

STIMULATION OF BRONCHUS - ASSOCIATED LYMPHOID TISSUE
(BALD) FOLLOWING INTRANASAL EXPOSURE OF
FORMALIN-KILLED *Pasteurella multocida* B:2 IN GOATS

FARRA AIDAH JUMUDDIN

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

STIMULATION OF BRONCHUS-ASSOCIATED LYMPHOID TISSUE (BALT)
FOLLOWING INTRANASAL EXPOSURE OF FORMALIN-KILLED
Pasteurella multocida B:2 IN GOATS

By:-

Farra Aidah Jumuddin

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
2005

This project should be cited as:

Farra, A.J. 2005. Stimulation of bronchus-associated lymphoid tissue (BALT) following intranasal exposure of formalin-killed *Pasteurella multocida* B:2 in goats. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 61p.

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.

1100036800



**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:....**Stimulation of bronchus-associated lymphoid tissue (BALT) following intranasal exposure of formalin-killed *Pasteurella multocida* B:2 in goats**...oleh ...**Farra Aidah B. Jumuddin**..., no. matrik: ...**UK 6576**... 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:

DR. MOHD. EFFENDY BIN ABD. WAHID
Nama: **DR. MOHD. EFFENDY BIN ABD. WAHID**
Associate Professor
Dept. of Biological Science
Faculty of Science and Technology
Cop Rasmi
University College of Science and Technology Malaysia
Mengahang Telipot
21030 Kuala Terengganu.

Tarikh: **March 3, 2005**

Penyelia Kedua (jika ada)

DR. MD. MAHFUZUL HOQUE
Nama: **DR. MD. MAHFUZUL HOQUE**
Cop Rasmi
LECTURER
Department of Biological Science
Faculty of Science & Technology
University College of Science and Technology Malaysia
21030 Kuala Terengganu.

Tarikh: **03.04.05**

Ketua Jabatan Sains Biologi

Nama:

PROF. MADYA DR. NAKISAH BT. MAT AMIN

Cop Rasmi:

Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: **5/4/05**

ACKNOWLEDGEMENT

Assalamualaikum w.b.t

Alhamdulillah, a great thanks to Allah S.W.T who giving me chance to finish my Final Year Project Report to fulfillment the requirements for the degree of Bachelor Degree in Biological Sciences.

First of all I would like to give my great thanks to my main supervisor Prof. Madya Dr. Mohd. Effendy B. Abdul Wahid and my co-supervisor Dr. Mohd. Mahfuzul Hoque who gave me so much of guidance and support to complete this project. Not forgettable, I would like to express my thankful to the master's students, Siti Tafsil Raudah, Siti Nur Tahirah and all the research laboratory assistants especially En. Mohammad Embong and Normazianti for all the cooperation given.

Last but not least, I would like to express my grateful thanks to my family especially to my parent, Jumuddin B. Mohd Daud and Manom Bt. Nordin, course mates and friends who gave me enormous support.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	i
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	1
1.1 Objectives	3
1.2 Hypothesis	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 <i>Pasteurella multocida</i>	5
2.2 Disease cause by <i>Pasteurella sp</i>	6
2.3 Immunology	8
2.4 The mucosa immune system	10
2.5 Bronchus-associated lymphoid tissue (BALT)	22
2.6 Lung	23
CHAPTER 3 METHODOLOGY	24
3.1 Animal studied	24

3.1	Experimental design	25
3.3	Statistical analysis	30
CHAPTER 4 RESULT		31
4.1	Comparison in area of in BALT (μm^2)	31
4.2	Comparison in number of lymphocytes in BALT	34
4.3	The nodular and aggregate of BALT	37
CHAPTER 5 DISCUSSION		42
CHAPTER 6 CONCLUSION		46
REFERENCES		47
APPENDIXES		50
CURRICULUM VITAE		61

LIST OF TABLES

Table	Page
Table 3.1 : Eperimental Design	27
Table 4.1 (a) : Average size of BALT	32
Table 4.1 (b) : Data analysis of size in BALT	32
Table 4.2 (a) : Average numbers of lymphocytes in BALT	35
Table 4.2 (b) : Data analysis of number of lymphocytes in BALT	35
Table 4.3 (a) : Total of nodular and aggregate occurred in BALT	38
Table 4.3 (b) : Data analysis of total nodular and aggregate occurred in BALT	38
Table C.1 : Data analysis of size of BALT	57
Table C.2 : Data of numbers of lymphocytes in BALT	57

