

SEVEN HUNDRED EIGHTY-EIGHT IN SEVENTEEN HUNDRED EIGHTY-EIGHT

EIGHTEEN HUNDRED EIGHTY-EIGHT

Parkerville, Newfoundland

ACADEMIC RECORD BOOK

FACULTY OF APPLIED TECHNOLOGY

UNIVERSITY OF NEWFOUNDLAND

2007

1100051137 Pe

Perpustakaan Sultanah Nur Zahirah (UMT)
Universiti Malaysia Terengganu

LP 24 FST 2 2007



1100051137

Serum Immunoglobulin G (IgG) level in goats following exposure to live attenuated *Pasteurella multocida* B2 / Joshua a Phillip William.



PERPUSTAKAAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

100051137

Lihat sebelah

HAK MILIK
PERPUSTAKAAN UMT

SERUM IMMUNOGLOBULIN G (IgG) LEVEL IN GOATS FOLLOWING
EXPOSURE TO LIVE ATTENUATED *Pasteurella multocida* B2

By

Joshua S/O Phillip William

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
UNIVERSITI MALAYSIA TERENGGANU
2007

1100051137

This project report should be cited as:

Joshua, P. W. 2007. Serum Immunoglobulin G (IgG) level in goats following exposure to live attenuated *Pasteurella multocida* B2. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, Terengganu. 47p.

No part of this project report may be reproduced 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
UNIVERSITI MALAYSIA TERENGGANU

UNIVERSITI MALAYSIA TERENGGANU

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

SERUM IMMUNOGLOBULIN G (IgG) LEVEL IN GOATS FOLLOWING EXPOSURE TO

TO LIVE ATTENUATED *Pasteurella multocida* B2

oleh **JOSHUA A/L PHILLIP WILLIAM** , no. matrik: **UK 10576** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)** , Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: /Verified by:

Penyelia Utama / Main Supervisor

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

Cop Rasmi:

Tarikh: **6^{hb} MEI. 2007**

PROF MADYA DR. MOHD. EFFENDY ABD WAHID
Pengarah
Institut Bioteknologi Marin
Universiti Malaysia Terengganu
21030 Kuala Terengganu, Terengganu.

Ketua Jabatan Sains Biologi/Head, Department of Biological Sciences

Nama: **DR. AZIZ AHMAD**

Cop Rasmi:

Tarikh: **6^{hb} MEI. 2007**

DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

ACKNOWLEDGMENTS

First and foremost, I would like to thank my Final Year Project Supervisor, Assoc. Prof. Dr. Mohd Effendy B. Abdul Wahid for his support and guidance throughout my project entitled “Serum Immunoglobulin G (IgG) level in goats following exposure to live attenuated *Pasteurella multocida* B2“. I would like to take this opportunity also to thank my Final Year Project Coordinators, Dr. Noraznawati Ismail and Mr. Wong Chee Ho and for their kind support.

I personally thank Dr. Aziz B. Ahmad (Department Head, Department of Biological Sciences), Dr. Cha Thye San, lecturers of Department of Biological Sciences, Cik Norazlina Bt. Abdul Aziz (Science Officer, Department of Biological Sciences), Pn. Ku Naiza Bt. Ku Nordin (Assistant Science Officer, Department of Biological Sciences), Mr. Mohammad B. Embong (Histology laboratory), Pn. Zarina and Pn. Mahidawati (Microbiology laboratory), Pn. Fatimah (Biochemistry laboratory), Raja Shairani Bt. Raja Soh and family, Weng Poh Leng, Devika Chinnayah, Vaanmathi Mayakrishnan, Raymond Dass s/o Yesudoss, Christopher Imbaraja s/o Rajakumar, Veterinary Department of Setiu District, Terengganu, Agro Livestock of Peladang Setiu Agro Resort, Terengganu, lecturers, All Starz, Chandramukhi, Snegithees and Hostel Kaigal for their helpful heart, comments and encouragements.

Finally, I would like to express my deepest gratitude and thanks to my parents and family members for their unfailing patience, support and encouragements to complete this study.

Thank you again.

TABLE OF CONTENTS

| | Page |
|--|-------------|
| ACKNOWLEDGEMENTS | ii |
| TABLE OF CONTENTS | iii |
| LIST OF TABLES | v |
| LIST OF FIGURES | vi |
| LIST OF ABBREVIATIONS | vii |
| LIST OF APPENDICES | viii |
| ABSTRACT | ix |
| ABSTRAK | x |
| | |
| CHAPTER 1 INTRODUCTION | 1 |
| 1.1 Introduction | 1 |
| 1.2 Objective of the Study | 2 |
| | |
| CHAPTER 2 LITERATURE REVIEW | 3 |
| 2.1 Antibodies | 3 |
| 2.2 Immunoglobulin G (IgG) | 3 |
| 2.2.1 Structural Features of IgG | 4 |
| 2.2.2 Biological Functions of IgG | 6 |
| 2.3 <i>Pasteurella multocida</i> | 6 |
| 2.4 <i>Pasteurella multocida</i> B2 | 7 |
| 2.5 Haemorrhagic Septicaemia | 7 |
| 2.6 Enzyme-Linked Immunosorbent Assay (ELISA) | 8 |
| | |
| CHAPTER 3 MATERIALS AND METHODS | 9 |
| 3.1 Animals | 9 |
| 3.2 Inoculum Preparation | 9 |
| 3.2.1 Biochemical Tests | 10 |
| 3.2.2 Formalin-killed Dead <i>Pasteurella multocida</i> B2 | 11 |
| 3.2.3 Live Attenuated <i>Pasteurella multocida</i> B2 | 13 |

