



UNIVERSITI PUTRA MALAYSIA

***IMMUNISATION EFFECT IN HOST FOLLOWING ORAL EXPOSURE
TO GRADED DOSES OF IMMUNOGENIC LIPOPOLYSACCHARIDE
EXTRACTED FROM PASTEURELLA MULTOCIDA TYPE B:2***

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EXTRACTED FROM *PASTEURELLA MULTOCIDA* TYPE B:2**

SARAH HELMY

**A project paper submitted to the Faculty of Veterinary Medicine, Universiti
Putra Malaysia**

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

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It is hereby certified that we have read this project paper entitled “Immunisation effect in host following oral exposure to graded doses of immunogenic lipopolysaccharide extracted from *Pasteurella multocida* type B:2”, by Sarah Helmy and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999-Project.

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DEDICATION

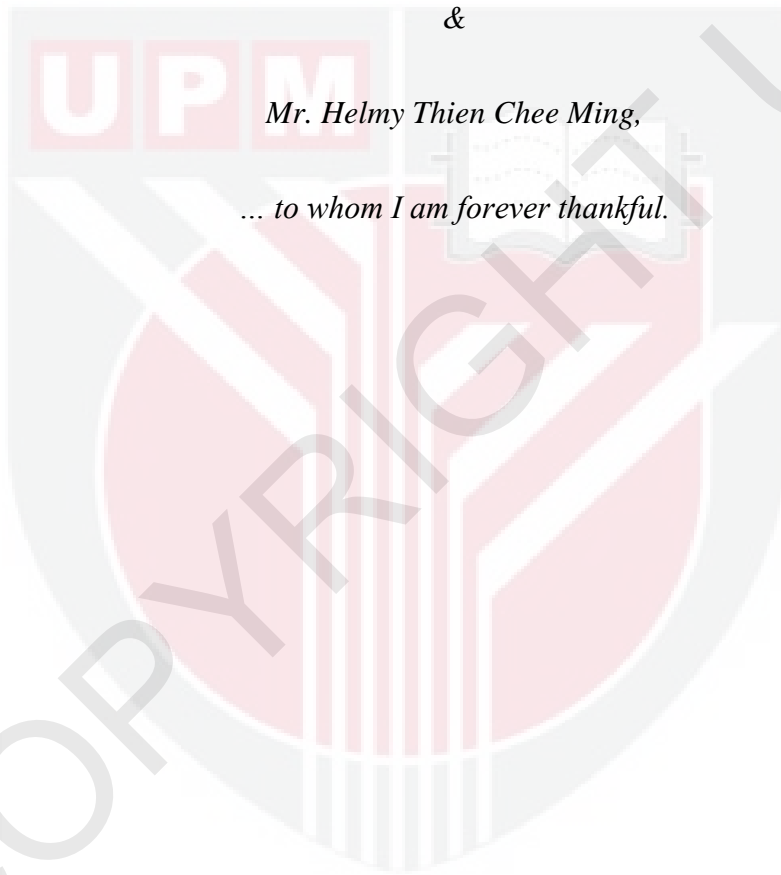
The love of my life,

Mrs. Salmah bt Abdullah

&

Mr. Helmy Thien Chee Ming,

... to whom I am forever thankful.



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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

**KESAN IMUNISASI TERHADAP HAIWAN BERIKUTAN INOKULASI
MELALUI ORAL DENGAN LIPOPOLISAKARIDA DARIPADA
PASTUERELLA MULTOCIDA JENIS B:2 YANG BERBEZA DOS**