LIST OF FIGURES

Figure		Page
Figure 2.1	: Mucosa associated lymphoid tissue (MALT)	12
Figure 2.2	: Image of a lymph node	13
Figure 2.3	: Lymphocytes homing pathways for the Common Mucosa Immune Systems (CMIS)	18
Figure 2.4	: Primary and secondary immune response	21
Figure 4.1	: Area of BALT (μm^2) in different Treatment (40x)	33
Figure 4.2	: Numbers of lymphocytes in different Treatment	36
Figure 4.3	: Nodular in BALT	39
Figure 4.4	: Aggregate in BALT	40
Figure 4.5	: The correlation between area and number of lymphocytes in BALT	41
Figure A.1	: 'Katjang' goats were used in the experiment	51
Figure A.2	: The lungs were fixed in 10% buffered formalin	51
Figure B.1	: Steps in Histological Technique	52
Figure B.2	: The Fixation process	53
Figure B.3	: Automatic Tissue Processing Machine	53

Figure B.4	: Embedding to form a solid block for sectioning	54
Figure B.5	: Sectioning by using Microtome in length of 3-5 μm	54
Figure B.6	: Waterbath	55
Figure B.7	: Staining jar	55
Figure B.8	: Sample was analyzed by using compound microscope (Leica DM LB2-Image Analyzer)	56

LIST OF ABBREVIATIONS

μm	-	micrometer
BALT	-	Bronchus associated lymphoid tissue
<i>P. m</i>	-	<i>Pasteurella multocida</i>
MALT	-	Mucosa associated lymphoid tissue
CMIS	-	Common mucosal immune system
PP	-	Payer's Patches

LIST OF APPENDICES

Appendix	Page
Appendix A : Animal used in the experiment	50
Appendix B : Histology Technique	52
Appendix C : Data analysis	57
Appendix D : Statistical Test	58

ABSTRACT

A study was conducted to determine the size and number of lymphocytes after stimulation with formalin-killed *Pasteurella multocida* B:2 in lung of goats. Fifteen clinically healthy goats were divided equally into three groups. Goats in Group 1 were exposed once with formalin-killed *Pasteurella multocida* serotype B:2 and slaughtered at day 15 after exposure. Goats in Group 2 were exposed intra nasally twice at 14 days intervals and slaughtered in days 28. Goats in Group 3 remained unexposed and considered as Control Group and slaughtered at days 15. After the goats were killed, the right apical lobes of the lungs were fixed in 10% buffered formalin to continue with histology techniques. The size of lymphocytes in bronchus associated lymphoid tissue (BALT) increased significantly ($p < 0.05$) in Group 1 but insignificantly ($p > 0.05$) in Group 2. While for numbers of lymphocytes in BALT, the three groups show significant level ($p < 0.05$). In addition, the interval gap for Group 2 should be increased to 3 weeks to get the rapid and great response in size and numbers of lymphocytes in BALT. There are also moderate correlation in Group 1, 2 and 3 ($r = 0.54$).

**RANSANGAN TISU LIMFOID BERKAIT BRONKUS (BALT) MELALUI
DEDAHAN INTRANASAL KEPADA *Pasteurella multocida* B:2 PADA
PEPARU KAMBING.**

ABSTRAK

Kajian yang dijalankan bagi mengkaji perubahan saiz dan bilangan limfosit di dalam BALT apabila dirangsang dengan *Pasteurella multocida* B2 pada paru-paru kambing. Lima belas ekor kambing dibahagikan samarata kepada tiga kumpulan. Setiap kumpulan mengandungi 5 ekor kambing. Kambing pada kumpulan 1 di dedahkan kepada *Pasteurella multocida* sebanyak sekali dan di sembelih pada hari ke 15. Manakala kambing dalam kumpulan 2 didedahkan kepada *Pasteurella multocida* sebanyak dua kali pada selang 15 hari. Kambing dalam kumpulan 3 pula tidak akan didedahkan kepada vaksin dan ia bertindak sebagai kumpulan kawalan dan disembelih pada hari ke 28. Setelah kambing tersebut disembelih, paru-parunya akan direndam di dalam 10% buffered formalin sebelum teknik Histologi dijalankan. Saiz limfosit di dalam BALT meningkat secara signifikan ($p < 0.05$) bagi kumpulan satu tetapi menjadi tidak signifikan ($p > 0.05$) pada Kumpulan 2. Sementara itu, bilangan limfosit di dalam BALT menjadi signifikan ($p < 0.05$) bagi ketiga-tiga kumpulan. Sebagai tambahan, selang masa bagi kumpulan 2 perlu ditingkatkan kepada 3 minggu bagi merangsang saiz dan bilangan limfosit di dalam BALT supaya menjadi banyak dan signifikan. Terdapat korelasi pada kadar yang medium di antara saiz dan bilangan limfosit di dalam BALT.