| | | |
|--|---|----|
| 3.3 | Experimental Procedure | 14 |
| 3.4 | Blood Sample Collection | 17 |
| 3.4.1 | Serum Sample Preparation | 17 |
| 3.5 | Indirect Enzyme-Linked Immunosorbent Assays (ELISA) | 19 |
| 3.6 | Statistical Analysis | 21 |
| CHAPTER 4 RESULTS | | 22 |
| 4.1 | Measurement of Serum Immunoglobulin G (IgG) Level | 22 |
| CHAPTER 5 DISCUSSIONS | | 27 |
| CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS | | 30 |
| REFERENCES | | 31 |
| APPENDICES | | 36 |
| CURRICULUM VITAE | | 47 |

LIST OF TABLES

| Table | Page |
|---|------|
| 3.1 The experimental design used in the study | 15 |
| 4.1 Paired-Samples T Test to compare differences between groups in the study to time (week): Group 1 (Negative control) - Group 2 (Positive control), Group 1 (Negative control) - Group 3 (Treatment), and Group 2 (Positive control) - Group 3 (Treatment). | 25 |
| 4.2 Paired-Samples T Test to compare differences within groups of the study to respective time (week): Group 1 (Negative control), Group 2 (Positive control) and Group 3 (Treatment). | 26 |

LIST OF FIGURES

| Figure | | Page |
|---------------|--|-------------|
| 2.1 | Antibody structure of an Immunoglobulin G (IgG) monomer. | 4 |
| 2.2 | The schematic diagram shows the domain structure of an IgG molecule. The light chain (red colour) has one variable domain (V_L) and one constant domain (C_L). The γ heavy chain (one yellow and one purple) has a variable domain (V_H) and three constant domains (C_H1 , C_H2 and C_H3). | 5 |
| 3.1 | Three out of nine goats of either sex aged 7-9 months old used in the study. Goats from different study groups (Negative control, Positive control and Treatment) are kept separated in a 2.0 m × 2.0 m pen. | 10 |
| 3.2 | Formalin-killed <i>Pasteurella multocida</i> B2 inoculum kept in sterile Eppendorf tubes (maximum capacity: 1.5 ml). | 12 |
| 3.3 | Serum (from blood) kept in a sterile Eppendorf tube (maximum capacity: 1.5 ml). | 18 |
| 3.4 | Colour changes observed in the microtiter immunoassay plate after addition of sulphuric acid (H_2SO_4) to stop peroxidase substrate (Tetramethyl Benzidine) reaction. | 20 |
| 3.5 | Microtiter immunoassay plate used was read by using an automated ELISA reader at the wavelength of 450 nm. | 20 |
| 4.1 | Mean values of Optical Density (OD) of serum IgG level in three different groups of goats (Group 1, Group 2 and Group 3). | 22 |
| 4.2 | Comparison of total mean IgG level of goats in Group 1 (Negative control), Group 2 (Positive control) and Group 3 (Treatment) detected throughout the 10 weeks of study period. | 24 |

LIST OF ABBREVIATIONS

| | | |
|-------------|---|--|
| BHI | - | brain heart infusion |
| BSA | - | bovine serum albumin |
| cfu | - | colony-forming unit |
| cfu/ml | - | colony-forming unit per milliliter |
| ELISA | - | enzyme-linked immunosorbent assay |
| etc | - | <i>et cetera</i> |
| Exp | - | example |
| Fig | - | Figure |
| HPR | - | horseradish peroxide |
| HS | - | Haemorrhagic septicaemia |
| Ig | - | immunoglobulin |
| IgG | - | immunoglobulin G |
| kDa | - | kilo Dalton |
| kg | - | kilogram(s) |
| m | - | metre(s) |
| MHC | - | major histocompatibility complex |
| ml | - | milliliter(s) |
| nm | - | nanometer |
| OD | - | optical density |
| PBS | - | phosphate-buffered saline |
| PBS-Tween20 | - | phosphate-buffered saline with Tween20 |
| rpm | - | revolutions per minute |
| SD | - | standard deviation |
| UMT | - | Universiti Malaysia Terengganu |
| % | - | percentage |
| µl | - | microlitre |

