

CHANGES OF ANT-ASSOCIATED LYMPHOID TISSUE
FOLLOWING INTRADERMAL EXPOSURE OF FORMALIN-
KILLED *Pastoralla multocida* B1 IN GOATS

SEEMALA A/P. MARIMUTHU

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

1/2113

1100036874

LP 33 FST 4 2005



1100036874

Changes of Gut Associated Lymphoid Tissue following intratracheal exposure of formalin-killed pasteurilla multocida B2 in goats / Shamala a/p Marimuthu.



PERPUSTAKAAN
KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100036874

| | | |
|------------|--|--|
| 1100036874 | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

CHANGES OF GUT- ASSOCIATED LYMPHOID TISSUE FOLLOWING
INTRATRACHEAL EXPOSURE OF FORMALIN-KILLED *Pasteurella multocida*
B2 IN GOATS

By

Shamala A/P Marimuthu

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Applied Science (Biodiversity Conservation and Management)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Marimuthu, S. 2005. Changes of Gut –Associated Lymphoid Tissue (GALT) following intratracheal exposure of formalin-killed *Pasteurella multocida* B2 in goats. Undergraduate thesis, Bachelor of Applied Science in Biodiversity Conservation and Management, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu, 66p.

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

1100036874



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:
 Changes of Gut Associated Lymphoid Tissue following Intratracheal Exposure of
 Formalin-killed *Pasteurella multocida* B2 in Goats.

..... Shamala A/P Marimuthu UK6422
 oleh, no. matrik: telah
 diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan
 kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan
 memperoleh ijazah **Bachelor Sains Gunaan (Pemuliharaan & Pengurusan Biodiversiti)**
 Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: **Prof. Madya Dr. Mohd Effendy b. Abd. Wahid**
 Pensyarah

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

Tarikh: **6 April 2005**

DR. MD. MAHFUZA HOQUE

Nama: **LECTURER**
Department of Biological Science
Faculty of Science & Technology
University College of Science and Technology Malaysia
 21030 Kuala Terengganu.

Tarikh: **6 April 2005**

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: **6 April 2005**

ACKNOWLEDGEMENTS

Here I would like to take this opportunity to express my appreciation to all the people who have helped me to finish this project. First of all, I would like to thank God following the success of this project. I would like to express my heartiest appreciation especially to my supervisor Associate Prof. Dr. Mohd. Effendy bin Abdul Wahid who has devoted a lot of his time for invaluable guidance especially on the planning of the study, constructive, suggestions and consistent motivation during the project. I also like to thank Dr. Mahfuzul Hoque for his guidance and helps. My thanks also go to Associate Prof. Liew Hock Chark to have helped me in the statistical analysis. My grateful thanks are also extended to Miss Siti Tafsil Raudah who helped to guide and teach me whenever my supervisor was not around. My thanks also goes to the laboratory assistants and research assistants especially Encik Mohammad and others who provided information about the laboratory, instructions on using the equipments and also for their cooperation. I am also grateful to my family members for their encourage, support and also for the permission to do the laboratory work during holidays. Here, I also wish to deliver my gratitude for the cooperation given by my group members during laboratory works. Talking about encouragement and support, I would like to thank my friends who cheers me up whenever the result is not that good. I appreciate your friendship and hope to keep in touch in future. All of your sacrifices and unreserved will forever last to be remembered.

TABLE OF CONTENTS

| | |
|-------------------------------------|------|
| ACKNOWLEDGMENTS | ii |
| LIST OF TABLES | vi |
| LIST OF FIGURES | vii |
| LIST OF ABBREVIATIONS | viii |
| LIST OF APPENDICES | ix |
| ABSTRACT | x |
| ABSTRAK | xi |
| CHAPTER 1 INTRODUCTION | 1 |
| 1.1 Objectives | 3 |
| 1.2 Hypotheses | 3 |
| CHAPTER 2 LITERATURE REVIEW | |
| 2.1 <i>Pasteurella multocida</i> | 5 |
| 2.1.1 Colonial Morphology | 5 |
| 2.2 Gut- Associated Lymphoid Tissue | 6 |
| 2.2.1 Peyer's patches | 9 |
| 2.2.2 Lamina propria Lymphocytes | 9 |
| 2.2.3 Intraepithelial Lymphocytes | 10 |
| 2.2.4 M-microfold cell | 10 |

| | | |
|-----|---|----|
| 2.3 | Common Mucosal Immune System | 11 |
| 2.4 | Gut-associated Lymphoid Tissue in Intestinal Infections | 15 |

CHAPTER 3 MATERIALS AND METHODS

| | | |
|-----|----------------------------|----|
| 3.1 | Animal | 16 |
| 3.2 | Preparation of Inoculum | 16 |
| 3.3 | Experimental Design | 17 |
| 3.4 | Histological Technique | 20 |
| 3.5 | Immunoperoxidase Procedure | 24 |

