240.
- Zamri-saad, M., Ernie, Z.A., and Sabri, M.Y. (2006). Protective effect following intranasal exposure of goats to live *Pasteurella multocida* B2. *Tropical Animal Health and Production*, 38: 541-546.
- Zamri-saad, M., Jasni, S., Nurida, A.B., and Sheikh-Omar, A.R. (1991). Experimental infection of dexamethasone-treated goats with *Pasteurella haemolytica* A2. *British Veterinary Journal*, 147: 565-568.