LIST OF APPENDICES

| Appendices | Page |
|---|-------------|
| Appendix 1 Preparation of Blood Agar Media | 36 |
| 1.1 Preparation of 5% Blood Agar Media | 37 |
| 1.2 Preparation of 4% of Blood Agar Media | 37 |
| 1.3 Preparation of 3% of Blood Agar Media | 38 |
| 1.4 Preparation of 2% of Blood Agar Media | 38 |
| 1.5 Preparation of 1% of Blood Agar Media | 39 |
| 1.6 Preparation of 0% of Blood Agar Media | 39 |
| Appendix 2 Gram Staining | 40 |
| Appendix 3 Buffers | 41 |
| 3.1 Coating Buffer (Carbonate/Bicarbonate, 0.05 M, pH 9.6) | 41 |
| 3.2 Washing Buffer (pH 7.4) | 41 |
| 3.3 PBS/ Bovine Serum Albumin/Tween 20 (pH 7.4) | 41 |
| 3.4 Substrate Buffer (pH 6.0) | 42 |
| 3.5 Preparation of 0.1 M Sodium Acetate/ Citric Acid Buffer | 42 |
| 3.6 2 M Sulphuric Acid | 42 |
| Appendix 4 Preparation of <i>Pasteurella multocida</i> B2 Antigen for ELISA | 43 |
| Appendix 5 Randomized Complete Block Design | 44 |

ABSTRACT

Pasteurella multocida B2 is the causative agent of Haemorrhagic septicaemia (HS), a disease that infects cattle and water buffaloes in the region of Asia. One of the best methods to increase the protection against HS is by inducing the production of Immunoglobulin G (IgG). Thus, a study was carried out to determine serum IgG level in goats following exposure to live attenuated *Pasteurella multocida* B2. Nine clinically healthy goats of either sex aged 7-9 months old were divided into three groups. Goats in Group 1 (Negative Control) were the unexposed control; Goats in Group 2 (Positive Control) were subjected to double intranasal exposures to formalin-killed *P. multocida* B2 while goats in Group 3 (Treatment) were subjected to double intranasal exposures to live attenuated *P. multocida* B2. Enzyme-linked immunosorbent assay (ELISA) was subjected to the serum samples collected once every week from each group of goats to determine the level of IgG. As the results, significant differences ($p < 0.05$) of IgG were obtained among all the groups in the study. The serum IgG levels of goats exposed to live attenuated *P. multocida* B2 in Group 3 showed a higher level of IgG as a reaction of immune response following exposure throughout the study and peaked at Week 6 compared to the IgG level of goats in Group 1 and Group 2 detected throughout the study. It was concluded that live attenuated *P. multocida* B2 induced a higher level of IgG in goats in Group 3 compared to the IgG level of goats in Group 1 and Group 2.

**TAHAP IMMUNOGLOBULIN G (IgG) SERUM KAMBING BERIKUTAN
PENDEDAHAN TERHADAP *Pasteurella multocida* B2 YANG TELAH
DILEMAHKAN**

ABSTRAK

Pasteurella multocida B2 adalah agen penyebab hawar berdarah, satu penyakit yang menjangkiti haiwan ternakan seperti lembu dan kerbau di negara-negara Asia. Satu cara yang terbaik untuk meningkatkan perlindungan daripada Hawar berdarah adalah dengan merangsang penghasilan Immunoglobulin G (IgG). Oleh itu, satu kajian telah dijalankan untuk menentukan tahap IgG dalam serum darah kambing berikutan pendedahan terhadap bakteria *Pasteurella multocida* B2 yang telah dilemahkan. Sembilan ekor kambing berumur di antara 7 - 9 bulan, tidak diutamakan jantina telah diagihkan sama kepada tiga kumpulan. Kambing di dalam Kumpulan 1 (Kawalan negatif) merupakan kambing yang tidak didedahkan terhadap sebarang bentuk bakteria *P. multocida* B2; kambing di dalam Kumpulan 2 (Kawalan positif) adalah kambing yang didedahkan dua kali secara intranasal kepada *P. multocida* B2 yang telah dimatikan menggunakan formalin manakala kambing di dalam Kumpulan 3 (Rawatan) adalah kambing yang didedahkan dua kali secara intranasal kepada *P. multocida* B2 yang telah dilemahkan. Enzyme-linked immunosorbent assay (ELISA) dijalankan terhadap sampel serum yang dikumpulkan sekali setiap minggu daripada setiap kumpulan kambing untuk menentukan tahap IgG. Keputusannya, perbezaan tahap IgG dalam serum darah kambing menunjukkan nilai kesignifikan ($p < 0.05$) diperoleh jika dibandingkan dengan ketiga-tiga kumpulan kambing. Tahap IgG kambing yang didedahkan kepada *Pasteurella multocida* B2 yang telah dilemahkan dalam Kumpulan 3 menunjukkan tahap IgG yang tinggi kesan tindakbalas imun sepanjang tempoh kajian dan memuncak pada minggu ke-6 jika dibandingkan dengan tahap IgG kambing dalam Kumpulan 1 dan Kumpulan 2 sepanjang tempoh kajian. Kesimpulannya, bakteria *P. multocida* B2 yang telah dilemahkan merangsangkan tahap IgG yang tinggi dalam serum darah kambing Kumpulan 3 berbanding tahap IgG dalam serum darah kambing Kumpulan 1 dan Kumpulan 2.