CHAPTER 4 RESULTS

| | | |
|-------|---|----|
| 4.1 | Comparison of Number of Lymphocytes | |
| 4.1.1 | Comparison of Number of Lymphocytes between Control and Treated Group | 25 |
| 4.1.2 | Comparison of Number of Lymphocytes at Different Parts of Small Intestine Between Control and Treated Group | 29 |
| 4.1.3 | Comparison of Number of Lymphocytes at Intraepithelial, Lamina propria and Crypts between Control and Treated Group | 29 |
| 4.1.4 | Comparison of Number of Lymphocytes in control Group | 31 |
| 4.1.5 | Comparison of Number of Lymphocytes in Treated Group | 31 |
| 4.2 | Correlation Between Number of Lymphocytes and Area of Peyer's patches | 33 |
| 4.2.1 | Correlation between Number of Lymphocytes and Size of Peyer's patches in Control Group | 33 |
| 4.2.2 | Correlation between Number of Lymphocytes and Size of Peyer's patches in Treated Group | 33 |
| 4.3 | Immunoperoxidase Procedure | 35 |

| | | |
|-------------------------|---|----|
| CHAPTER 5 | DISCUSSION | 36 |
| CHAPTER 6 | CONCLUSION | 42 |
| REFERENCES | | 43 |
| APPENDICES | | |
| A | Histological Technique | 49 |
| B | Apparatus for Histological Technique | 52 |
| C | Immunoperoxidase Procedure | 55 |
| D | Chemicals | 56 |
| E | Monitoring Schedule | 58 |
| F | Table of Comparison of Mean Number of Lymphocytes | 59 |
| G | Statistical Analysis | 61 |
| CURRICULUM VITAE | | 66 |

LIST OF TABLES

| Table Number | | Page |
|---------------------|--|-------------|
| 4.1 | Mean number of lymphocytes among the goats between treated and control | 25 |
| A.1 | Tissue processing steps by using Automatic Tissue Processor | 50 |
| A.2 | The reagent used and the duration for the complete staining process | 51 |
| E.1 | Monitoring schedule of the goats | 58 |
| F.1 | Mean number of lymphocytes at different parts of small intestine between treated and control group | 59 |
| F.2 | Mean number of lymphocytes at intraepithelial, lamina propria and crypts between treated and control group | 59 |
| F.3 | Mean number of lymphocytes in intraepithelial, lamina propria and crypts at different parts of small intestine in control group. | 60 |
| F.4 | Mean number of lymphocytes in intraepithelial, lamina propria and crypts at different parts of small intestine in treated group | 60 |

LIST OF FIGURES

| Figure Number | | Page |
|---------------|--|------|
| 2.1 | Organization of Gut-Associated Lymphoid Tissue | 8 |
| 2.2 | Diagram of the cellular components of the mucosal immune system | 13 |
| 2.3 | Common mucosal immune system | 14 |
| 3.1 | The goats were kept in a pen | 18 |
| 3.2 | Food compartments of the goats in the pen | 18 |
| 3.3 | Inoculum of formalin-killed <i>Pasteurella multocida</i> B2 | 19 |
| 3.4 | Inoculum of formalin-killed <i>Pasteurella multocida</i> B2 was injected intratracheally | 19 |
| 3.5 | Samples were collected and washed with PBS before fix in 10% buffered formalin | 21 |
| 3.6 | Samples were fixed in 10% buffered formalin | 21 |
| 4.1 | Lymphocytes in the crypts at small intestine after 2 weeks post-exposure | 26 |
| 4.2 | Intraepithelial lymphocytes at small intestine after 2 weeks post-exposure | 26 |
| 4.3 | Lamina propria lymphocytes at small intestine after 2 weeks post-exposure | 27 |
| 4.4 | Follicle lymphocytes at Peyer's patches after 2 weeks post-exposure | 27 |
| 4.5 | Differences of number of lymphocytes between control and treated group | 28 |

| | | |
|-----|---|----|
| 4.5 | Differentiation of number of lymphocytes between treated and control group | 28 |
| 4.6 | Comparison of mean number of lymphocytes at different parts of small intestine between control and treated group | 30 |
| 4.7 | Comparison of mean number of lymphocytes at intraepithelial, lamina propria and crypts between control and treated group | 30 |
| 4.8 | Comparison of mean number of lymphocytes in intraepithelial, lamina propria and crypts at different parts of small intestine in control group. | 32 |
| 4.9 | Comparison of mean of number of lymphocytes in intraepithelial, lamina propria and crypts at different parts of small intestine in treated group. | 32 |
| 5.0 | Correlation between number of lymphocytes and size of Peyer's patches in control group | 34 |
| 5.1 | Correlation between number of lymphocytes and size of Peyer's patches in treated group | 34 |
| 5.2 | Specific Ig A binds at lymphocytes | 36 |
| A.1 | Simplified digramme of histological technique | 49 |
| B.1 | Automatic tissue processor | 52 |
| B.2 | Embedding machine | 52 |
| B.3 | Microtome | 53 |
| B.4 | Waterbath | 53 |
| B.5 | Image Analyze Computerized System | 54 |

