

THE EFFECTS OF THE INGESTION OF  
THE BACTERIA PASTEURELLA  
MULTOCIDA IN WHITE RATS

BY  
D. S. S. S. S. S.

DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY  
FACULTY OF MEDICAL SCIENCES, UNIVERSITY OF MALAYA  
2000



DEVELOPMENT OF BRONCHUS-ASSOCIATED LYMPHOID TISSUE (BALT)  
FOLLOWING INOCULATION OF DUST PREPARATION OF  
*PASTEURELLA MULTOCIDA* B:2 IN WHITE RATS

By

Mohd Azrul bin Lokman

Research Report submitted in partial fulfillment of  
the requirements for the degree of  
Bachelor of Science (Biological Sciences)

Department of Biological Sciences  
Faculty of Science and Technology  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA  
2006

This project should be cited as:

Azrul-Lokman, M. 2006. Development of Bronchus-associated Lymphoid Tissue (BAL) Following Inoculation of Dust Preparation of *Pasteurella multocida* B3 in White Rats. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 59p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.



**JABATAN SAINS BIOLOGI  
FAKULTI SAINS DAN TEKNOLOGI  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN  
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: DEVELOPMENT OF BRONCHUS-ASSOCIATED LYMPHOID TISSUE (BALT) FOLLOWING INOCULATION OF DUST PREPARATION OF *PASTEURELLA MULTOCIDA* B:2 IN WHITE RATS, oleh Mohd Azrul bin Lokman, no. matrik: UK 7919 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama **PROF. MADYA DR. MOHD. EFFENDY ABD WAHID**  
Pegawai

Cop Rasmi: **Pusat Bioteknologi Marin  
Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu.**

Tarikh: 14 Mei 2006

Ketua Jabatan Sains Biologi

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**  
Ketua

Cop Rasmi: **Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
(KUSTEM)  
21030 Kuala Terengganu.**

Tarikh: .....

## ACKNOWLEDGEMENTS

Assalamualaikum and peace upon all,

Thank you to Allah the Almighty for blessing me to complete this project.

On this opportunity, I would like to thank all the people who have helped me through out this project. A special thanks to my respective supervisor, Assoc. Prof. Dr. Mohd Effendy Abd. Wahid for his guidance, advices, and support that he gave to me during the completion of this project. Thanks for your constructive comments too and I will appreciate that.

A million thanks to the Science Officer and the staff of histology laboratory and microbiology laboratory of KUSTEM, Miss Norazlina Abd. Aziz, Mr. Muhammad Embong, Mrs. Zarina, Mrs. Mahidawati, and Kak Zie for allowing me to use the laboratory and its facilities. Further thanks to Master students, Ooi Keng Wooi and Siti Nurtahirah who have help me a lot.

I also wish to thanks my family and my beloved parent. Tn. Hj. Lokman Samsuri and Mdm. Salmiah Absar for their love and support. Not forgetting my project partners. Firdaus, Meor, Rawaidah, Chiah and Zaireen for their help. Lastly, I would like to thank all the people who have involved directly or indirectly in completing this project especially my kind-hearted adopted brother. May Allah bless us all. Thank you.

## TABLE OF CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENTS</b>	ii
<b>LIST OF TABLES</b>	vi
<b>LIST OF FIGURES</b>	vii
<b>LIST OF ABBREVIATIONS</b>	viii
<b>LIST OF APPENDICES</b>	ix
<b>ABSTRACT</b>	x
<b>ABSTRAK</b>	xi
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Introduction	1
1.2 Importance of Study	2
1.3 Objective of Study	3
<b>CHAPTER 2: LITERATURE REVIEW</b>	4
2.1 <i>Pasteurella multocida</i>	4
2.1.1 Classification	4
2.1.2 Morphology	5
2.1.3 Growth	6
2.1.4 Capsule serotyping	7
2.2 Related Diseases	7
2.2.1 Haemorrhagic septicaemia	7

2.2.2	Atrophic rhinitis	8
2.2.3	Fowl cholera	8
2.2.4	Other diseases	9
2.3	Bronchus-associated Lymphoid Tissue (BALT)	9
2.3.1	Characterization of BALT	10
2.3.2	Structure of BALT	10
2.3.3	Function of BALT	10
<b>CHAPTER 3: MATERIALS AND METHODOLOGY</b>		12
3.1	Animal	12
3.2	Lyophilized Crude Preparation	14
3.3	Experimental Design	15
3.4	Histological Technique	17
3.4.1	Specimen fixation	17
3.4.2	Tissue processing	17
3.4.3	Embedding	19
3.4.4	Cutting	19
3.4.5	H & E staining and DPX mounting	19
3.5	Data Analysis	21
<b>CHAPTER 4: RESULTS</b>		22
4.1	The Area of BALT	22
4.2	The Number of Lymphocytes	26



<b>CHAPTER 5: DISCUSSION</b>	28
<b>CHAPTER 6: CONCLUSION</b>	34
<b>REFERENCES</b>	36
<b>APPENDICES</b>	39
<b>CURICULUM VITAE</b>	58

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
3.1	The process for the tissue processing with their chemicals and immersing duration	18
3.2	Procedure for the H&E Staining process	20