Oleh

Sarah Helmy

2016

Penyelia: Dr. Faez Firdaus Jesse Abdullah

Di Malaysia, penyakit hawar darah atau Haemorrhagic Septicaemia (HS), iaitu sejenis penyakit yang mempunyai kadar kematian yang tinggi di kalangan kerbau dan lembu, adalah disebabkan oleh bakteria *Pasteurella multocida* jenis B:2. Lipopolisakarida (LPS) merupakan salah satu faktor penting untuk *P. multocida* menghasilkan tindak balas imunisasi. Oleh itu, eksperimen ini dijalankan bagi mempelajari kesan imunisasi terhadap mencit yang diberi lipopolisakarida daripada *P. multocida* B:2 melalui mulut. Sebanyak 25 ekor mencit dibahagikan ke dalam lima kumpulan, dimana setiap kumpulan mempunyai lima ekor mencit. Kumpulan terkawal telah diberi 0.2ml Phosphate Buffer Saline (PBS) pH 6.8 melalui oral, manakala Kumpulan 1, Kumpulan 2, Kumpulan 3 dan Kumpulan 4 masing-masing diberi 0.2ml lipopolisakarida daripada 10^3 , 10^5 , 10^7 dan 10^9 cfu *Pasteurella*

multocida jenis B:2, melalui oral. Haiwan-haiwan tersebut diperhatikan setiap hari selama 14 hari, untuk tanda-tanda penyakit yang penting. Selepas tujuh belas hari, haiwan-haiwan tersebut diberikan sebanyak 0.2 ml bakteria *Pasteurella multocida* yang mempunyai konsentrasi sebanyak 10^7 cfu. Sekali lagi, haiwan-haiwan tersebut diperhatikan untuk tanda-tanda penyakit penting. Selepas tujuh hari, mencit yang masih hidup, dimatikan dan organ di simpan untuk pemeriksaan histopathologi dan untuk mengasingkan dan mengesan kehadiran bakteria. 60% daripada haiwan daripada semua kumpulan mengalami cirit-birit sebelum mati, 38.5% daripadanya pula mempunyai luluhan okular dan 100% daripadanya mengalami kesukaran untuk bernafas. Terdapat sedikit sehingga sederhana lesi histopathologi di dalam kesemua organ yang dikaji yang merupakan jantung, paru-paru, hati, limpa, buah pinggang, usus besar, usus kecil dan perut di dalam kesemua kumpulan. Secara ketara ($P < 0.05$), Kumpulan 1 mempunyai sedikit sel kerandangan di dalam limpa (1.4) dan usus kecil (0.7), manakala Kumpulan 4 mempunyai sedikit nekrosis di dalam buah pinggang (1.1) dan nekrosis sederhana di dalam perut (2.2). *P. multocida* telah dijumpai dari jantung, paru-paru, hati, limpa, buah pinggang, usus dan perut daripada kesemua kumpulan. Kadar kematian haiwan menunjukkan tiada perbezaan ketara ($P > 0.05$) diantara kumpulan terkawal (40% kematian), kumpulan konsentrasi rendah (Kumpulan 1 dan Kumpulan 2) (33.33% kematian) dan kumpulan konsentrasi tinggi (66.67%). Hal ini menunjukkan lipopolisakarida daripada *P. multocida* B:2, samada konsentrasi rendah atau konsentrasis tinggi gambar untuk memberi imunisasi untuk melindungi mencit daripada penyakit HS.

Kata kunci: Hawar darah, Pasteurella multocida jenis B, mencit, lipopolisakarida, lesi histopatologi



ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

**IMMUNISATION EFFECT IN HOST FOLLOWING ORAL EXPOSURE OF
GRADED DOSES OF IMMUNOGENIC LIPOPOLYSACCHARIDE EXTRACTED
FROM PASTEURELLA MULTOCIDA TYPE B:2.**

by

Sarah Helmy

2016

Supervisor: Dr. Faez Firdaus Jesse Abdullah

In Malaysia, *Pasteurella multocida* serotype B:2 is the causative agent of haemorrhagic septicaemia (HS), a highly fatal disease in buffalo and cattle. Lipopolysaccharide (LPS) is one of the major immunogen of *P. multocida*. This study aims to determine the protective effect due to oral inoculation of LPS extracted from *P. multocida* B:2 against HS disease. Twenty-five healthy mice were divided into five groups consisting of five animals each. The control group was inoculated orally with 0.2ml of Phosphate Buffer Saline (PBS) pH 6.8, whereas Group 1, Group 2, Group 3 and Group 4 were inoculated orally with 0.2ml lipopolysaccharides extracted from 103, 105, 107 and 109cfu of *Pasteurella multocida* serotypes B:2, respectively. The experimental animals were observed for clinical signs for seventeen days. All the groups were subjected for challenge with 0.2 mL of 107 wild type