LIST OF ABBREVIATIONS

| | |
|------------------------|------------------------------------|
| $^{\circ}\text{C}$ | Degree Celsius |
| % | Percentage |
| BHI | Brain Heart Infusion |
| PBS | Phosphate Buffered Saline |
| cfu/ml | Colony forming unit per milliliter |
| g | Gram |
| min | Minute |
| rpm | Revolution per minute |
| ml | milliliter |
| H_2O_2 | Hydrogen peroxidase |
| μ^2 | Micrometer square |
| Pp | Peyer's patches |
| IELs | Intraepithelial lymphocytes |
| LPLs | Lamina propria lymphocytes |

LIST OF APPENDICES

| Appendix | | Page |
|-----------------|-------------------------------------|-------------|
| A | Histological Technique | 49 |
| B | Histological Apparatus | 52 |
| C | Immunoperoxidase Procedure | 55 |
| D | Chemicals | 56 |
| E | Monitoring Schedule | 58 |
| F | Table of Mean Number of Lymphocytes | 59 |
| G | Statistical Analysis | 61 |

ABSTRACT

A study was conducted to determine the role of Gut-Associated Lymphoid Tissue (GALT) in gastrointestinal tract of goats following intratracheal exposures to formalin-killed *Pasteurella multocida* B2. Six clinically healthy goats were divided into two groups; goats group1 were subjected to intratracheal exposures to formalin-killed *P. multocida* B2 while group 2 was remained unexposed control. Prior to two weeks intervals post-exposure, all the goats were slaughtered and the samples of duodenum, jejunum and ileum were taken and fixed in 10% formalin. The fixed tissues were examined histologically. Number of lymphocytes was significantly increased ($P<0.05$) in treated group compared to control group. There was also a significant increase in number of lymphocytes at intraepithelial, lamina propria and crypts ($P<0.05$) between the groups. But, there was no significant increase in number of lymphocytes in duodenum, jejunum and ileum between the groups ($P>0.05$). There was a high correlation ($r=1$) between number of lymphocytes and size of Peyer's patches in treated group. Immunoperoxidase procedure was carried out to identify Ig A producing cells in GALT. According to the result, production of antibody- forming cells after an antigenic challenge are due to the presence of immune memory cells that are recruited and developed. The responses are parallel to the time of antibody responses. Intratracheally-stimulated vaccination had induced gastrointestinal immunity.

PERUBAHAN TISU LIMFOID BERKAIT USUS PADA KAMBING YANG DIDEHAHKAN DENGAN *Pasteurella multocida* B2 YANG DIMATIKAN DENGAN FORMALIN MELALUI INTRATRAKEA.

ABSTRAK

Kajian ini telah dijalankan untuk mengetahui peranan tisu limfoid berkait usus (Gut) di dalam saluran usus kecil kambing. Sebanyak 6 ekor kambing telah dipilih dan dibahagikan kepada 2 kumpulan. Kumpulan kedua merupakan kawalan manakala kumpulan pertama disuntik melalui trakea dengan inokulum *Pasteurella multocida* B2 yang dimatikan dengan formalin. Selepas pendedahan dua minggu, semua kambing telah disembelih dan sampel-sampel duodenum, jejunum dan ileum diambil serta diawet dalam 10% formalin. Tisu-tisu ini telah dikaji dengan cara histologi. Keputusan menunjukkan bilangan limfosit meningkat di kumpulan dirawat berbanding kumpulan kawalan ($P < 0.05$). Bilangan limfosit juga meningkat dalam intraepithelial, lamina propria dan crypts di kumpulan dirawat berbanding kumpulan kawalan ($P < 0.05$). Tetapi, tiada perbezaan dalam bilangan limfosit di antara duodenum, jejunum dan ileum ($P > 0.05$). Terdapat juga korelasi yang tinggi ($r=1$) diantara saiz Peyer's patches dengan bilangan limfosit di kumpulan dirawat. Selain itu, Immunoperoxidase prosedur telah dijalankan untuk mengetahui pembentukan sel antibodi. Keputusan menunjukkan masa selama dua minggu mencukupi untuk pembentukan sel antibodi selepas didedahkan antigen disebabkan oleh pembentukan sel ingatan. Kajian ini juga menunjukkan bahawa rangsangan melalui trakea ke atas tisu limfoid berkait usus dapat merangsangkan gastrointestinal immunity.