## LIST OF FIGURES

Figure		Page
2.1	The taxonomy of <i>P. multocida</i>	5
3.1	White rat ( <i>Rattus norvegicus</i> ) type Sprague-dawley	12
3.2	White rats were housed 10 per cage	13
3.3	White rats had free access to food and water during the entire experiment	13
3.4	The lyophilized crude of <i>P. multocida</i> B:2	14
3.5	Lyophilized crude of <i>P. multocida</i> B:2 was inoculated into intranasal of white rat	16
3.6	Slaughtering process of white rat to collect lung sample	16
4.1	The average of BALT area in control and treatment group of white rats for day-14 and day-28	22
4.2	BALT area in white rat in control group for day-14	23
4.3	BALT area in white rat in treatment group for day-14	23
4.4	BALT area in white rat in control group for day-28	24
4.5	BALT area in white rat in treatment group for day-28	24
4.6	The average of number of lymphocytes in control and treatment group of white rats for day-14 and day-28	26

## LIST OF ABBREVIATIONS

%	Percent
\$US	US Dollar
BALT	Bronchus-associated Lymphoid Tissue
µm	Micron meter
°c	Degree Celsius
mm	Milimeter
GALT	Gut-associated Lymphoid Tissue
NALT	Nasopharyngeal-associated Lymphoid Tissue
VALT	Vagina-associated Lymphoid Tissue
g	Gram
ml	Mililiter
rpm	Rotation per Minute
mg	miligram
BHI	Brain Heart Infusion
<	Less than
>	More than
SD	Standard deviation
MHC	Major Histocompatibility Complex
APC	Antigen Presenting Cell
Th cell	Helper T cell

## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
Appendix A	The Apparatus	40
Appendix B	Raw Data	44
Appendix C	The Average / Mean with Standard Deviation	48
Appendix D	Statistical Test	52

## ABSTRACT

Twenty clinically healthy white rats (*Rattus norvegicus*) from type Sprague-dawley were equally divided into two groups; group 1 as the control untreated group while group 2 was inoculated with lyophilized crude of *Pasteurella multocida* B:2 on day-0 and day-14. On day-14, five white rats from each group were euthanized and lung samples were collected. On the same day, the crude was readministered to the rest of the animals in group 2. On day-28, all the rest of white rats were slaughtered and lung samples were collected. All lung samples were prepared for histological examination. The objective of these experiment is to determine the response of bronchus-associated lymphoid tissue (BALT) after inoculation of lyophilized crude of *Pasteurella multocida* B:2. From observation, the average of BALT areas for the treatment group on day-14 and day-28 are  $1.36 \times 10^{-5} \pm 58011.00 \mu\text{m}^2$  and  $1.97 \times 10^{-5} \pm 95669.61 \mu\text{m}^2$ , higher than the range of control group. The average of number of lymphocytes for the treatment group on day-14 and day-28 are  $9528 \pm 4259.24$  and  $16803 \pm 9607.99$ , higher than the range of control group. There are significant differences between all the groups of individual and groups of experiment except one – analyzed by ANOVA Two-Factor with replication. Therefore, the BALT that was inoculated by lyophilized crude of *Pasteurella multocida* B:2 is response well.

**PERKEMBANGAN TISU LIMFOID BERHUBUNG-BRONKUS (BALT)  
SELEPAS INOKULASI *PASTEURELLA MULTOCIDA* B:2  
DALAM BENTUK SERBUK PADA TIKUS PUTIH**

**ABSTRAK**

Sebanyak 20 ekor tikus putih (*Rattus novvegicus*) dari jenis Sprague-dawley yang sihat dibahagikan sama rata kepada dua kumpulan; kumpulan 1 sebagai kumpulan kawalan tanpa sebarang rawatan dan kumpulan 2 yang dirawat dengan serbuk *Pasteurella multocida* B:2 yang tidak ditapis pada hari-0 dan hari ke-14. Pada hari ke-14, lima ekor tikus putih dari setiap kumpulan disembelih dan sampel paru-paru dikumpulkan. Pada hari yang sama, serbuk tersebut diberi sekali lagi kepada baki tikus putih dalam kumpulan 2. Pada hari ke-28, kesemua tikus putih disembelih dan sampel paru-paru dikumpulkan. Semua sampel paru-paru diperiksa secara histologi. Tujuan kajian ini ialah untuk menentukan tindakbalas BALT selepas didedahkan dengan serbuk *Pasteurella multocida* B:2 yang tidak ditapis. Daripada pemerhatian, didapati purata keluasan kawasan BALT untuk tikus putih dari kumpulan rawatan pada hari ke-14 dan hari ke-28 adalah  $1.36 \times 10^{-5} \pm 58011.00 \mu\text{m}^2$  dan  $1.97 \times 10^{-5} \pm 95669.61 \mu\text{m}^2$ , lebih tinggi dari tikus putih dari kumpulan kawalan. Purata bilangan limfosit untuk tikus putih dari kumpulan rawatan pada hari ke-14 dan hari ke-28 pula adalah  $9528 \pm 4259.24$  dan  $16803 \pm 9607.99$ , lebih tinggi dari tikus putih dari kumpulan kawalan. Kesemua perbezaan yang dianalisis dengan ANOVA 2-Hala dengan replikasi adalah ketara kecuali satu. Jadi, selepas diberi serbuk *Pasteurella multocida* B:2 yang tidak ditapis, BALT memberi tindakbalas yang baik.