Pasteurella multocida B:2 after 17 days post LPS inoculation. Then, the groups were observed for clinical signs for seven days. After seven days, surviving mice were euthanised and the organs were collected for histopathological examination and bacterial isolation and identification. Before death, 60% of the animals from all group had diarrhoea, 38.5% had severe ocular discharge and 100% had laboured breathing. Mild to moderate histopathological lesions were observed in heart, lungs, liver, spleen, kidney, small intestine, large intestine, small intestine and stomach of all groups. Significantly ($P < 0.05$), Group 1 had mild presence of inflammatory cells in spleen (1.4) and small intestine (0.7), whereas Group 4 had mild to moderate degeneration and necrosis of kidney (1.1) and stomach (2.2). *P. multocida* was isolated from heart, lung, liver, spleen, kidney and gastrointestinal tract of all groups. Rate of animal mortality showed no significant different ($P > 0.05$) between control group (40% mortality), low concentration group (Group 1 and Group 2) (33.33% mortality) and high concentration group (Group 3 and Group 4) (66.67% mortality). This showed that oral inoculation of LPS extracted from *P. multocida*, both in low and high concentration, in mice, failed to give immunity to the host.

Keywords: Haemorrhagic septicaemia, *Pasteurella multocida* serotypes B:2, lipopolysaccharides, mice, histopathological lesions

1.0 INTRODUCTION

World Organisation for Animal Health (OIE) defined Classical Haemorrhagic Septicaemia (HS) as a disease caused by *Pasteurella multocida* serotypes B:2 or E:2 (Mosier, 2014). HS is an acute, febrile and lethal disease, causing death in susceptible animals in less than 36 to 48 hours of exposure to the organism (Jamal, 2013). It is seen mainly in cattle and water buffalo with progressive clinical signs from dullness and fever to death within hours, and is transmitted via ingestion or inhalation of the organism (Spickler & Roth, 2009). HS causes high economic losses due to animal mortality.

Oil-Adjuvanted Vaccine (OAV), applied parenterally, is the main prophylactic agent used in Malaysia against HS infection (Chung *et al.*, 2015). However, only 17% of Malaysian buffaloes were vaccinated due to difficulty of vaccine administration (Zamri-saad, 2013). Animal needs to be individually restrained to apply parenteral vaccination, making vaccine application difficult. Studies by Abu Bakar *et al.* (2011) had shown that oral route inoculation of whole bacteria of *P. multocida* in buffaloes causes less severe clinical signs, compared to other routes, indicating that oral route might be a readily available route for effective vaccination administration (Chung, *et al.*, 2015).

In addition, lipopolysaccharide of *Pasteurella multocida* type B was found to be protective against experimentally induced pasteurellosis in mice (Muniandy *et al.*, 1998). They are also good immunogens and can be used for subunit vaccine development (Sarangi, *et al.*, 2014). However, there is still a lack of understanding in the tissue changes of immune system organs with *P. multocida* infections and hence

limited knowledge regarding the protective effect conferred by oral route inoculation of lipopolysaccharide of *Pasteurella multocida* type B:2 against HS disease.

Therefore, the aim of this study is to determine the protective effect on host upon oral inoculation with graded doses of immunogenic L extracted from *Pasteurella multocida* type B:2 against HS disease. The results and knowledge obtained from this study may serve as a future reference in improving the preventive measures and treatment of HS disease, and the development of oral subunit vaccine against HS applied in animal feed. Such application will be of more convenient to the farmers, and may greatly improve prophylactic measures and immunisation against HS in Malaysia.

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